Seed Oil Determination Without Weighing and Drying the Seeds by Combined Free Induction Decay and Spin-Echo Nuclear Magnetic Resonance Signals

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ABSTRACT: The free induction decay (FID) and echo signals in a spin-echo pulse sequence have been used for seed oil determination without weighing and drying the seeds with nuclear magnetic resonance (NMR) equipment that has a magnetic field of low homogeneity. Earlier known methods, based on use of the FID signal to determine seed oil, become inapplicable when the magnetic field homogeneity is poor, because the angular position of seed significantly affects the signal. The present method, which elegantly eliminates the angular dependence, involves sampling the FID signal at 10 μs after a 90° pulse and the subsequent echo signal at 100 μs formed by applying a 180° pulse at 50 μs. Such short pulse spacing in spin-echo sequence produces almost a full oil signal. It also eliminates the effects of sample-to-sample variation in T_2 and molecular diffusion on oil signal. The oil values obtained by this method are in good agreement (correlation for mustard: 0.952; linseed: 0.99; and for peanut: 0.912) with the values obtained by the well established and accurate pulsed NMR method, which is based on the measurement of the FID signal of oil in dried and weighed seeds.

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KEY WORDS: Nondestructive analysis, oil content, oilseeds, pulsed NMR, spin-echo.

The importance of a rapid and nondestructive method for determining oil content in oilseeds for breeding high oil-yielding varieties, germplasm evaluation, and marketing is well known. Using low-field, continuous-wave, wide-line nuclear magnetic resonance (NMR) and pulsed NMR techniques, methods have been developed for quick determination of oil content in oilseeds (1–6). The pulse technique is superior to the earlier, wide-line NMR method of seed oil determination in terms of lower requirement of magnetic field homogeneity, speed of analysis, and elimination of angular dependence (2–6). Furthermore, it has the unique advantage that seeds need not be weighed or dried, which makes the analysis much faster and truly nondestructive (3,5,6). NMR methods for seed oil determination are based on the differences in the proton spin-spin relaxation time (T_2) of the various constituents of the seeds. The T_2 of oil that exists in the liquid phase of seed is much larger than that of solid components, i.e., protein and carbohydrates, or of moisture. Therefore, it is possible to measure the oil signal without interference from other constituents of the seeds.

In principle, it is possible to determine the oil content in seeds by using any of the available pulse sequences (4), namely free induction decay (FID), spin-echo (SE), or Carr-Purcell-Meiboom-Gill. In FID, the simplest of the three sequences, a short 90° radio frequency pulse is applied to the sample. The oil signal from the sample decays exponentially with time constant T_2^*, where T_2^* is given by the equation:

$$1/T_2^* = 1/T_2^{\mathrm{oil}} + 1/T_2^{\mathrm{field}} \qquad [1]$$

Because in actual practice $T_2^{\mathrm{field}} << T_2^{\mathrm{oil}}$, decay of the oil signal is governed almost entirely by magnetic field inhomogeneity, which may be different in different portions of the field. Therefore, the signal from different portions will decay at different rates, resulting in the signal being dependent on the angular position of the sample.

The method in which the FID signal is used for seed oil determination was developed in early 1970 after an exhaustive study of the various parameters that affect the signal, as well as taking steps to eliminate the possible sources of errors in the measurement (2). The seed oil values determined by this method are accurate and agree well with values obtained by chemical extraction methods (2). Though the method is quite accurate, it requires that the seeds be dried and weighed, making the analysis considerably slower and not truly nondestructive. To overcome these limitations, another method was developed, which does not require weighing or drying of seeds (3). In this method, the signal measured at 10 μs (just after the dead time of the receiver) represents partly decayed solid signal and full liquid signal. And signal measured at 70 μs gives only full liquid signal because the proton signal from solids has decayed completely by then. It has been reported that the effect of angular position of seed on FID signal increases with an increase in signal sampling times (2,4,5). At 70 μs, the seed orientation effect on the FID signal, measured by a Bruker Minispec (Bruker, Karlsruhe, Germany) instrument, is negligible (3,5). The oil content values automatically calculated by

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the instrument (with the average correction factor, F, to account for solid signal decay, moisture content, and proton density difference in solid and liquid constituents of seeds) have been shown to be in good agreement with those obtained by the quite accurate method based on measurement of FID signal of oil in dried and weighed seeds (2,3).

However, the FID signal method becomes inaccurate if the magnetic field is less homogeneous because the angular dependence would be quite significant, even at shorter sampling times. In the present study, we have employed an SE pulse sequence to eliminate the effect of angular orientation of sample, even at longer signal sampling times for determining oil in oilseeds in an NMR instrument of low-magnetic field homogeneity.

EXPERIMENTAL PROCEDURES

A pulsed NMR Instrument (Minispec p20i; Bruker) with double coil arrangement, operating at 20 MHz, was used for studying angular dependence. Its T_2^*, which represents magnetic field inhomogeneity, was about 600 μs. To study the dependence of signal on angular position of the sample, a single peanut seed was placed eccentrically in the sample tube of the NMR spectrometer. The sample tube was rotated roughly by 90, 180, and 270° from the initial position taken as 0°. At each orientation, the SE signal was recorded with the transient recorder at different pulse spacing.

Another pulsed NMR instrument, fabricated by the Instrument Design and Development Centre, Indian Institute of Technology (Delhi, India), which had a lower magnetic field homogeneity ($T_2^* \approx 300$ μs), was used to determine oil content in different kinds of oilseeds by the SE sequence and measuring the echo signal formed at 100 μs, and the FID signal after the 90° pulse in the same sequence at 10 μs (just after the dead time of the receiver). The instrument was programmed to calculate the oil content automatically by using the F-factor as described earlier (3,5). Because the angular dependence for peanut seeds has been reported to be more than that of other oilseeds (2), the oil content of its six samples was measured at four angular positions (0, 90, 180, and 270°) by this method to find the precision of the measurement. In addition, oil contents of ten samples each of mustard, linseed, and peanut were also measured at only one angular position by the present method in the NMR instrument of lower magnetic field homogeneity, and also by the earlier-reported FID signal method in the Bruker Minispec PC 20 instrument. The oil contents of six peanut samples were measured four times by the FID signal method to find the precision of the measurement.

Theory of the method. In the SE sequence, the 90° pulse is followed after a time interval, τ, by a 180° pulse, which refocuses precessing spins to form an echo at time 2τ. The echo amplitude, *A*, at time 2τ is given by:

$$A_{(2\tau)} = A_o\{\exp(-2\tau/T_2) - 2/3D\,\gamma^2 G^2 \tau^3\} \qquad [2]$$

where A_o is the initial amplitude, *G* the magnetic field gradient, *D* the diffusion coefficient, and γ the gyromagnetic ratio of protons. The angular dependence observed in the FID signal due to magnetic field inhomogeneity is eliminated by refocusing the spins in this method. The effect of molecular diffusion during the time interval, τ, in decreasing the intensity of SE signal can be minimized by using a very short τ, which reduces the second term in the Equation 2 to a negligible value. A very short τ also makes $A_{(2\tau)}$ practically equal to A_o.

The pulse sequence used in this method for seed oil determination without weighing and drying the seeds is shown schematically in Figure 1. Because the T_2 of solid components is about 12 μs, it would have decayed almost completely by 100 μs, the time at which the signal intensity (*L*) was measured. Therefore, *L* would consist of the full signal due to oil and the left-over moisture signal. In addition, the FID after the 90° pulse in SE sequence is sampled at 10 μs (immediately after dead time of the receiver), which is a measure of partly decayed solid signal (*s*) and full liquid signal (*L*). The full solid signal (*S*) is obtained by applying the appropriate correction to the observed signal "*s*" along similar lines, as described earlier (3). The oil content in seeds is obtained by the equation:

$$\text{oil \%} = \{L/(L + S)\} \times 100 \qquad [3]$$

where $S = \mathrm{F} \cdot s$, and F is a correction factor, which accounts for the decay of solid signal, moisture content, and proton density difference between solid and liquid constituents of the oilseeds. The F factor is determined by using samples of known oil content as described earlier (3). It is assumed that sample-to-sample variations in the moisture content, proton density difference, and solid phase T_2 are insignificant. The solid phase T_2 values of several oilseeds have been reported as nearly the same (3). The variation in moisture content of the sample can be made insignificant by storing the samples under similar conditions before the analysis.

RESULTS AND DISCUSSION

The angular orientations of a single peanut seed affecting the FID signal after a 90° pulse and the subsequent SE signal after a 180° pulse at 1 ms are shown in Figure 2. It clearly shows that, although the echo width changes with the orientation of the seed, the height remains unchanged, thereby eliminating the angular dependence due to magnetic field inhomogeneity. The higher pulse spacing of 2 ms produced a fairly similar result. However, the angular position effect at longer pulse spacings of 10 ms showed a small scatter of echo positions and heights, which may be due to incomplete elimination of the effect of magnetic field inhomogeneity at high τ values.

While the FID signal decays by T_2^*, the SE signal decays by T_2 of the sample. T_2 of the oil in seeds varies from sample to sample, which may affect the oil signal. This effect is insignificant in the present measurement because the SE signal is measured at 100 μs, whereas the value of the oil T_2 was about 100 ms. The oil content of six peanut samples determined by combined use of the FID signal at 10 μs and the SE

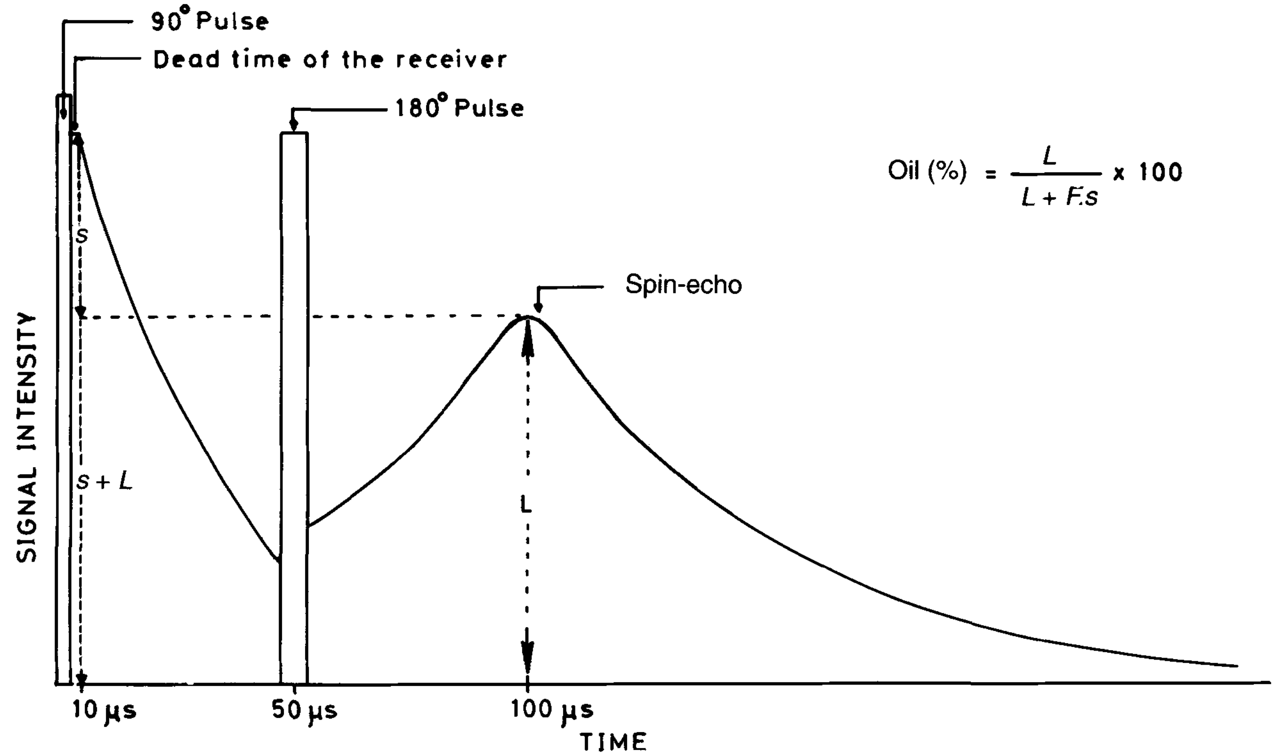

FIG. 1. The schematic diagram of the spin-echo pulse sequence used in the present work for seed oil determination without weighing and drying the seeds. The full liquid signal (*L*) and partly decayed solid signal (*s*) are shown by arrows.

signal at 100 µs with the NMR instrument of lower magnetic field homogeneity ($T_2^* \approx 300$ µs) at four angular positions of each sample, along with the mean value of the oil content and standard deviation, are given in Table 1. The data show that precision of oil determination by the present method is good (average SD: ±0.316) and that the effect of the angular position of samples on the measurement has become insignificant in the present method. The precision of oil determination by the FID signal method in the Bruker Minispec PC-20 is given in Table 2 (average SD: ±0.290). The precision of measurement by the two methods is nearly the same.

The oil content of ten samples each of mustard, linseed, and peanut, measured by the present method and the well-established FID signal method (2), are given in Table 3. The agreement between the oil contents measured by the two methods is good. Because the oil values measured by the FID signal method have been reported to be in good agreement with the values measured by the standard chemical extraction method (2), and because the present method agrees well with the FID signal method, the oil values measured by the present method also will agree well with the values measured by the chemical extraction method.

The various pulse sequences for seed oil determination have been compared and reviewed (4,7,8). The choice of pulse sequence would depend upon the purpose of the determination and the quality of the magnetic field. For an accurate determination in a magnetic field of good homogeneity ($T_2^* \geq 600$ µs), the FID signal method (2) may be used. The SE signal method (4) will have to be used if the magnetic field homogeneity is

TABLE 1
Oil Content of Peanut Seeds Measured at Four Angular Positions of the Samples by the Present Method

Sample	Oil content (%) sample position[a] 0°	90°	180°	270°	Average oil content (%)	SD (±)
1	51.0	50.9	51.1	51.4	51.1	0.216
2	49.5	49.9	49.2	49.7	49.6	0.299
3	51.7	50.8	50.6	51.5	51.2	0.532
4	51.0	51.8	51.4	51.5	51.4	0.330
5	51.5	51.2	51.8	52.0	51.6	0.350
6	52.3	52.3	52.4	52.0	52.3	0.173
Average SD						±0.316

[a] The initial position of the tube was take arbitrarily as 0° for each sample.

TABLE 2
Oil Content of Peanut Samples Measured Four Times by the Free Induction Decay Method

Sample	Oil content (%)				Average oil (%)	SD (±)
1	48.8	48.2	48.8	48.9	48.7	0.320
2	48.5	48.6	48.5	49.3	48.7	0.386
3	50.2	49.6	49.8	50.0	49.9	0.258
4	47.8	47.3	47.0	47.5	47.4	0.337
5	46.5	46.2	46.6	46.9	46.6	0.289
6	49.6	49.4	49.6	49.3	49.5	0.150
Average SD						±0.29

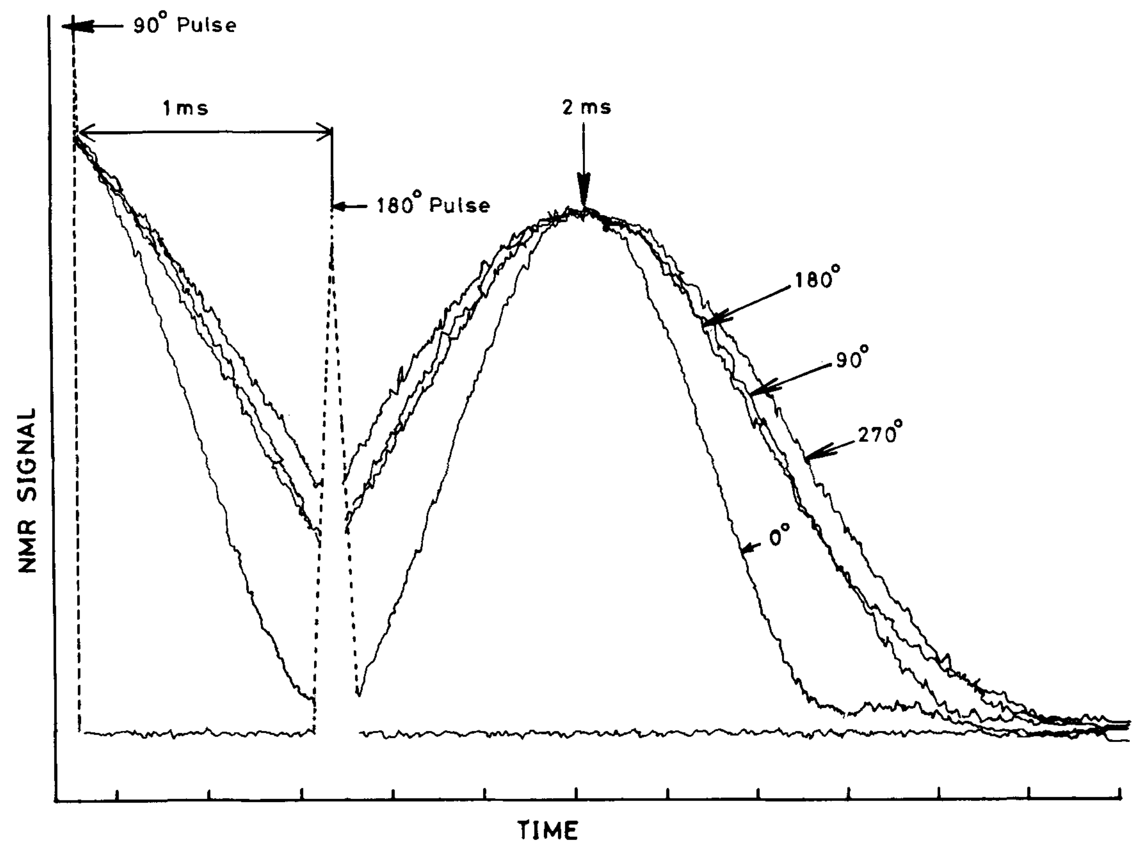

FIG. 2. The variation in free induction decay and spin-echo signal of the same single peanut seed at different angular orientations. The initial position of the seed in the nuclear magnetic resonance (NMR) tube was taken arbitrarily as 0°, and the tube was rotated by 90° each time.

poor ($T_2^* \approx 300\ \mu s$). However, in both methods (2,4), the seeds will have to be weighed and dried, which makes the analysis somewhat slow and not truly nondestructive, as the viability of the seed is lost by drying at higher temperature.

For oilseed breeding work, a method for rapid and truly nondestructive determination of oil content is required. In such a case, the method (3) based on measurement of the FID signal of solid and liquid phase combined and liquid phase signal alone may be used if the magnetic field homogeneity is fairly good ($T_2^* \geq 600\ \mu s$). The present method may be used for such work if the magnetic field homogeneity is poor ($T_2^* \leq 300\ \mu s$).

TABLE 3
Comparison of Oil Content (%) Measured by the Present Method and the Earlier Reported Free Induction Decay (FID) Method

	Mustard		Linseed		Peanut	
Sample number	Present method	FID method	Present method	FID method	Present method	FID method
1	42.6	42.7	43.0	42.8	51.7	51.5
2	40.5	41.0	47.3	47.0	53.6	54.2
3	41.6	41.1	38.1	37.9	48.3	50.4
4	38.9	38.5	43.8	43.3	49.2	49.8
5	40.5	41.1	43.1	42.6	52.2	52.5
6	38.7	38.2	45.3	44.8	48.7	48.8
7	39.6	39.3	42.7	42.0	54.7	53.8
8	40.1	39.6	43.4	43.9	51.8	50.4
9	40.4	40.0	44.3	43.8	54.5	54.8
10	39.8	39.0	—	—	52.9	53.7
Correlation	0.952		0.990		0.912	

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