Nondestructive Single-Seed Oil Determination of Meadowfoam by Near-Infrared Transmission Spectroscopy

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ABSTRACT: Near-infrared transmission spectroscopy (NITS) was explored for single-seed oil determination of meadowfoam. Two calibration sets were determined by principle component analysis of recorded spectra. Nuclear magnetic resonance (NMR) spectroscopy was used for oil content determination. The calibrations had standard errors of cross-validation of 3.6 and 4.4%, respectively, with an oil content ranging from 0.8 to 45.7%. This error is similar to that reported for NITS of single maize kernels, relative to the respective oil content ranges. Although not as accurate as NMR spectroscopy, NITS does provide a fast, efficient, and nondestructive method of predicting oil content of individual meadowfoam seeds.

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The objective of this research was to assess the utility of near-infrared transmission spectroscopy (NITS) for nondestructive single-seed oil determination of meadowfoam.

Meadowfoam (*Limnanthes* spp.) is a new oilseed crop that has been recently domesticated at Oregon State University and commercialized in Oregon by the Oregon Meadowfoam Growers Association (1–3). The triglyceride seed oil is of unique composition, a virtually pure raw oil source of long-chain fatty acids (4) with perhaps the greatest oxidative stability of all known vegetable oils (5). The plant is a herbaceous winter–spring annual, typically seeded in October in the Willamette Valley of Oregon, where approximately 2600 acres were harvested in 1996.

Initial commercial sales have been primarily for high-value personal-care product markets. Numerous other industrial market applications may develop as factors such as price, utilization experience, derivative development, and supply logistics are established. Profit drives the commercialization process, and oil yield per acre is a prime objective. Increasing the oil content of seeds is one important component of improving oil yield, and this may be enhanced through single-seed selection.

It has been demonstrated that the rate of oil content gain in maize (*Zea mays*) can be improved through single-seed selection, as opposed to composite sample analysis (6). Recently, single-kernel oil determination in maize by NITS has been reported (7). Like maize, meadowfoam breeding and selection for increased oil content may be augmented by nondestructive single-seed analysis for oil content. Unlike maize kernels, the relatively small mass (10 mg average) and large physical variation observed in meadowfoam seeds (3–32 mg) are ambitious limits to accommodate for a NITS calibration.

NITS analysis is fast, efficient, nondestructive, and amenable to substantial automation compared to traditional chemical analysis. It has potential to advance the rate of progress in meadowfoam oil yield improvement and to facilitate breeding programs and physiology studies where further use of live seeds is necessary.

MATERIALS AND METHODS

Individual meadowfoam seed spectra were obtained with a Tecator Infratec 1255 scanning monochronometer, which was modified with a custom single-seed adapter (Perstorp Analytical, Inc., Silver Spring, MD). The custom single-seed adapter and control mechanism can automatically position up to 23 individual seeds for scanning. Absorbance values, log (1/T) (T = transmission), were recorded at 2-nm intervals between 850 and 1050 nm. Seeds were manually placed with the embryo up, as this was the most stable orientation in the single-seed adapter.

Two calibration models were constructed by using Infratec Calibration Maker software (Perstorp Analytical, Inc.) with partial least-squares regression. Principle component analysis (PCA) is the accepted and established procedure in spectroscopy for efficiently selecting a population of diverse spectra (8,9), and it was used to determine the calibration seed sets. Cross validation with 20 segments was used as the validation method of the resulting calibrations.

For the first calibration, a total of 966 seeds from 21 individual sources, from our breeding program, representing a variety of genetic origin and 7 growing seasons, were randomly selected for PCA. One NIT spectrum was recorded for each of the selected seeds. PCA was performed to yield a calibration set of 75 seeds.

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Larger seeds were included in the second calibration because increased seed size has contributed to increased oil yield per acre (10). These larger seeds were selected from quantities of bulk seed samples by screening and by using an air blower from E.L. Erickson Products (Brookings, SD). The average mass of the resulting seeds was 20.6 mg, an average increase in mass of 104% over the seeds used in the first calibration. Of these larger seeds, 368 were randomly selected, and NIT spectra were recorded. These spectra were combined with the 966 spectra from the first calibration, and PCA was performed on the resulting 1334 spectra to produce a calibration set of 100 seeds.

Traditional wet-chemistry methods, used for oil determination with large seeds such as soybean or maize, are much less practical for the small meadowfoam seed with oil contents that may range as low as 0.5 mg per seed or less.

Pulsed nuclear magnetic resonance (NMR) can accurately determine oil content of oilseeds below 10% moisture content, and methods of simultaneous determination of oil and moisture content have been developed (11,12). A low average moisture content of 8.1% for the meadowfoam seeds in this study was found gravimetrically by heating a representative bulk sample of seeds to 103°C for 17 h. High-frequency (187 MHz) pulsed NMR has been used to determine the oil content of single maize kernels, with a reproducible single-kernel oil content standard deviation of 0.3% (7). This error is sufficiently small to warrant the use of high-frequency pulsed NMR as a reference method, and it was therefore used for oil determination of the calibration seeds.

Individual seed oil content determinations were obtained with a 200-MHz Bruker ACF-200 series FT NMR spectrometer by Numare Spectralab, Inc. (Berkeley Heights, NJ). The 5-mm VSP-200 micro-probe was baked at 152°C for 24 h to remove adsorbed moisture. A HAHN spin-echo pulse sequence was used with a spectral width of 8064 Hz and signal averaging of 128 scans of 2048 data points each. A calibration curve was constructed by correlating the average of three NMR measurements with weighted samples of super-refined meadowfoam oil from Croda Japan (Croda, Inc.; Yorkshire, England), and the linear regression fit had a correlation coefficient of 1.00 (Fig. 1). Every fifth determination was repositioned and repeated three times, resulting in a reproducible average seed oil content standard deviation of 0.07%.

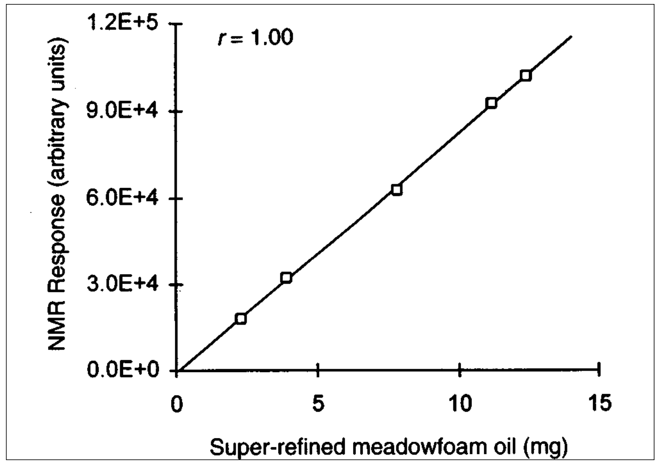

FIG. 1. Plot of nuclear magnetic resonance (NMR) calibration linear regression.

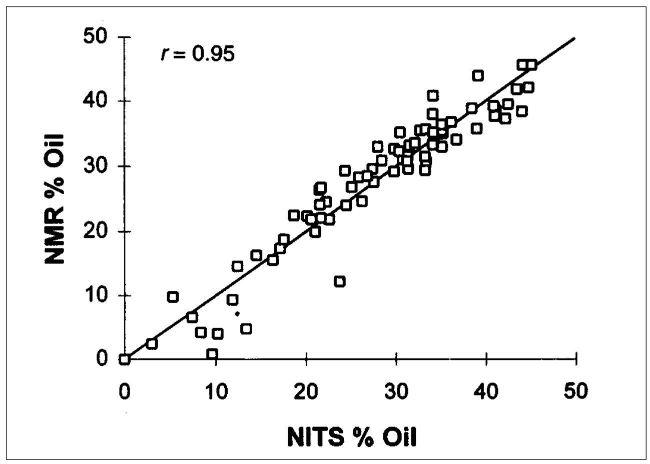

FIG. 2. Plot of NMR-predicted oil content vs. near-infrared transmission spectroscopy (NITS)-predicted oil content with linear regression by cross-validation of the first calibration set (75 seeds). See Figure 1 for other abbreviation.

RESULTS AND DISCUSSION

The calibration set of 75 seeds, selected by PCA from a random set of 966 seeds, had an average seed mass of 10.1 mg, with a standard deviation of 4.0 mg, and a mass range of 3.0 to 21.9 mg. The average oil content was 28.5%, with a standard deviation of 10.9%, ranging from 0.8 to 45.7%. This calibration had a correlation of 0.95 (r^2 = .90), and a standard error of cross-validation (SECV) of 3.6% (Fig. 2).

The calibration set of 100 seeds had a correlation of 0.88 (r^2 = 0.77), and a SECV of 4.4% (Fig. 3). This broader calibration spans the same oil content range as the first calibration with an average oil content of 27.1% and a standard deviation of 8.5%. The average mass was 16.4 mg with a standard deviation of 7.1 mg and a range of 3.0 to 32.6 mg. By cross-validation, both NITS calibrations consistently overpredicted the oil content of seeds weighing less than 5 mg (Figs. 4 and 5) vs. the reference method.

In constructing the 100-seed calibration, only 39 seeds (4.0%) from the original 966 seeds were selected by PCA, while 61 seeds (16.6%) from the 368 larger seeds were selected. This indicates that the larger seeds were much more likely to be selected by PCA due to more unique characteristic spectra from increased absorbance. The average mass of these larger seeds was 20.6 mg, with a mass range of 9.3 to 32.6 mg. NMR analysis determined that the average oil content was 28.1%, similar to the smaller seeds from the first calibration set, with a range of 4.8 to 38.8%. The SECV of a calibration is expected to decrease with an increasing calibration

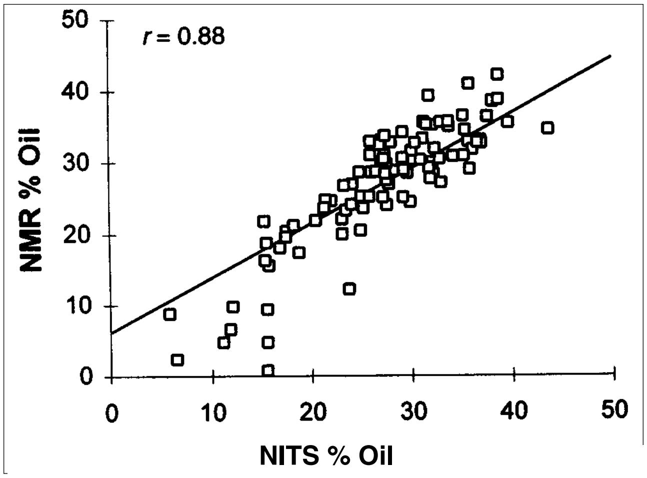

FIG. 3. Plot of NMR-predicted oil content vs. NITS-predicted oil content with linear regression by cross-validation of the second calibration set (100 seeds). See Figures 1 and 2 for abbreviations.

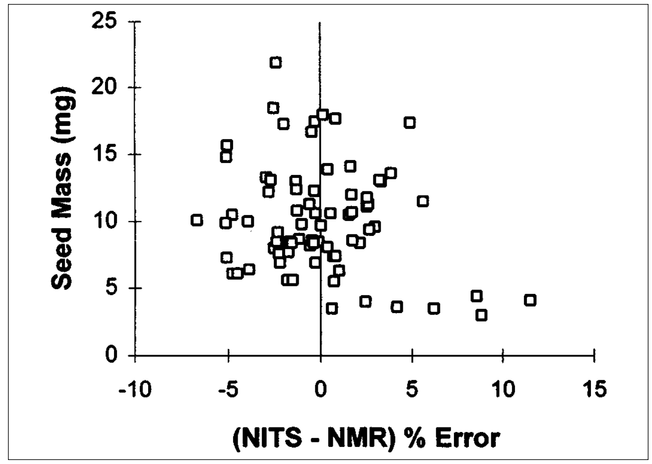

FIG. 5. Plot of (NITS-NMR) % oil prediction error vs. seed mass by cross-validation of the second calibration set (100 seeds). See Figures 1 and 2 for abbreviations.

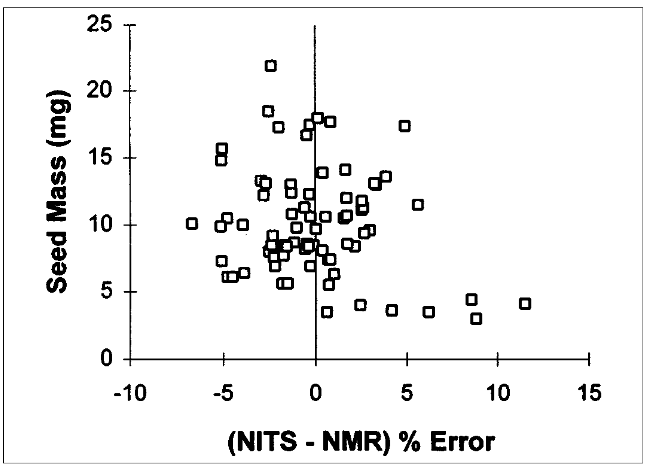

FIG. 4. Plot of (NITS-NMR) % oil prediction error vs. seed mass by cross-validation of the first calibration set (75 seeds). See Figures 1 and 2 for abbreviations.

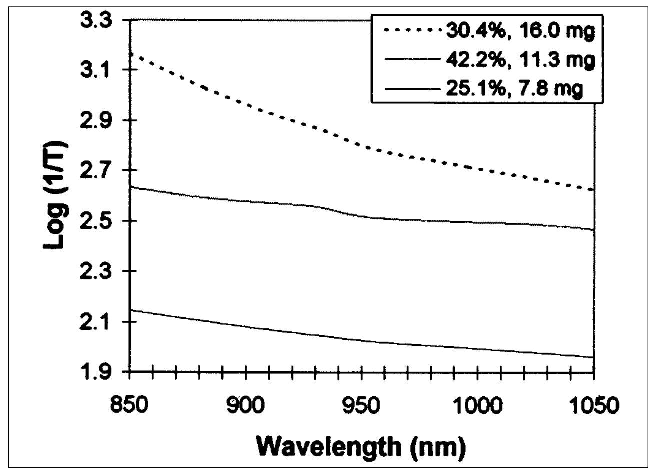

FIG. 6. Plot of log (1/T) vs. wavelength in nanometers of three meadowfoam seeds. NMR % oil content given. Transmission decreases with seed mass, while overall transmission is not influenced by oil content.

set size. However, while increasing the size of the calibration set, the physical variation represented was dramatically increased, which resulted in a higher SECV.

One initial concern was whether the mass of meadowfoam seeds was too small for NITS to predict oil content successfully. An examination of several NITS meadowfoam seed log (1/T) spectra revealed a significant influence by seed mass (Fig. 6), and it was determined that a positive correlation of 0.65 exists between seed mass and the average log (1/T). Second derivatives of the same spectra reveal that influence of meadowfoam seed chemical composition on transmission is detectable (Fig. 7), and the relatively high correlations of the two calibrations (0.95 and 0.88) with oil content demonstrate that NITS can estimate oil content in small samples.

The SECV of the two calibrations, 3.6 and 4.4%, may seem disappointing when initially compared with the best calibration found for single-kernel maize, which had an SECV of 1.2% with the same instrument and calibration software (7). However, the oil content range of the meadowfoam calibrations is 44.9%, yielding relative standard errors of 8.0 and 9.8% of the oil range, while the oil range of the maize calibration was 13.5%, yielding a relative standard error of 8.8% of the oil range. Therefore, the SECV of the meadowfoam and maize calibrations are similar, relative to the oil content range.

NITS does provide a fast, efficient, and cost-effective method of sorting quantities of individual seeds by oil content, for applications such as breeding programs. Although not presently able to predict oil content in meadowfoam seeds as accurately as NMR, the prospects of being able to sort individual meadowfoam seeds by oil content with NITS are good. The NITS instrument is portable, requires little maintenance, routine upkeep, or operating expense, and is much faster than present high-frequency NMR spectroscopy instru-

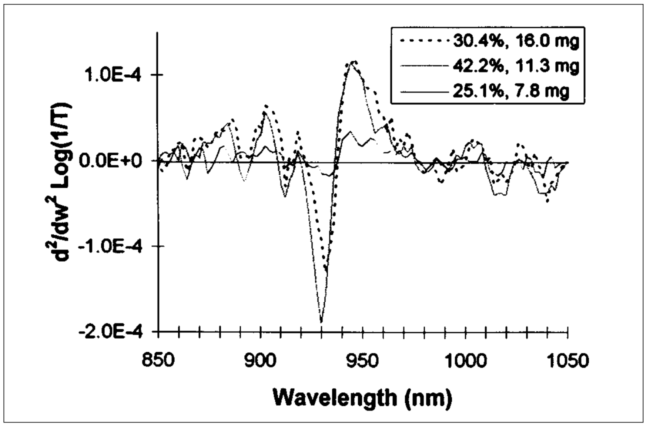

FIG. 7. Plot of d^2/dw^2 log (1/T) vs. wavelength in nanometers of three meadowfoam seeds. NMR % oil content given. Examination of the second derivative reveals influence of chemical composition on transmission.

ments, which may take several minutes for a single determination. In less than one minute, the custom single-seed adapter and control mechanism can automatically position up to 23 individual seeds for NITS analysis. With minimal training, it is possible to sort hundreds of meadowfoam seeds by oil content with an NITS instrument in one hour. NITS is anticipated to advance the rate of progress in meadowfoam oil yield improvement.

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