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ORIGINAL ARTICLE

Single Soybean Seed NMR Calibration for Oil Measurement Using Commercial Cooking Oils

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Abstract Development of high oil soybeans would reduce the cost of soybean oil production for biodiesel or edible oil applications. An accurate determination of soybean seed oil concentration is essential especially when developing superior cultivars with increased seed oil content. The objective of this study was to develop an oil measurement method for single seeds using NMR spectrometry. An NMR spectrometer was calibrated using commercial cooking oil. Fifteen cultivars of known mean oil content were used to evaluate the calibration curves. The calibration curves developed had a correlation coefficient of 0.99. It was found that soybean and corn oil gave identical results over the calibrated interval.

Keywords Soybean · Oil content · NMR spectrometer · Single seed oil measurement

Introduction

Increasing soybean oil content would reduce the production cost of soybean oil for biodiesel or edible oil applications. Improving soybean oil concentration has historically received less attention than improving protein content hence there are few studies with the aim of increasing soybean oil content [1–3].

An accurate determination of soybean seed oil concentration is essential for the development of superior cultivars especially when the aim is increasing seed oil content. Like near infra-red spectrometry (NIRS), nuclear magnetic resonance spectrometry (NMR) is an accurate, rapid and non-destructive method of measuring oil content in living seeds [4] and may be used to determine the oil content in either a single seed or bulk samples [5, 6]. Determination of oil content in a single seed could facilitate and accelerate the breeding and development of cultivars with higher oil content [7]. Previously, a small number of studies have applied NMR to oil measurements of single seeds, including those by Conway (maize, 1960) [8], Alexander et al. (maize, 1967) [5] and Collins et al. (soybean, 1967) [9]. All three used continuous wave (CW) spectrometers that could not discriminate between water and oil within the seed, unlike the more recent pulsed NMR spectrometers that use the 'spin-echo' method [10]. They thus required seeds to be dried to 3–4% moisture first [9].

Calibration of the NMR spectrometer is critical for obtaining accurate, meaningful results that may be useful to both breeders and end-users such as crushing facilities. In previous reports, calibration of the NMR was achieved by adding precise amounts of oil to de-fatted crushed seed [5]; gravimetric extraction and analysis of a single seed from each of several matched pairs, with the intact seed becoming a known standard [9] and, calibration with pure oil recently extracted from seed [11].

The objective of this study was to develop a single seed oil measurement method using NMR spectrometry that could be used by plant breeders to select single seeds of high oil content, with the intent of developing high oil soybean cultivars.

G. R. Ablett is deceased.

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Materials and Methods

Plant Material

Twelve soybean cultivars having a range in oil contents from approximately 16–22% were selected using NIRS. Several cultivars were grown in more than 1 year, to give a total of 15 samples (Table 1).

NIRS Seed Oil Analysis

Seed samples (~300 g) of a range of cultivars were analyzed for oil content by NIRS with a Grain-spec C1126 (Foss Electric, Denmark). Results of this analysis were used to select 15 cultivars (Table 1) having a range in oil content from approximately 16–22%.

NMR Bulk Seed Oil Analysis

Four five-gram sub-samples of each cultivar and year were analyzed for oil content by pulsed NMR spectrometry (Bruker Minispec Mq10; Bruker, Karlsruhe, Germany) using the calibration curve described and validated by Weir et al. [4].

NMR Single Seed Calibration

Calibration for oil content was performed with the gain at 97 dB, 100 scans, 1.50 s recycle delay, narrow band width and 40.00 °C magnetic box temperature. Start SW used was 6.713 ms and Stop SW was 7.313 ms. To calibrate the NMR using cooking oil, both pure soybean oil and corn oil were used to create separate curves. Oil masses of approximately 22.5, 26.25, 30.0, 33.75, 37.5, 41.25, 45.0 mg were placed at the bottom of 14-mm NMR tubes. These oil masses were used to simulate seed oil contents of 15–30%, respectively in 2.5% increments, with the "seed weight" entered in the NMR adjusted accordingly within a range of 140–170 mg. Two curves were created for soybean oil. In the second instance, the oil was not placed directly into the tubes, but first some shredded Kimwipes® were placed in the bottom of the tubes as recommended by Rubel [11]. The oil was dropped on top and allowed to soak in for approximately 10 min.

Data analysis including line regression and correlation was performed by the software of the Minispec NMR and re-analyzed manually by comparing NMR percentage readings with those of the input percentages. Further

Table 1 Percentage oil concentration by NIRS oil measurement and NMR for both bulk and single seeds

Cultivar	NIRS[a]	NMR (5 g)[b]	NMR (single seed)[d]	Difference column 3, 4
Kamichis	17.7	18.3 ± 0.6	19.0 ± 2.0	0.7
OAC Lakeview 2002[e]	22.0	23.0 ± 0.5	22.9 ± 1.6	0.1
OAC Lakeview 2006	21.0	23.0 ± 0.4	23.1 ± 1.8	0.1
OAC Wallace 2003	22.0	21.3 ± 0.2	21.7 ± 2.1	0.4
OAC Wallace 2005	22.0	22.4 ± 0.2	22.2 ± 2.0	0.2
OAC Wallace 2006	22.0	23.8 ± 0.6	24.5 ± 1.7	0.7
ADV Windfall	19.8	20.7 ± 0.5	21.5 ± 1.6	0.8
HDC 2701	18.6	20.2 ± 0.2	20.1 ± 4.0	0.1
RD 714	16.8	18.3 ± 0.5	18.5 ± 2.5	0.2
Secan 04-22	19.7	19.6 ± 0.2	20.1 ± 2.2	0.5
OAC 01-26	20.6	21.6 ± 0.4	22.2 ± 2.3	0.6
OAC 02-02	21.3	22.6 ± 0.6	22.2 ± 2.9	0.4
OT 03-16	18.2	19.3 ± 0.5	18.7 ± 2.2	0.6
OT 04-11	20.0	21.7 ± 0.2	22.9 ± 4.4	1.2
TO 207	–[c]	23.7 ± 0.2	24.2 ± 3.2	0.5
Mean values	20.1	21.1[f]; 21.3	21.6	0.4

All values are on a dry-weight basis

[a] Near infrared spectroscopy (~300 g/sample)

[b] Four replicates, each weighing 5 g were used. Mean and range are recorded

[c] Unknown oil content

[d] Ten single seeds were tested individually using the standard curve generated without using Kimwipes® and the mean and range recorded

[e] 2002 etc., refers to the year in which this seed was produced

[f] When TO 207 is removed, the average is 21.1

analysis included correlations between the main treatments and, for the NMR data only, range in mean oil contents.

Single seed moisture content. To prepare a single seed moisture calibration curve, seeds with a high moisture content were placed in the oven/dryer at 170 °C for 30, 60, 90, 120, 150 min and 24 h to produce seeds having moisture contents ranging from near zero to approximately 20%. Each seed was placed in a 14-mm NMR tube with five glass boiling beads below them so that the seed remained immobile and central in the tube. The calibration was performed with the gain at 97 dB, 100 scans, 1.50 s recycle delay, narrow band width and 40.00 °C magnetic box temperature. Start SW used was 0.050 ms and Stop SW was 0.060 ms. The NMR signal data for each seed were plotted against moisture contents to give a calibration curve via regression analysis.

Results and Discussion

NMR Bulk Seed Oil Analysis

It was found that NIRS and NMR values for seed oil contents were highly correlated ($r = 0.908$, Fig. 1). The four replicate NMR samples of each of the selected cultivars gave on average 1% higher oil readings than the NIRS (Table 1). It is possible that calibration of either or both machines may contribute to the discrepancy as the annual calibration is only validated to ±0.4% oil on either machine. We also attempted an independent oil analysis by means of Soxhlet extractions of 2 g seed samples, but found that correlations were poor ($r = \sim 0.6$), even when we extended extraction times to 10 h as suggested by previous researchers [12]. Thus this approach was abandoned.

NMR Single Seed Calibration Using Cooking Oils

From the seed oil data (Table 1) it can be seen that there is considerable variability between individual seeds of any given cultivar, typically in the order of ±2%, but some showing as much as 4% either side of the mean. Sources of variability include position of the seed on the plant and environmental influences, as well as plant genetic variability [13] making selection of single seeds of "known" oil content impossible. Rubel [11] had previously reported that the Bruker Minispec may be calibrated for multi-seed samples using pure vegetable oil of the same seed type as that to be tested according to the draft standard for CW NMRs [14]. In this instance we used the same principle to create a calibration curve for single seeds.

We achieved an excellent linear correlation of the data, with $Y = 0.48 + 8.86X$ ($r = 0.995$) for the soy oil and $Y = 0.12 + 8.89X$ ($r = 0.998$) for the corn oil curves (without Kimwipes®; Fig. 2). Comparison by means of the Students t test found that the intercepts and slopes were not significantly different ($p = 0.01$) when calculated to 2 dp and, in practice, oil values obtained using either curve differ by less the 0.1% over the calibrated interval. When the same calibration was repeated using soy-oil soaked Kimwipes®, the intercept became negative and the slope of the curve was a little less steep: $Y = -0.3 + 8.80X$, $r = 0.996$ (Fig. 2). This was found to be significantly different to the other two curves ($p = 0.01$), with the average calculated oil percentages for any given reading within the calibrated range being between 0.2 and 0.3% lower. This reduction, if applied to the mean values of the single seeds in Table 1 would make their average value between 0 and 0.1% higher then that obtained for the 5 g samples, indicating a high degree of precision in obtaining single seed oil data by this method. It therefore appears advisable to use the soaked Kimwipes rather then calibrating with oil only, for improved accuracy. Moreover,

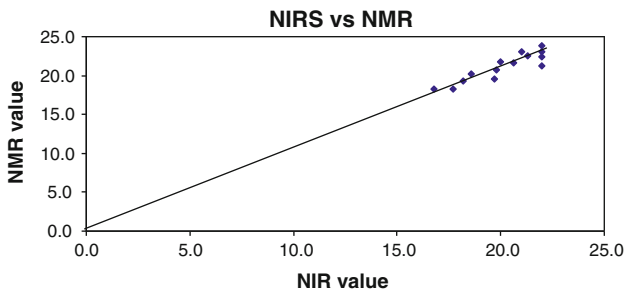

Fig. 1 Relationship between NIR and NMR oil reads (percent) for 300 and 5 g samples, respectively. The slope of the line is 1.049 X with an intercept of 0.189. The correlation $r = 0.908$ over the interval 16.8–22% oil content

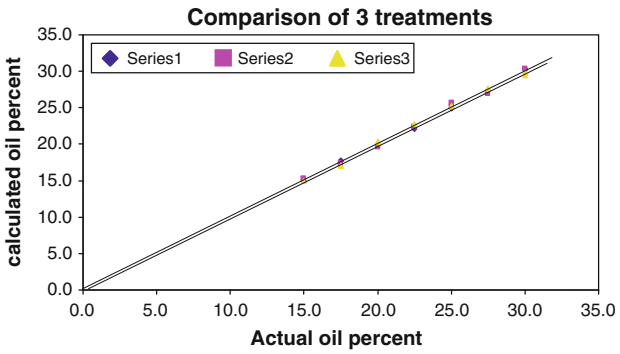

Fig. 2 Regression line for oil content and NMR signal after conversion of signal strength to percentage values. Data points are plotted at 2.5% intervals from 15 to 30%. The slope of the regression line for the pure soy and corn oils is 8.9 (1 dp), with an intercept of 0.5 and 0.1, respectively. Correlation over the interval of 15–30% oil is 0.998 for corn oil, 0.995 for the soy oil data (*upper line*). When soaking oil into Kimwipes®, the intercept became negative (−0.3) and the slope decreased slightly to 8.8 (*lower line*). *Series 1* corn oil, *series 2* soy oil, *series 3* soy oil in tissue

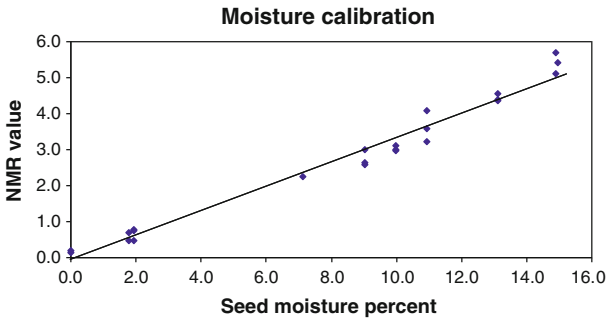

Fig. 3 Regression line for moisture content and NMR signal after conversion of signal strength to percent values. The slope of the line is 0.335, with an intercept of −0.017. Correlation over the interval 0–15% was 0.986

since the corn and soy oil behave in identical manner, just the one curve may be used when testing either corn kernels or single soybean seeds for oil content.

Moisture Calibration

In constructing a moisture calibration curve, it was assumed that after 24 h at 170 °C all moisture seed moisture would have been removed, giving a baseline dry weight. Re-hydrating and then progressively drying the seeds gave an NMR regression correlation of 0.986, with a slope of 0.335 and an intercept of −0.017 (Fig. 3), indicating a good fit of line to the data over the range of 0–15% seed moisture content. This curve was integrated with the oil curve on the NMR, enabling a direct reading of oil contents on a dry-weight basis as presented in Table 1. Although seeds already stored inside for several months or more are likely to be uniform in moisture contents, this additional calibration would be invaluable when testing recently harvested materials having a large range in moisture contents, removing the need for long periods of slow drying in an oven.

In conclusion, NMR spectrometry has been calibrated for single seed measurements by means of precisely weighed masses of oil soaked in shredded tissue. It was found that corn oil and soy oil produced curves that were essentially the same, making either oil acceptable for calibrating the NMR. This technique will enable the use of NMR in single seed selection for high oil in future breeding programs.

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