High-Throughput Determination of Oil Content in Corn Kernels Using Nuclear Magnetic Resonance Imaging

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ABSTRACT: A new high-throughput method for measuring oil content in intact, single corn kernels is demonstrated using nuclear magnetic resonance imaging (MRI) methods. This nondestructive technique enables the evaluation of relative oil content in up to 2,592 corn kernels in less than 40 min using a 1.5 T clinical MRI scanner. Custom software was developed to process and analyze 3-D magnetic resonance (MR) image data rapidly. The precision and accuracy of the MR method for measuring oil content are discussed. The precision of the MRI results is shown to be dependent on MR scanner noise. The MRI results show very good relative accuracy compared with low-field NMR, NIR transmission, and accelerated solvent extraction measurements. Minor differences between the MRI and low-field NMR experimental protocols were shown to be inconsequential to the oil content measurement. Extending the MRI method to the analysis of other oilseeds and/or the use of other magnetic field strengths is discussed, as is a comparison of this MRI method relative to other high-throughput magnetic resonance screening techniques.

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KEY WORDS: High-throughput, intact seed, magnetic resonance imaging, MRI, nondestructive, proximate oil.

Quantitative analysis of oil content in single seeds is a valuable analytical method for monitoring the success of selective breeding and transgenic engineering programs focused on increasing oil content in commercial crops. Conventional analytical procedures, such as solvent extraction (1), accelerated solvent extraction (ASE) (2,3), supercritical fluid extraction (4–6), microwave-assisted extraction (7), and Soxtherm extraction (8), provide both high accuracy and high precision for measuring oil content. Unfortunately, these methods are time consuming, laborious, and involve hazardous solvents. The minimum sample quantities required using these methods often preclude measurements of single seeds, or at best provide single-seed measurements with reduced accuracy and precision. Most importantly, these methods destroy the sample, an undesirable approach if intact seeds are required for subsequent breeding experiments or for genetic investigations of the heritability of a high-oil trait.

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NIR transmission (NIRT) spectroscopy is a nondestructive method for evaluating bulk oil content in samples containing large numbers of seeds (9,10). Oil content is calculated using a nonlinear chemometric approach, which depends heavily on the creation of a hyperspectral model basis set. Robust NIRT results are achieved when the seeds being tested are similar in composition to the seeds used to construct the chemometric model. Trait differences between test and model seeds, i.e., differences in oil, protein, and/or starch content, can lead to poor measurement accuracy/precision for bulk samples and create difficulties using NIRT for measurement of single seeds. Since trait modification is the precise goal behind many selective breeding and transgenic programs, new NIRT chemometric models would have to be developed to account for differences in test and model seeds. An alternative approach, however, would be to use model-independent procedures that possess high accuracy and precision for analyses of single seeds.

Low-field pulsed ^{1}H nuclear magnetic resonance (NMR) relaxometry provides another nondestructive approach for evaluating oil content in intact seeds (11–16). These ^{1}H NMR methods do not suffer from the model-dependent disadvantages inherent to NIRT spectroscopic approaches. The signal amplitude of the Hahn echo detected in the NMR experiment directly correlates with the oil level within the seed. The NMR signal amplitudes are converted to relative oil content numbers using a standard calibration curve that correlates the NMR response of seeds with known oil contents. Ultimately, the percent oil content (w/w) for a single seed is calculated from the relative oil content using the total seed weight. Low-field NMR measurements that follow this normalization protocol are robust, accurate, and precise. These methods can measure single corn seeds with oil contents as low as 0.3% (w/w). The NMR measurement is not influenced by novel compositions of protein or starch and can be linearly extrapolated with good confidence beyond the calibrated range for unusually high- or low-oil-containing seeds.

Low-field NMR is a rapid analytical method, typically requiring about 30 s per sample. This level of throughput is still insufficient to meet the tremendous demands of selective breeding and transgenic engineering programs, which potentially would like to evaluate millions of individual seeds within one given breeding cycle. Higher throughput can be achieved by converting a serial measurement examining single samples in a sequential fashion to a parallel process that examines multiple samples

simultaneously. Parallel processes can be established by increasing analytical resources dedicated to making the measurements, or by improving the analytical efficiency of a given method. As with any high-throughput analytical screen, quality controls must be implemented to track seed identity during separate measurements. Quality controls also must include performance tests, comparisons, and cross validations of multiple instruments if they are used to screen and compare large numbers of single seeds.

This report describes a new, high-throughput magnetic resonance imaging (MRI) method that is capable of analyzing more than 15,500 single corn kernels within 4 h. This method uses standard protocols available on clinical 1.5 T MRI scanners such as those currently available in most hospitals. The use of a single MRI scanner equipped with a large-bore magnet, like those used to image the human body, enables simultaneous oil analysis of thousands of individual kernels. The identity of each seed is maintained *via* the collection of 3-D MRI data that spatially localizes the NMR signal for each seed placed within the active imaging volume of the MRI scanner. These MRI procedures are based on the same physical principles as those used in low-field NMR studies with the added benefit of tremendous improvements in data collection efficiency. The MRI results have been compared with results obtained using NIRT, low-field NMR, and ASE methods to validate the accuracy and the precision of the MRI procedure. The MRI method discussed herein has been implemented in a production laboratory environment and has been successfully applied to conventional and transgenic breeding programs by rapidly selecting individual seeds possessing high oil contents.

EXPERIMENTAL PROCEDURES

Preparation of seed and calibration samples. Corn kernels were obtained from selective breeding programs of Monsanto Company and its subsidiaries. The MRI corn samples were prepared by placing a weighed corn kernel in each well of a bar-encoded 24-well microtiter plate. Pure corn oil used for oil calibration standards was purchased from a local retail grocery store. The MRI oil calibration samples were prepared by placing 100 ± 10 mg of pure corn oil in 200-μL vials, weighing the oil content to ±0.2 mg, and placing sealed vials in each well of a bar-encoded 24-well microtiter plate.

MRI samples were prepared by arranging 12 of the 24-well plates on a Plexiglas™ tray in a single layer consisting of 3 rows and 4 columns. Nine of these layers were stacked to form a rectangular sample cube. The sample cube was placed on a Plexiglas base and covered with a Plexiglas cover. The base and the cover were fastened together using four threaded nylon rods, which served to align and lock the 24-well plates in the sample cube in place. A schematic drawing of a final, completed sample cube is shown in Figure 1. In all cases, individual corn kernels or standard oil samples were identified from their well position (Cartesian coordinate) in the sample cube and were tracked during data analysis using processing software that identified the sample based on its individual well position, the plate barcode label, and the layer within the sample cube.

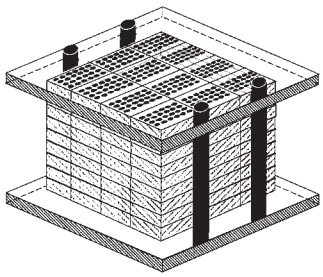

FIG. 1. Schematic drawing of a completed magnetic resonance (MR) sample cube. The MR sample cube contains 108 different 24-well microtiter plates arranged in nine layers of 12 plates arranged in 3 rows and 4 columns. Except for the microtiter plates, all components were constructed in-house from Plexiglas™ and nylon materials.

MRI. Experiments were performed using either a 1.5 T MAGNETOM™ VISION magnetic resonance (MR) scanner or a 1.5 T SYMPHONY MR scanner (Siemens, Erlangen, Germany) using the standard whole-body resonator (radio frequency coil). Both systems were equipped with 1-m diameter bore magnets. All MR data were obtained using vendor-supplied spin-echo protocols with an excitation flip angle of 90° and a refocusing flip angle of 180° for the radio frequency pulses. The gradient strength was set to 25 mT/m on all axes. With the gradient booster, the full strength ramp time was 312 μs. The receiver bandwidth was 130 Hz/pixel. All corn and oil calibration sample cubes were equilibrated at room temperature prior to analysis. The repetition time (TR) was set to 1920 ms, and the echo time (TE) was set to 17 ms, which was the minimum value attainable on the MR scanners. At this TE value the ^1H_2O MR signals (T2 ≈ 1 ms) from individual kernels had completely decayed.

The position of a sample cube within the magnetic field was determined using axial and saggital scout MR images. The final image data set acquired for each cube contained multiple coronal slices, acquired using the interleaved option. The data matrix for each coronal slice consisted of 256 × 256 pixels covering a field of view of 450 × 450 mm, which provided sufficient resolution to unambiguously identify and isolate the localized signal intensity for each corn kernel within each layer. For the corn oil calibration standards two scans are obtained, with the receiver gain set to 97.98 dB and FFT scale factor set to 0.1. For the corn-seed samples, four scans are obtained, with the receiver gain set to 116.98 dB and the FFT scale factor set to 0.007114. Manually setting these acquisition parameters for all runs helped optimize sampling (and avoid signal clipping) during the analog-to-digital conversion of the time-domain MR data. Maintaining these settings across all samples also allowed relative integrated signal intensities to be directly compared from cube to cube. The total data acquisition time was 18 min for each corn oil standard cube and 32.5 min for each corn sample cube. Representative images showing a single layer of corn kernels and a single layer of oil standards are shown in Figure 2. These images demonstrate that samples in the microtiter

plate wells have minor positional variability and magnetic field distortions, i.e., slight skewing is observed in the upper and lower rows of the image.

Each coronal MR image was assigned to each layer within the sample cube. A noise threshold was determined from the first summed image that corresponded to 2.5 times the SD of the noise level. All summed images were segmented using this noise-threshold value. The integrated MR signal intensity was then calculated for each well in each layer within a given sample cube. All MRI data were processed using IDL-based software programs (Research Systems, Inc., Boulder, CO) developed in-house.

The amount of oil in each corn kernel (% oil by weight) was automatically determined by normalizing the integrated MR signal amplitude for each kernel using the integrated MR signal amplitude and known weight for the oil standard that had occupied the same well position in the calibration standard cube, and then dividing by the initial weight of the kernel. Normalizing data for corn kernels with data from pure oil standards has been shown to be the most accurate calibration method for detection of MR signals (13). However, pure corn oil can differ from oil in intact seeds as a result of differences in nuclear relaxation processes, translational diffusion, and potential differences in oil composition caused by oxidation or the extraction process. Therefore, at this stage in the analysis, these measurements represent *relative* oil content for the individual intact seeds. These relative oil measurements were directly compared with the measurements obtained using low-field NMR and NIRT methods described below. Additionally, the absolute oil content of each kernel was calculated by further normalizing the mean of the relative oil content values either to the average oil value determined from the NIRT measurement of a bulk sample of corn kernels or to the low-field NMR results obtained from a subset of the corn kernels. Both the NIRT and low-field NMR measurements were calibrated to provide absolute oil content *via* calibration against ASE results (*vide infra*). The absolute oil content for each kernel was used as the screening criterion to support breeding and transgenic engineering efforts. All of the oil content calculations were performed using Visual Basic programs developed in-house.

NIRT spectroscopy. To evaluate the MRI results, bulk quantities of corn consisting of 30 to 50 mL of corn kernels (i.e., 100–150 kernels) were analyzed for average oil content using NIRT methods on a Tecator Infratec 1221 scanning monochromator (Foss North America, Eden Prarie, MN), with a 30-mm pathlength sample cuvette. Absorbance values, as log (1/T) where T represents transmission, were recorded at 2-nm intervals between 850 and 1048 nm. These absorbance values were translated to oil content measurements using Infratec Corn Analysis Model #CO980811 for the measurement of kernels with 2.9 to 10.7% oil contents. The commercial Analysis Model and standard operating procedure were validated relative to ASE results prior to this analysis; the SD of accuracy was shown to be less than 0.6% oil relative to dry sample weight.

Low-field NMR relaxometry. To evaluate the MRI results, further single corn kernels were analyzed for oil content using a Maran-23 Ultra low-field NMR spectrometer (Resonance Instruments, Whitney, United Kingdom), operating at 23.4 MHz proton frequency and a magnet temperature of 40.0°C. The measurement protocol followed AOCS Method AK 4-95, measuring the signal amplitude sum of a 50 μs acquisition window centered about the Hahn echo generated at 7.0 ms (15). Signal amplitudes of sample kernels were automatically converted to % oil measurements by the MultiQuant software package (Resonance Instruments), based on a linear calibration determined from low-field NMR and ASE results. The linear calibration was established using corn kernels that possessed an oil content that ranged from 1.6 to 14.5%. All low-field NMR measurements were determined from the sum of eight sequential acquisitions, which provided the best combination of accuracy and throughput required for this analysis. The SD of NMR result accuracy relative to ASE results was 0.66%. The sample was measured at room temperature for 12 s, and no temperature effects were observed during this brief analysis period.

Low-field NMR relaxometry was also performed to investigate the effect of translational diffusion within intact corn kernels. A series of exponentially decaying NMR signal amplitudes of single corn kernels were acquired using a Carr-Purcell Meiboom-Gill (CPMG) pulse sequence. The TE parameter was varied from 200 μs to 125 ms, and the number of echoes was set so that the total acquisition time was 2.0 s. Spin-spin relaxation time constants (T2) were determined from the sum of the even echoes of 32 sequential acquisitions by a least-squares fitting of these signal amplitudes to a single decaying monoexponential function.

ASE. To calibrate low-field NMR relaxometry results with oil content, single corn kernels were analyzed for oil content using a Dionex ASE 200 Accelerated Solvent Extraction System. Single kernels were first ground to a consistent mesh size

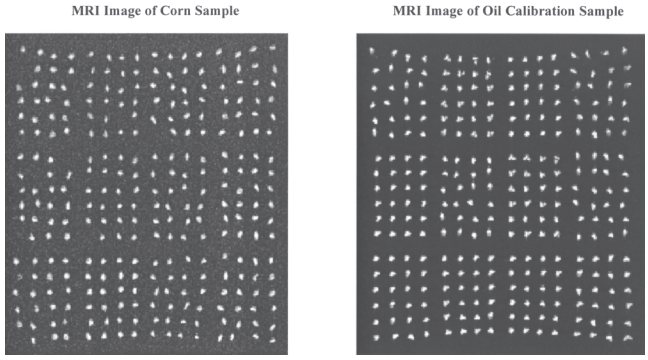

FIG. 2. Representative MR images of corn kernels and oil calibration standards. Magnetic resonance imaging (MRI) images of layer 5 of an MRI corn sample cube and layer 5 of an MRI oil calibration sample cube were acquired and processed using standard procedures. These images are scaled differently to demonstrate the magnitudes of the signal amplitudes relative to the noise floor for each image. Layer 5 of the corn sample cube contained a single corn kernel in each well, except for the well in the lower left corner, which was intentionally left empty. Corn kernels in several other well positions showed little signal intensity relative to the noise; subsequent analysis of these corn kernels using low-field NMR showed that these corn kernels contained less than 1.5% oil.

using a grinding apparatus developed in-house. Oil was extracted from 100 ± 2.5 mg of ground corn using 2 mL of petroleum ether for 5 min at 105°C at 1000 psi (6890 kPa); the extraction process was repeated three times per sample. The SD of reproducibility was 0.22% oil for this method. Two 100-mg samples were tested for each corn kernel, and if results differed by more than 0.7% oil (3 times the SD) for a particular corn kernel, the ASE results for that kernel were discarded.

RESULTS AND DISCUSSION

Precision of MRI signal amplitude detection. Maximum throughput for the MRI seed analyses method is achieved by closely matching the physical dimensions of the sample cube to the useful dimensions of the main magnetic field and radiofrequency coil (homogeneous volumes), and the magnetic gradient field gradients (volume of linearity). This active sample volume was mapped by comparing 16 separate sets of MR image data, i.e., all 9 layers, for a single MRI corn sample cube (Fig. 3). Oil content of the individual corn kernels ranged from 1.2 to 19.9% oil. Regions toward the center of the sample volume tended to show smaller SD than those toward the periphery. Only 7 of 2,592 well positions within the sample cube display a SD greater than 8% for MRI signal amplitude reproducibility. These 7 wells are located at the extreme periphery of the sample cube, which was an anticipated consequence of fitting a rectangular sample cube into a roughly elliptical useful volume. Larger-sized MRI sample cubes display significantly greater errors (data not shown) at the periphery regions than those shown in Figure 3. The SD

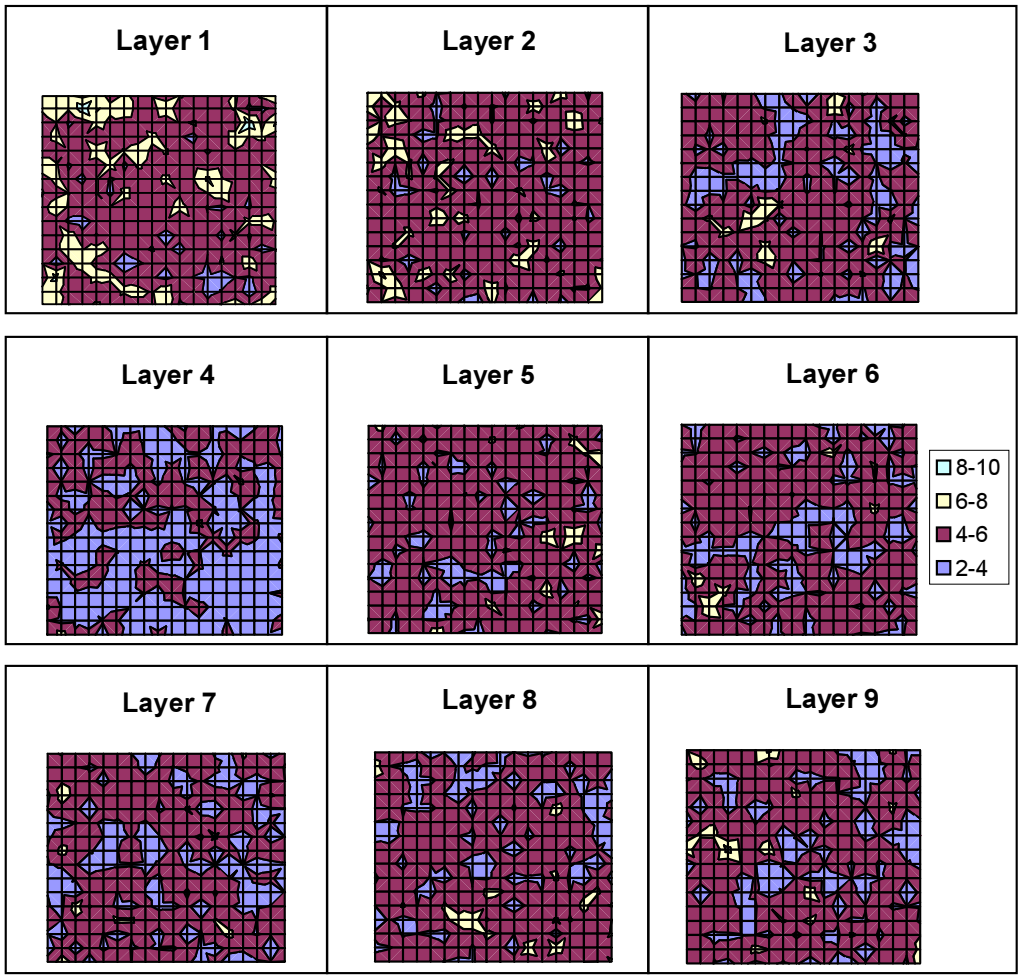

FIG. 3. Map of uncertainty in corn kernel oil ^{1}H MR signal amplitudes. The map of uncertainty in corn kernel oil ^{1}H MR signal amplitudes was generated by sequentially acquiring and processing 16 sets of MRI images of an MRI sample cube that contained single corn kernels at each well position and by calculating the SD of the MRI signal amplitudes at each well position. Each grid point of each map represents a well position within a cube layer. For clarity, the SD have been converted to a percentage of the average MRI signal amplitude of all well positions in the cube. Contour lines are drawn at 2–4, 4–6, 6–8, and 8–10% SD. Layers are numbered starting at the top of the cube and progressing sequentially through to the bottom layer. For abbreviations see Figures 1 and 2.

of the signal amplitudes did not show a correlation with the amplitude values.

The SD of MRI signal amplitude measurements matched a Gaussian distribution for all wells within the sample cube. Further analyses of the 16 sets of MR image data showed that the precision of MRI signal amplitudes correlated with the square root of the number of scans but did not correlate with the magnitude of the signal amplitudes. Both of these results indicate that the precision of the MRI measurement was dependent on stochastic, phase-incoherent spectrometer noise contributions to the signal amplitude that do not scale with the signal amplitude (17,18). This is an expected statistical property for background noise using Fourier-transform NMR techniques.

Because of nonidealities in scanner hardware, the MRI signal amplitude per gram of oil differed marginally for each well position within the sample cube. Fortunately, spatial dependencies can be removed because the MRI signal amplitude at each well position is relatively invariant between MRI experiments. Thus, the MRI image of each corn kernel can be normalized relative to the MRI image of an oil calibration sample at the same well position. In addition, a comparison of the MRI results for the same oil calibration sample obtained using two different MRI scanners showed excellent correlation, which demonstrated that spatial differences between different MRI scanners can also be accounted for by this process (Fig. 4).

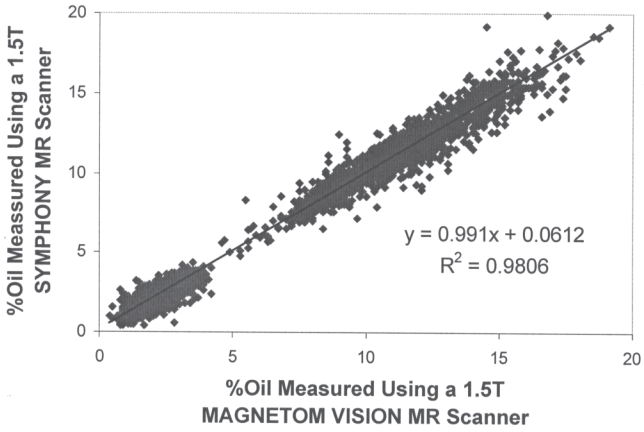

FIG. 4. Comparison of oil content measurements using different 1.5 T MRI scanners. An MRI sample cube containing 2,496 corn kernels was scanned using a 1.5 T MAGNETOM™ VISION MR scanner and a 1.5 T SYMPHONY MR scanner (both from Siemens, Erlangen, Germany). The data were acquired and processed following standard procedures described herein. The SD of the differences between each measurement was 0.68% oil. For abbreviations see Figures 1 and 2.

Accuracy of MRI oil measurements. The accuracy of the MRI method for determining oil content was measured by comparing the MRI data with the results obtained using low-field NMR relaxometry, ASE, and NIRT methods. The analysis of a single MRI image of 2,592 single corn kernels was compared with single-seed analyses of the same corn kernels using low-field NMR relaxometry (Fig 5). This evaluation shows an excellent linear correlation ($R^2 = 0.97$) and a very good relative correspondence (slope = 0.93). A strong correlation also is observed between the MRI and NMR results for corn kernels analyzed from different MRI sample cubes on different days (Fig. 6). Further validation of the MRI results was achieved by comparing MRI results with ASE measurements. In this assessment, 72 single corn kernels that were analyzed using ASE also showed good linear correlation and relative correspondence (Fig. 7) with the MRI results. Similarly, MRI results correlated well with NIRT results (Fig. 8). For this comparison, corn kernels from 34 different pedigrees were combined to form 34 different bulk samples, which were analyzed using NIRT methods. The oil value of these bulk samples agreed well with the average oil value determined from single-kernel MRI measurements. A difference in weight basis accounts for the high underdetermination of oil content by MRI vs. NIRT (*x*-intercept <0% oil), relative to the underdetermination of oil contents by MRI vs. the other methods as shown in Figures 5–7. The SD of the differences of the MRI and NIRT measurements was 0.90% oil.

The comparison of MRI results with low-field NMR, NIRT, and ASE results demonstrates that MRI methods systematically underestimate the oil content. The slight underestimate is attributable to the normalization of the MRI results relative to pure corn oil. The T2 value for oil contained in intact corn kernels is shorter than the T2 value for pure corn oil (<100 ms vs. ~200 ms, respectively). This difference translates into a loss in MRI signal amplitude for an intact kernel relative to the same amount of pure oil when a TE of 17 ms is used in the spin-echo MRI pulse sequence. In addition, slight variations in T2 values can occur with changes in temperature and presumably with parameters such as different pedigrees of corn and kernel growth conditions. Although all sample temperatures were maintained within the instrumentation specifications of ±0.3°C, a change in temperatures between methods might lead to inaccuracies between MRI and

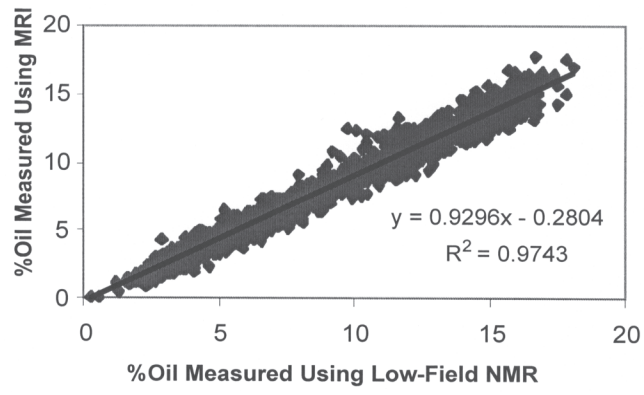

FIG. 5. MRI of seeds analyzed on the same day compared with low-field NMR. MR images of a sample cube containing 2,278 corn kernels was acquired and processed using standard procedures to measure the oil content of each corn kernel. Each corn kernel was then analyzed using low-field NMR relaxometry within 2 wk of the MRI data acquisition. The same weight of each kernel was used for both the MRI and NMR analyses, which did not appreciably change during the 2-wk period. The SD of the differences of the MRI and low-field NMR measurements was 0.68% oil. For abbreviations see Figures 1 and 2.

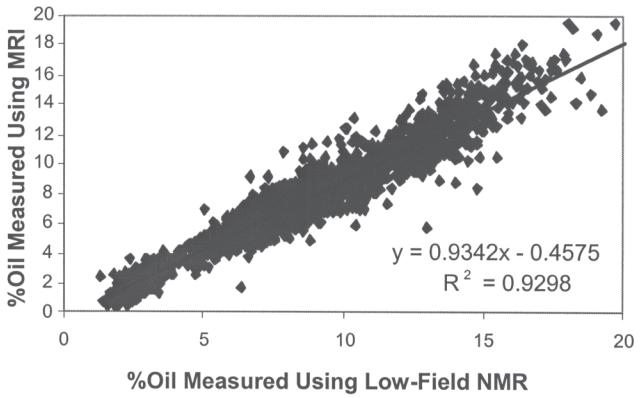

FIG. 6. Low-field NMR results compared with MRI results of seeds analyzed on different days. One hundred thirty-two MRI sample cubes were analyzed using the MRI method over the course of 6 wk. Eighteen corn kernels were randomly selected from each sample cube, and these 2,376 corn kernels were each analyzed using low-field NMR relaxometry within 2 wk of the MRI data acquisition. The same weight of each kernel was used for both the MRI and NMR analyses. The SD of the differences of the MRI and low-field NMR measurements was 0.97% oil. For abbreviation see Figure 2.

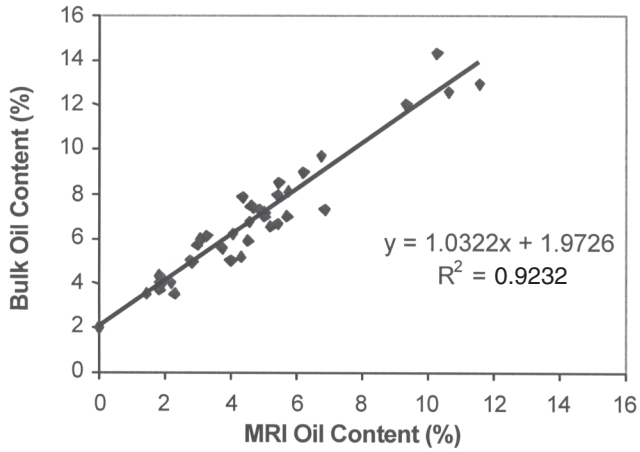

FIG. 8. Comparison of NIR transmission (NIRT) results with MRI results. Oil contents of a volume of 30 to 50 mL of corn kernels from 34 pedigrees were measured using the MRI method. The corn kernels from each pedigree were each combined to form 34 bulk samples, which were then analyzed for oil content using NIRT. The weight of each corn kernel was used to determine the average oil content of the bulk sample as measured by the MRI method. The dry weight (total kernel weight excluding weight of the moisture within the kernel) was used to determine the average oil content as measured using NIRT. For other abbreviation see Figure 2.

the other methods. Hence, as previously mentioned, MRI methods measure relative oil content. The MRI-derived oil content of a particular corn kernel must only be considered with respect to the oil contents of other corn kernels measured during the same study. Relative oil values are acceptable for corn breeding programs, where only the kernels with highest oil contents are selected for further investigation. Yet, if desired, the absolute oil values can be obtained by normalizing the MRI measurements relative to another method. A further trivial refinement would be to quantify the "typical" oil T2 for corn pedigrees of interest and simply correct for the differential spin echo oil MR signal attenuation between kernel and standard samples using the simple ratio, $[\exp(-TE/T2_{kernel})/\exp(-TE/T2_{standard})]$.

The SD of the difference between low-field NMR and the MRI results for corn kernels in a given sample cube was 0.68% of the measured oil content. This value compares favorably with the SD of accuracy of the low-field NMR method. This SD increased to 0.97% oil when low-field NMR and MRI were used to co-analyze a series of different sample cubes on different days, indicating that day-to-day variance also affects accuracy of the MRI method. The SD of the difference between the low-field NMR and the MRI results improved from 0.97 to 0.63% oil for corn kernels analyzed with four sequential MRI measurement repetitions. Additional sequential repetitions did not further improve this level of accuracy, suggesting that the variances responsible for this SD must be due not to variances within the MRI scanner (i.e., due to spectrometer noise) but instead to variances associated with the sample. For example, small positional differences of the MRI sample cubes or of the individual samples within the microtiter plate wells, or minor differences in the materials used to construct MRI cubes that lead to differences in magnetic susceptibilities, may cause variance in the detected MRI signal amplitude of the same sample. Similarly, minor differences could exist in the T2 values for different seeds in a given pedigree. Further improvements to the MRI method to reduce sample-dependent variances were considered, such as the use of plates with smaller wells, or the use of the same or susceptibility-matched MRI sample cubes. These procedural modifications were deemed impractical for high-throughput screening. Because the value of this high-throughput method is a function of both accuracy and throughput, the minor decrease in accuracy relative to other oil analysis methods is more than offset by the tremendous increase in sample throughput when meeting high demands of breeding and transgenic engineering programs.

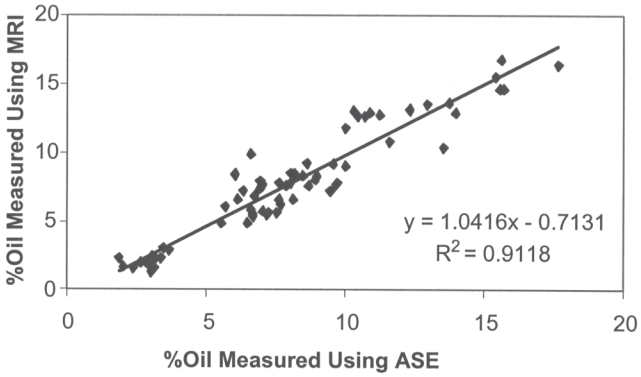

FIG. 7. Comparison of accelerated solvent extraction (ASE) results with MRI results. Of the 2,376 corn kernels analyzed in Figure 6, 72 were randomly selected for ASE oil content analysis. The same weight of each kernel was used for both the MRI and ASE analyses. The SD of the differences of the MRI and ASE measurements was 1.25% oil. For abbreviations see Figures 2 and 6. For abbreviation see Figure 2.

Choice of spin TE and experiment (TR). The choice of TE

and TR parameters in the MRI experiment affects the accuracy of the oil measurement for intact corn kernels. Standard AOCS methods recommend using TE = 7 ms to ensure that the water ^{1}H NMR signal in the kernel has sufficiently decayed prior to detection of the oil ^{1}H NMR signal. A short TE also minimizes the loss in the oil signal due to oil T2 relaxation processes. Unfortunately, the minimum TE attainable using the current MR scanner hardware was 17 ms. Use of a longer TE in the MRI experiment better suppresses the water signal (a marginal improvement given the ^1H_2O T2 in kernels of ~1 ms), but it also exacerbates the T2-induced oil signal loss leading to further degradation in the accuracy of the oil measurement. Such signal loss is well recognized in the field and, given the use of identical acquisition parameters for all sample cubes, should not significantly affect the *relative* comparison of individual kernel oil content measured in this experiment. Water and oil T2 values in corn kernels were determined to be 600–800 ms and 200–400 ms, respectively, as measured for bulk corn samples using low-field NMR relaxometry. Thus, at a TE of 17 ms the loss in oil detection sensitivity is only 2.5 to 5% relative to standard AOCS methods. Also, the TR was set to 1920 ms, which ensured that greater than 99% of the sample's magnetic polarization had returned back to thermal equilibrium between NMR signal excitations. Selection of appropriate TR value is critical for quantitative analyses and is well described by NMR relaxation theory.

The MRI oil signal can also suffer from attenuation losses due to translational diffusion of oil in the presence of internally or externally generated magnetic field gradients during TE. This diffusion effect could be substantially greater at 17 ms than it would be at 7 ms. To investigate this potential problem, T2 values for a series of CPMG experiments with TE spanning 200 ms to 125 ms were performed using low-field NMR relaxometry on a random selection of available corn kernels. Translational diffusion of the oil within the kernel, if significant, would manifest itself as a decrease in the apparent T2 value for experiments with longer TE (19,20). No change in the T2 time constant was observed for any of the samples, suggesting that translational diffusion of oil in the kernels in the presence of internal magnetic susceptibility-induced field gradients does not significantly affect signal amplitudes at least on a timescale between 200 μs and 125 ms at low magnetic-field strengths. A similar experiment cannot be performed using the MRI scanner when a minimum TE of 17 ms is needed. Even though the MRI scanner field strength is almost three times stronger than the low-field NMR field strength, the effects of translational diffusion are expected to be similar when comparing the high-field TE of 17 ms vs. the low-field TE of 125 ms. Whereas possible deleterious effects of diffusion in the externally applied MRI field gradients were not present in this low-field experiment, the displacement motion of oil within the corn kernel is expected to be highly restricted/hindered and thus, *a priori*, to be ineffectual in modulating the oil's MRI spin echo signal amplitude at TE of 17 ms.

Minimum MRI-detectable oil content. For the given MRI data acquisition and processing parameters, single corn kernels must contain a minimum oil content of approximately 1.5% oil to generate a detectable MRI signal. Below this level, the MR signal amplitude is below the noise-threshold segmentation value set for the method. The exact minimum detectable oil content depends on the weight of the kernel, the position of the kernel within the MRI sample cube, and the influence of other sample-related variances during the measurement. Figure 2 shows an example of an MRI corn image that contains some corn kernels with less than 1.5% oil. On occasion, kernels with oil contents as high as 2.7% have generated insufficient MRI signal amplitudes for measuring oil content. These situations usually occur in the periphery of the MRI cube samples where the precision and accuracy are lowest. Fortunately, the qualitative identification of low-oil-content corn kernels is sufficient for studies focused on identifying high-oil-containing kernels. The detectable oil limits could be raised by reducing the noise-threshold value. Increased signal-averaging and/or higher-field MR scanners also could be used to improve the signal-to-noise ratio and increase the detectable oil levels. Preliminary results at magnetic field strengths of 4.7 T were comparable with the results obtained using the 1.5 T MR scanner, as has been observed with other types of MRI analyses (21). The improved sensitivity at higher fields, however, can be offset by losses due to increased magnetic susceptibility. Modifications of the MR method, such as these, could alter both data collection times and corn kernel throughput and justify further investigation.

Establishment of a new maximum standard for oil measurement throughput of intact seeds. In general, the slow repolarization rate of ^{1}H magnetization (~1 s) and the low signal sensitivity of NMR (relative to other types of spectroscopic techniques) require relatively long data acquisition times per sample. In practice, for samples with strong signals, NMR requires roughly 10 min per sample for spectrometer preparation, sample changing, data acquisition, and data analysis, but only a small fraction of this time is actually spent acquiring NMR signals. The development of flow-NMR techniques, whereby samples are automatically transferred to and from a sample cell within the magnet using liquid handling pumps, has improved throughput to approximately 5 min per sample (22–25). These methods require substantial quality controls to ensure that sample purity and other environmental conditions are maintained during sample transfer and NMR data acquisition (22). The fastest sustainable throughput achieved using automated flow-NMR techniques was roughly 1.5 min per sample, which was optimized only for qualitative screening purposes (25). Further improvements in sample throughput using these destructive serial analytical NMR approaches are unlikely.

The nondestructive and quantitative MRI methods described herein are routinely applied in Monsanto's laboratories to analyze over 15,500 corn kernels within 4 h. Without robot-assisted sample preparation, roughly 3.5 h is needed to prepare the sample cube, about 35 min is needed to set up and acquire the MRI data, and only ~5 min is needed to process the multiple-slice MRI data. Thus, over the long term, the slow steps in this process are the weighing and cube construction steps, which can be easily overcome by using more weighing and cube-constructing

machines. High-throughput screening at a rate of less than 1 s per kernel is achieved using the described parallel analytical method, which is almost two orders of magnitude faster than the fastest high-throughput serial analytical flow NMR method. Improved quality control is attained using the MRI method relative to destructive analytical methods, since environmental conditions are uniform, and sample handling is minimized for all kernels within the MR sample cube. This analysis rate is the fastest ever demonstrated for biochemical screening for oil content in oilseeds and represents a new practical standard for the maximum throughput that can be achieved for other biochemical screening methods using magnetic resonance techniques. The current MRI methods can be modified and applied to studies of other intact oilseeds. Preliminary studies of soybeans and canola seeds demonstrate even higher throughput because more seeds can be placed in an MR sample cube and positioned within the active imaging volume of the MR scanner. The precision, accuracy, and throughput for other oilseeds will depend upon the oil content of the seeds, as well as the factors described in this report.

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