Rapid and Nondestructive Determination of Seed Oil by Pulsed Nuclear Magnetic Resonance Technique

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ABSTRACT

The pulsed NMR technique for rapid and nondestructive determination of oil in oilseeds has been developed. The effects of spin-lattice relaxation time, spin-spin relaxation time, seed moisture, angular position of the seeds, sample tube thickness, and sample height upon the magnitude and reproducibility of the NMR signal were studied. Based upon these studies, various parameters for seed oil analysis have been fixed. The oil content of Brassica, peanut, and sunflower seeds was determined. The reproducibility of the measurement is ± 1%. The technique was tested by measuring the oil content of the same seeds by the cold percolation method (CCl_4 extraction). It was further tested by determining the oil content of 60 Brassica seed samples independently at three laboratories. The results of these tests are given.

INTRODUCTION

Most of the oil crop breeding programs are aimed at producing more oil/day/hectare. The total quantity of oil produced by an oil crop is the product of its yield and percentage of oil. The breeder uses visual methods for evaluation of high yields and other characters, such as resistance to diseases, pests, and arid conditions; the ability of the crop to withstand wind and rain; and its ability to reach early maturity. However, he faces enormous difficulties when selecting seeds and plants for higher percentage of oil among thousands of progenies on the basis of chemical analysis, which is slow and destructive. The destructive nature of the analysis does not permit direct propagation of seeds.

This paper deals with the development of the pulsed NMR technique for rapid, nondestructive, precise determination of oil in oilseeds. The effects of spin-lattice relaxation time, T_1; spin-spin relaxation time, T_2; seed moisture; angular position of the seed; sample tube thickness; and sample height upon the magnitude and reproducibility of the NMR signal have been studied. Based upon these studies, various parameters have been determined for the seed oil analysis. Practically, no sample preparation other than cleaning and drying the seeds is required. The analysis has been checked against the established chemical methods of oil analyses. The measurement of the pulsed NMR signal takes less than 10 sec. This makes the pulsed NMR technique an order of magnitude faster than the wide-line NMR technique for oil analysis (1-4); further, the requirement of magnetic field homogeneity is not critical for the pulsed NMR technique. The dependence of the signal upon the angular position of the single seeds of maize, sunflower, and soya that has been found (5) in the transient wide-line NMR technique can be eliminated easily by the pulsed NMR method.

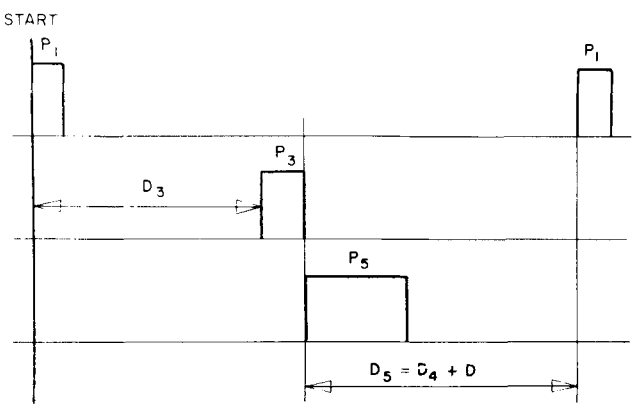

FIG. 1. Pulse programe. D = 0.3-10 sec, D_3 = 10-990 μsec, D_4 = 0.1-9.9 sec, P_1 = 3-30 μsec, P_3 = 10-90 μsec, and P_5 = 0.1 sec.

BASIC PRINCIPLE

The theory of the pulsed NMR is found in several standard texts on NMR (6, 7). The basic difference between the wide-line NMR and the pulsed NMR is that, in the former, a continuous radiofrequency magnetic field is applied on the samples, whereas a pulsed (discontinuous) radiofrequency magnetic field is applied in the latter. The radiofrequency magnetic field applied to the sample at the resonance frequency tips the magnetization vector away from its equilibrium direction. A pulse which can turn the magnetization vector perpendicular to the uniform magnetic field is referred to as the 90° or the $\pi/2$ pulse.

After a 90° pulse, the magnetization vector decays exponentially, being proportional to exp. $(-t/T_2)$; T_2 is the spin-spin or transverse relaxation time, and t is the time from the end of the pulse. T_2 depends strongly upon the mobility of the magnetic nucleus in the sample. T_2 increases with mobility (8). In oilseeds, hydrogen which gives the strongest NMR signal is present mainly in four forms: oil, moisture (boundwater), carbohydrates, and proteins. The oil hydrogen is the most mobile among them. As a consequence of this, the pulsed NMR signal from the oil will be present up to a time when all the other signals are dead. This makes it possible to measure the oil signal even in the presence of the other constituents of the seeds. This signal is converted into the quantity of oil with the help of a calibration graph, prepared by using the pressed oil of the same crop. The use of such a calibration is based upon the fact that the variation in hydrogen percentage of oil from different varieties of the same crop is generally not significant. Some error will be introduced in the analysis, if this variation is significant. This error may be minimized by preparing the calibration graph using the oil of intermediate iodine value from the crop which is to be analyzed.

PULSED NMR SPECTROMETER

The coherent pulsed NMR spectrometer obtained from Josef Stefan Institute, Ljubljana, Yugoslavia, has been used for the present work. It works at two fixed frequencies, 16 MHz and 32 MHz. The coil to be used with 16 MHz has the inside diameter of 24 mm and that with 32 MHz has the inside diameter of 11 mm. Consequently, the 16 MHz coil can analyze larger samples; the 32 MHz coil is suitable for the analysis of smaller samples. The coil serves alternatively as transmitter of radiofrequency magnetic field and as receiver of free-decay signal.

The magnitude of the signal is displayed on a 4 digit digital voltmeter. There are two modes of operations, mode 100 and mode 1. On mode 100, the spectrometer gives the

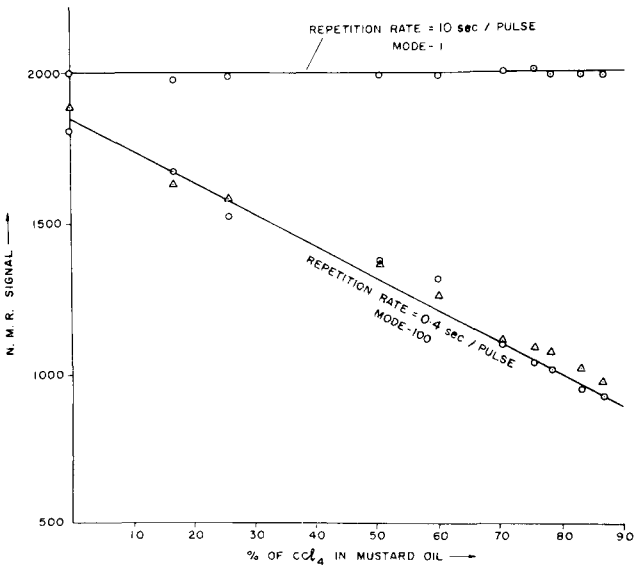

FIG. 2. Variation in NMR signal of a fixed amount of mustard oil mixed with different percentages of CCl_4. Wt of oil = 0.791 g, frequency = 16 MHz, delay time = 250 μsec, temperature = 31 C.

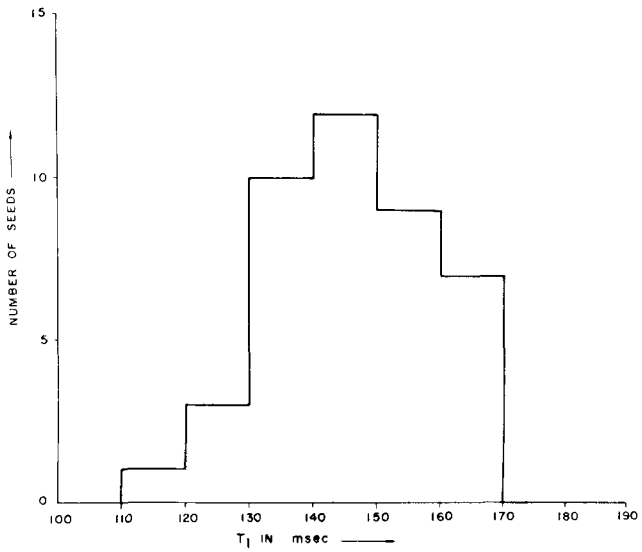

FIG. 3. Seed to seed variation in T_1 of peanut at 22 C and 32 MHz.

average of 100 signals. On mode 1, it gives the value of a single signal. Obviously, the weak signals from the smaller samples or single seeds can be measured on the mode 100 with better reproducibility than on the mode 1.

The 90° pulse program shown in Figure 1 is used for the determination of oil in the oilseeds. P_1 is 90° pulse. To make 90° pulse, the width of the pulse is adjusted to get the maximum signal. D_3 is the delay time after which the sampling pulse P_3 measures the height of the signal. The D_3 can be varied from 10-990 μsec in 99 steps, and the P_3 can be varied from 10-90 μsec in 9 steps. P_5 is the count time which has a fixed value of 100 msec. D is the display time which can be varied continuously from 0.3-10 sec. As shown in Figure 1, $D_5 = D_4 + D$. It is equal to the time between the P_5 pulse and the next P_1 pulse. The D_4 can be varied from 0.1-9.9 sec in 99 steps. On the mode 100, the display time is zero during the averaging. Therefore, the separation between consequective P_1 pulses or the repetition rate on the mode 100 is governed mainly by the D_4. On the mode 1, the repetition rate is governed by the D_4 and the D as well.

EFFECT ON SPIN-LATTICE RELAXATION TIME UPON NMR SIGNAL

The determination of oil in the oilseeds is based upon the measurement of hydrogen content of the seed oil. For the validity of the method, it is essential that the pulsed NMR signal from a given amount of oil remains independent of its relaxation times T_1 and T_2. To check this point, the NMR signal of a fixed amount of mustard oil mixed with different percentages of CCl_4 was measured on the mode 100 and at 16 MHz, keeping D_4 = 0.4 sec and D_3 = 250 μsec. The result is shown in Figure 2. The signal decreases with the increase in the percentage of CCl_4. To explain the decrease in the NMR signal with increase of CCl_4 in the oil, the T_1 value of each mixture was measured at 16 MHz by the π_2-π_2 pulse method. As expected T_1 was found to increase with the increase in the percentage of CCl_4. The increase in T_1 explains the decrease in NMR signal, because the hydrogen nuclei with larger T_1 take longer time (higher pulse separation) to reach the same level of equilibrium magnetization than the nuclei of smaller T_1 values. The points shown by triangles in Figure 2 represent the calculated values of NMR signal. The observed points are shown by circles. The agreement between them is quite good. The NMR signal of a fixed quantity of the oil is found to decrease with increase in T_1. This means that the determination of oil content at this setting (mode 100, D_4 = 0.4 sec, D_3 = 250 μsec) is not valid if there is sample to sample variation in T_1. Figure 3 shows that there is a large seed to seed variation in T_1 of the same peanut variety. The different varieties of Brassica are found to have different values of T_1.

The pulsed NMR signal can be made independent of T_1 provided sufficient time is given between the consecutive 90° pulses so that the samples having different T_1 values attain equilibrium magnetization. Table I gives the time required for 99.9% equilibrium magnetization of nuclei with different values of T_1. The time required for 99.9% equilibrium magnetization of nuclei with T_1 = 500 msec is 3.45 sec. To account for the samples that might have T_1 values even higher than 500 msec, we have used 10 sec interval (repetition rate) between the consecutive P_1 pulses. The upper line in Figure 2 shows that, at this setting, the NMR signal from the fixed amount of oil does not decrease with the increase of percentage of CCl_4. It may be noted that the observation is taken on the mode 1 and not on the mode 100. If the mode 100 is used with 10 sec pulse interval, the time taken for each analysis will be more than 16 min. This will make the analysis slow. To keep the analysis rapid and valid, it is necessary to use the mode 1. The use of mode 1 poses the problem of reproducibility of the NMR signal, especially from small samples and single seeds. An overall improvement in the sensitivity of the spectrometer may be able to solve this problem to a large extent.

EFFECT OF SPIN-SPIN RELAXATION TIME UPON NMR SIGNAL

The pulsed NMR signal of a fixed quantity of the mustard oil was measured at two delay times, 200 and 990 μsec by changing the T_2 of oil by adding different quantities of CCl_4 to it. No change in the NMR signal was observed with change in the T_2 of the oil. This is because

TABLE I

Time Required for the Fixed Magnetization

Spin-lattice relaxation time T_1 in msec	Pulse interval (repetition rate) in sec required for 99.9% equilibrium magnetization
500	3.45
200	1.38
100	0.69

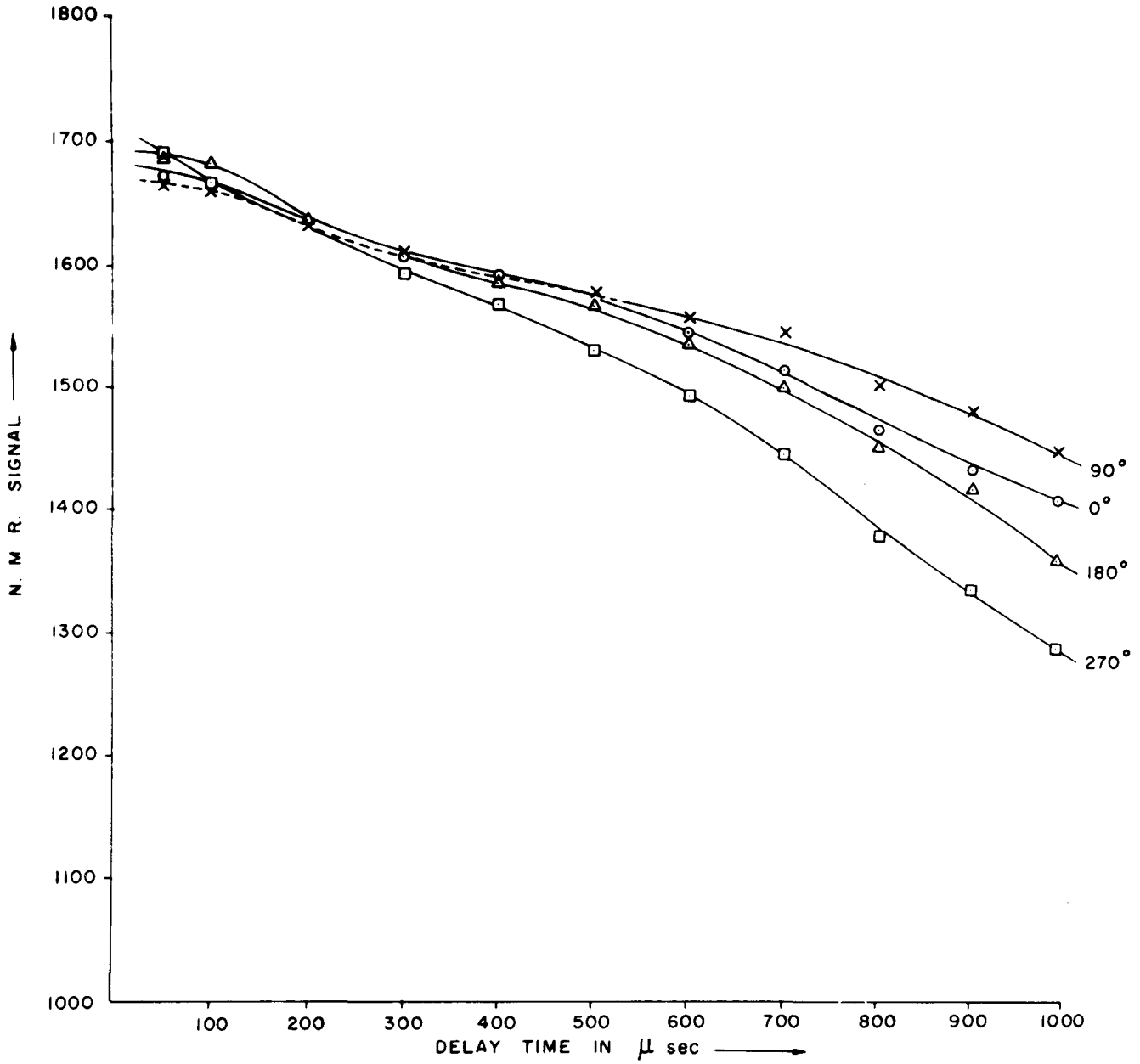

FIG. 4. Angular dependence of NMR signal for peanut single seed.

TABLE II

Oil Percentage in Brassica

Sample no.	Pulsed NMR technique		Cold percolation (CCl_4 extraction)	
	I	II	I	II
1	47.8	48.7	46.3	48.3
2	46.8	46.9	44.8	45.4
3	45.8	46.0	45.3	43.5
4	42.8	43.3	41.6	42.9
5	43.9	43.0	41.5	44.8
6	45.4	45.3	45.4	45.1
7	29.4	29.2	29.2	28.9
8	46.3	45.8	46.3	45.7
9	40.4	39.5	39.5	38.1
10	48.6	48.4	48.9	48.0
11	39.4	39.9	40.8	
12	47.0	47.2	46.9	47.7
13	39.6	39.4	40.2	40.7
14	39.7	39.6	40.8	
15	39.3	38.6		39.3
16	45.7	46.4	47.9	46.5
17	40.2	39.2	41.1	40.1
18	44.4	43.7	44.9	43.4
19	39.4	39.1	39.8	38.8

the decay of the signal after the $\pi/2$ pulse is not governed by the T_2 of the oil but by the T_2* which is given by:

$$1/T_2^* = 1/T_2 \text{ (oil)} + 1/T_2 \text{ (field)}.$$

The T_2 (field) is governed by the magnetic field inhomogeneity. In actual practice, the T_2 (field) is small as compared to the T_2 (oil). Therefore, for all practical purposes the T_2* depends upon the T_2 (field). Consequently, the NMR signal after the $\pi/2$ pulse does not depend upon the T_2 (oil). It is the magnetic field inhomogeneity which enables the magnitude of the pulsed NMR signal to remain independent of the T_2 of the oil.

EFFECT OF SEED MOISTURE UPON NMR SIGNAL

The variation in the NMR signal of 105 C, 60 C, and the room dried peanut and Brassica seeds was measured at different delay times. It was observed that beyond the delay time of 250 μsec, the NMR signals of the seeds dried at 105 C are the same as that of 60 C dried seeds. This means that the moisture in the seeds remaining after 60 C drying does not give any signal beyond 250 μsec. It was

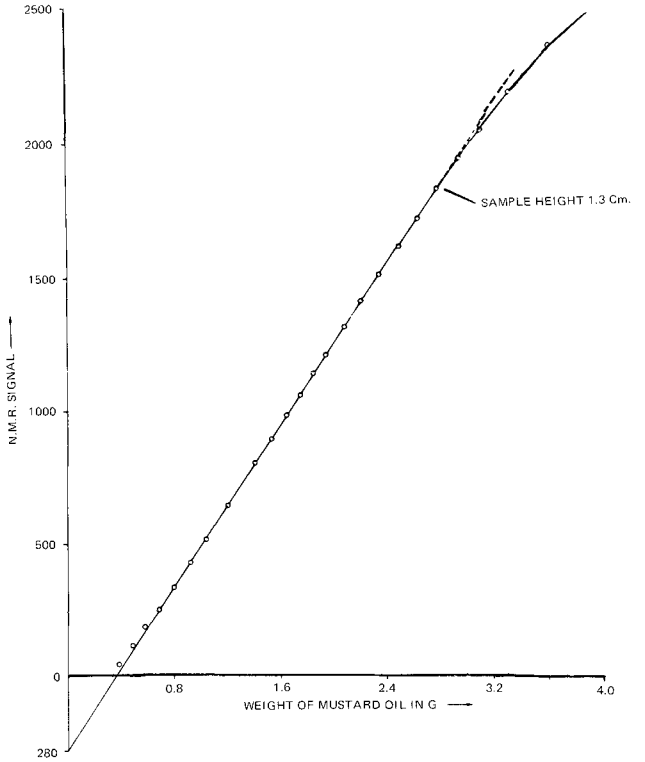

FIG. 5. A typical calibration curve. Mode - 1, Rep rate = 10 sec/pulse, gain = 2.80, D_3 = 250 μsec, frequency = 16 MHz, temperature = 27 C.

found that the signal from the room dried and 60 C dried Brassica and peanut seeds is the same beyond delay time of 900 μsec. It was further noted that the ratio of the NMR signals of the moist and 60 C dried Brassica and peanut seeds at the delay time of 990 μsec is unity up to ca. 5% moisture. From this, one might conclude that it is not necessary to dry the seeds if the moisture content as determined by 60 C drying is less than 5%. But as discussed below, this is not applicable in practice because of the angular dependence of the NMR signal.

ANGULAR DEPENDENCE OF NMR SIGNAL

While measuring the oil content of single seeds of maize, sunflower, and soya by the transient wide-line NMR technique, an angular dependence has been observed (5). Such a dependence was not found in rapeseeds. To study the angular dependence of the pulsed NMR signal, a single seed of peanut was placed eccentrically in the sample tube which was inserted in the 16 MHz coil. The initial position of the tube was taken to be the 0° position. It was rotated by 90°, 180°, and 270° from the initial position. At each position the NMR signal was recorded at different delay times. The angular dependence is shown in Figure 4. The angular dependence is pronounced at larger delay times. All the four curves meet at the delay time of ca. 200 μsec. This means that for eliminating the angular dependence, it is necessary to make the measurement around 200 μsec. This is contradictory to the requirement of the moisture signal elimination. For eliminating the moisture signal either the seeds are to be dried, or the signals should be recorded at the delay time of 900 μsec or beyond. Since it is essential to record the signal at lower delay time for eliminating the angular dependence, the seeds are dried to constant wt at 60 C for eliminating the interference of the moisture in the oil signal. The signals are measured at the delay time of ca. 250 μsec for eliminating the angular dependence. The viability of 60 C dried seeds is 100%.

The angular dependence of the NMR signal was found to be less for those samples which could be uniformly filled in the sample tube. For example, in case of mustard seeds, it was small; and, for the liquid oil, it was completely absent. This is because, in such samples, the distribution of magnetic field, with respect to the sample, remains unchanged by rotating the sample tube. However, for the samples which cannot be filled uniformly, the field distribution is different for different angular position of the sample tube. This results in different values of the T_2* for the same sample as shown in Figure 4. The pulsed NMR offers an unique advantage over the wide-line NMR in easily eliminating the angular dependence by recording the signal at the lower delay time D_3.

TABLE III

Oil Percentage in Peanut

Sample no.	Pulsed NMR technique		Cold percolation (CCl_4 extraction)	
	I	II	I	II
1	50.4	48.6	49.3	47.5
2	51.8	48.3	50.9	47.3
3	52.3	47.5	51.1	
4	49.7	51.6	46.1	50.5
5	49.8	44.5	48.8	43.8
6	51.1	48.4	52.6	49.6
7	42.5	42.2	43.5	44.6
8	49.5	45.3	48.5	47.2
9	48.4	45.4		46.9
10	46.3	47.2	47.6	47.6

TABLE IV

Oil Percentage in Sunflower

Sample no.	Pulsed NMR technique		Cold percolation (CCl_4 extraction)	
	I	II	I	II
1	44.9	45.6	45.2	46.8
2	38.5	40.1	38.9	44.8
3	43.9	45.4	39.1	45.6
4	43.3	40.7	42.6	40.7
5	41.4	42.1	41.1	41.5
6	37.0	34.1	38.3	36.5
7	32.5	34.1	34.4	33.7
8	44.5	44.1	45.1	
9	35.7	37.6	36.4	37.2
10	45.0	45.3	45.4	45.7
11	35.6	39.6	36.5	36.2
12	32.7	32.2	32.5	32.5

MAXIMUM PERMISSIBLE SAMPLE HEIGHT

The maximum permissible sample height is equal to the length of radiofrequency magnetic field coil or sample tube within which the NMR signal is linear. To determine this, the height of a small quantity of Brassica sample was varied in the sample tube by floating it in CCl_4. At each height, the NMR signal was measured. It was noted that the signal remains constant within the length of 1.3 cm for the 16 MHz coil. Therefore, the maximum permissible sample height is 1.3 cm for the 16 MHz coil. For seed oil determination, the glass tube containing the sample, not exceeding 1.3 cm height is inserted in the radiofrequency coil. The position of the sample tube is adjusted to get the maximum signal. A Teflon collar is fixed on the sample tube for its quick positioning in the r_f coil.

DEPENDENCE OF NMR SIGNAL UPON SAMPLE TUBE THICKNESS

The NMR signal is found to decrease with the increase in the thickness of the sample tube. Taking this point into

TABLE V

Brassica Oil Percentage Values from the Three Laboratories

Sample name	Steel tube method at SSA			Pulsed NMR technique at NRL			Soxhlet extraction technique at BARC		
	I	II	III	I	II	III	I	II	III
DS 17D	45.9	45.2	46.3	44.4	44.3	44.9	43.9	42.0	43.2
Toria T-19	49.4	49.6	49.6	47.6	47.8	48.2	44.6	44.0	44.3
Sulphala DS 17M	42.5	43.0	42.3	40.5	39.8	40.8	38.6	38.2	43.7
Brassica carinata	41.7	42.1	42.1		40.3	40.5	40.6	40.2	40.6
Brassica napus	41.3	41.8	42.9	40.9	40.5	40.8	37.7	40.4	40.0
Brassica Nigra	42.0	42.2	42.1	40.9	40.7	40.3	37.9	38.1	38.0
Rai T-11	47.9	47.8	47.5		46.3	45.7	43.7	42.7	42.2
Brassica Sativa	37.4	37.6	37.3	36.2	36.3	35.9	36.5	36.5	37.3
Toria TTY-1	49.8	42.8		49.0	49.2	49.0	44.5	43.3	44.3
Brown Sarson		42.4	49.2	41.1	40.8	41.2	40.0	39.5	39.8
Rai T-59	49.5	49.6	42.6	47.4	48.4	47.5	45.0	45.0	44.3
Assam massselection	46.6	46.3	46.9	44.4	45.3	45.0	42.8	43.7	43.6
N-21	44.5	45.1	45.0	43.9	43.3	43.4	39.7	41.7	42.7
N-12	39.8	39.8		37.6	38.9		37.4	38.6	
N-17	40.4	40.5		38.1	38.8		38.7	36.9	
Rai K	47.6	47.8		45.6	45.7		43.1	42.2	
N-9	42.1	41.8	42.0	40.7	40.6	39.8	36.8	37.7	39.1
Brassica Hirta	42.8	42.9		41.2	41.8		42.2	43.2	
Br. 13	45.3	44.0	45.4	43.8	43.8	44.1	40.6	42.3	43.8

[a]SSA = Swedish Seed Association, NRL = Nuclear Research Laboratory, BARC = Bhabha Atomic Research Center.

account in the analysis, several glass tubes giving the same NMR signal were selected in the beginning of the analysis so that if one glass tube broke, another could be used without affecting the accuracy of the measurement. It is undesirable to use a sample tube of thickness more than necessary for sufficient mechanical strength so that it does not break easily.

SEED OIL DETERMINATION

To determine the quantity of oil in oilseeds of a particular crop, a calibration curve was drawn by recording the NMR signals of the different quantities of pure oil from the same crop. The signals were measured after the $\pi/2$ pulse on the mode 1, keeping the pulse separation (repetition rate) $D + D_4 = 10$ sec and delay time $D_3 = 250$ μsec. Since the digital voltmeter reading becomes nonlinear above 2200, the gain of the amplifier was adjusted so that the digital voltmeter reading for the samples under analysis did not exceed 2200. A typical calibration curve is shown in Figure 5. The curve is nonlinear beyond the maximum permissible sample height of 1.3 cm. The quantity of the sample and the amplifier gain are adjusted to keep the signal in the linear portion of the calibration curve, preferably above 500 for less error in the analysis.

At the time of taking the observation for the calibration curve, the NMR signal of a standard also was measured. The oilseeds of the same crop which were to be analyzed were used as standard. The standard was dried at 60 C and sealed in a glass tube. The quantity of the standard was such that it gave the signal in the region of 1500. The quantity of the sample was similar to that of the standard. The glass tubes used for drawing the calibration curve and measuring the standard and sample signals were identical.

The samples to be analyzed were cleaned and dried at 60 C. The NMR signals of the dried samples were recorded at the same setting of the spectrometer that was used for drawing the calibration curve. The signal of the standard also was recorded after every 10 samples. The sample signal was corrected by multiplying it with the ratio of the standard signal taken at the time of drawing the calibration curve to its value observed at the time of sample analysis. The quantity of oil in the sample can be read against its corrected signal from the calibration curve. We have used the formula for the straight line for directly calculating the percentage of oil by the computer. For an overall check on the large scale analysis, an oilseed sample with known oil content was measured at regular intervals during the course of the analysis.

TESTING THE TECHNIQUE

The pulsed NMR technique for the seed oil determination was subjected to two tests. In the first test, the oil content of Brassica, peanut, and sunflower seed samples, each weighing ca. 2 g, was determined in duplicate by the pulsed NMR technique. The same samples (since the NMR technique is nondestructive) were analyzed by the cold percolation method (CCl_4 extraction). The results are given in Tables II, III, and IV. It may be noted that the pulsed NMR values for the duplicate Brassica samples generally agree within $\pm$ 1%. The agreement is not as good for the duplicate samples of peanut and sunflower, because 2 g is not a good representative sample for them. The blank spaces in the tables correspond to the samples on which the chemical analysis could not be completed because of the oxidation and the flask breakage. The oil values, belonging to one row, marked I under pulsed NMR technique and CCl_4 extraction are for the same samples, first analyzed by the pulsed NMR technique and then by the CCl_4 extraction. The same is true for the values marked II also.

In the second test, 60 Brassica seed samples were independently analyzed for the oil content at our laboratory by the pulsed NMR technique; at the Bhabha Atomic Research Center, Bombay, by the Soxhlet extraction method; and at the Swedish Seed Association, Svalov, by the steel tube gravimetric method (9). The 60 samples sent to each of the above laboratories were drawn from 20 Brassica crops in triplicate. The results obtained from the laboratories are given in Table V. The blank spaces in the table correspond to the samples for which no values were reported.

The reproducibility of the pulsed NMR measurements is better than the other two methods. The oil values determined by the steel tube method are signficantly higher than the Soxhlet method; the pulsed NMR values lie between them. The possibility of an analytical error is almost negligible in the NMR technique because the procedure is simple and automatic. In the case of any doubt, the analysis can be instantaneously repeated on the same sample because the technique is nondestructive.

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