Simultaneous Determination of Oil and Water Contents in Different Oilseeds by Pulsed Nuclear Magnetic Resonance[1]

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Routine analysis of oil and water contents in different oilseeds with the Bruker Minispec pulsed nuclear magnetic resonance (NMR) analyzer (Bruker Analytische Mestechnik, Rheinstetten, Germany) is rapid (16 s measurements), precise, accurate and nondestructive. In 1991, subcommittee SC2 (oil seeds) of the Technical Committee Number 34 [organized by the International Standards Organization (ISO), Geneva, Switzerland] organized an international collaborative study for pulsed NMR analysis of oil content in whole rapeseed. An additional study was performed in 1993 to extend the method to sunflower, linseed and soy seeds. The Draft International Standard ISO DIS/10565 (*Draft International Standards*, International Standards Organization, Geneva, 1993) describes the procedure of the pulsed NMR method for determination of oil content in rapeseed and presents the interlaboratory comparison results based on the calculations described in the Normative ISO 5725 (*Draft International Standards*, International Standards Organization, Geneva, 1993). The standard was approved in 1992 and will be published in 1993. The interlaboratory collaborative studies showed that the analyses of oil and moisture by pulsed NMR were either comparable or more repeatable than measurements done by the traditional methods. No statistical differences between determinations by the traditional and pulsed NMR methods were found. Simultaneous determination of percent moisture and percent oil content in whole seeds is possible with pulsed NMR by the spin-echo method. In addition, multiple components of the oil can be detected and quantitated by T2 analysis from the Carr–Purcell–Meiboom–Gill pulse sequence. The instrument is easy to calibrate with whole oilseeds, and the calibration can be checked periodically with the same seeds because the measurement is nondestructive. Pulsed NMR provides a rapid alternative to the long, laborious, traditional methods of analysis and offers substantial long-term savings of both time and money. Minimal operator training is required once the technique has been established for routine use.

KEY WORDS: Instrumentation, moisture content, NMR, oil content, oilseeds, pulsed NMR.

A quick method for determination of the oil and water contained in all commercial oilseeds is necessary because of the large amounts of oilseeds of differing quality that are involved in commercial transactions around the world.

The pulsed nuclear magnetic resonance (NMR) method has many advantages when compared to conventional chemical methods of analysis: (i) analysis by pulsed NMR is fast (the measurement takes 16 s) and can easily be repeated in the event of unexpected results; (ii) the method is nondestructive, the analyzed seeds can be directly planted after measurement; (iii) the calibration procedure is easy, and the calibration line is stable. A set of three rapeseed calibration samples, certified by the BCR (Bureau Communautaire de Référence, Brussels, Belgium) can be purchased, and other reference seed samples are planned; (iv) reproducibility of the results is excellent; (v) the simplicity and the rapidity of the technique allows for the screening of a large number of samples by relatively unskilled personnel; (vi) pulsed NMR does not require wet chemistry, flammable solvents or drying of the seeds to constant weight; and (vii) the method is applicable to whole commercial seeds.

In 1991, subcommittee SC2 (oil seeds) of the Technical Committee (TC) Number 34 [organized by the International Standards Organization (ISO), Geneva, Switzerland] organized an international collaborative study for pulsed NMR analysis of the oil content in whole rapeseed. An additional study was performed in 1993 to extend the method to sunflower, linseed and soybean seeds. Unfortunately, only Bruker Minispec (Bruker Analytische Mestechnik, Rheinstetten, Germany) users participated in this collaboration.

The Draft International Standard ISO DIS/10565 (1) describes the procedure of the pulsed NMR method as a standardized method for the determination of the oil content in rapeseeds, and it presents the interlaboratory comparison results based on the calculations described in Norm ISO 5725 (1).

An earlier Normative Standard (Norm ISO 5511, Ref. 2) and the Federation of Oil and Fats Association (FOSFA) text (3) described the use of CW (continuous wave) NMR. However, CW NMR has the disadvantage of taking $5 \times 32 = 160$ s for one result under standard conditions, and it requires time-consuming drying of the seeds (an entire night at 103°C), after which the seeds are no longer viable for planting. In addition, the norms describing the CW NMR method give no details about the test results.

MATERIALS AND METHODS

The normative reference methods are as follows:

Oilseeds—determination of hexane extract (or light petroleum extract), called "oil content" (4). This method involves two determinations on 10 g out of 200 g seeds with a Soxhlet extraction apparatus. Extraction takes approximately 6–8 h. The difference between the two results must be less than 0.4% in oil content. This method is time-consuming, needs qualified chemists and requires flammable solvents. In France, a rapid hexane extraction method (4 h, NF V03908-1988) is also used.

Oilseeds—determination of moisture and volatile matter content (5). This method involves dehydration of approximately 10 g of seeds in a 70-mm diameter metal cup placed in an oven at 103°C for 3 h, followed by a cool-down in a desiccator equipped with P_2O_5 and weighing. The sample is then heated in the oven for one hour, cooled down and weighed a second time. This procedure is repeated several times until a constant weight is attained. This method is also time-consuming. Use of fresh P_2O_5 in the desiccator is important to avoid rehydration, which gives an NMR signal, and to assure reproducible measurements. These dried samples cannot be used as 0% water samples for NMR because some rehydration is un-

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avoidable. In France, a rapid method is also used (15 h at 103°C, NF V03909/1988).

Oilseeds sampling (6). This involves preparation of the laboratory sample.

Oilseeds—reduction of the laboratory sample (7). This requires the reduction of the bulk laboratory sample with the appropriate equipment to obtain 200 g rapeseeds or 500 g sunflower seeds for the different analyses. The sample reduction is an important step because the seeds are not always homogeneous in a sample batch, and therefore the sample must be collected carefully to yield a representative sample. Differences found between NMR results and reference methods results are mainly due to this sample reduction, especially when no appropriate reduction apparatus was available.

Principles of the pulsed NMR method. Gambhir (8) has recently reviewed different pulsed NMR methods for the determination of oil content. In oilseeds, protons (^1H) that produce the NMR signal are present mainly in four forms—oil, moisture, carbohydrate and proteins. Differences in the mobility of the ^1H nuclei in the various hydrogen-containing constituents give spin-spin relaxation times (characterized by the relaxation time constant T2) that are quite different.

Figure 1 shows the free induction decay of the NMR signal after a 90° RF (radio frequency) pulse. The signal from the "solid" components (carbohydrates and proteins) decays rapidly, and, after 70 μs, only the signal from the "liquid" components (water in an adsorbed state and small liquid droplets of oil) remains. The signal voltage measured at this point (S1) is proportional to the water and oil contents of the seeds.

The T2 relaxation time constant of the adsorbed water is in the range of a few milliseconds, whereas T2 from the oil is in the order of a few hundreds of milliseconds. As a consequence, the contribution of water and oil protons to a pulsed NMR signal can be separated by the spin-echo method.

Spin-echo method in low-resolution pulsed NMR. Figure 2 shows the spin-echo signal (S2), measured after a 180° pulse, which refocuses only the oil protons. The signal S2, measured in volts, is proportional to the number of oil protons present in the seeds. The difference (S2 − S1) is proportional to the adsorbed water content. To determine the oil content of unknown seed samples, the seeds must be weighed and the NMR signal must be calibrated against a known amount of oil under identical experimental reference conditions.

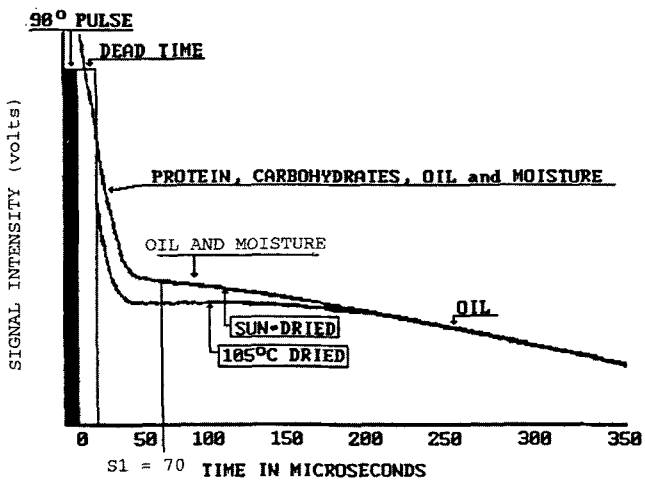

FIG. 1. Free induction decay of mustard seeds dried in the sun and at 105°C.

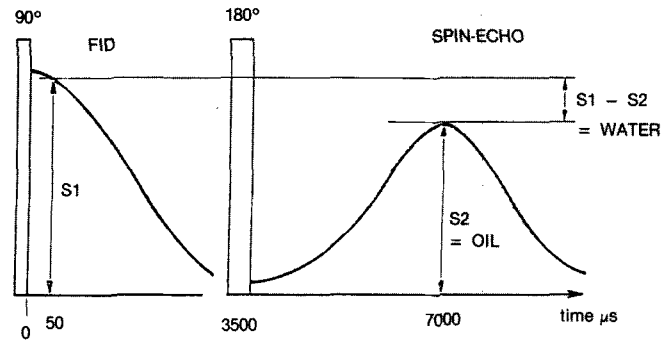

FIG. 2. The spin-echo pulse sequence showing measuring points S1 and S2.

The signal S2 depends on the oil's proton density (composition), on the T2 of the oil and on the quantity of excess water (absorbed liquid water or external moisture). Effectively, if it rains, the seeds become physically wet with absorbed water. Excess absorbed water has a T2 relaxation time similar to the oil phase and, therefore, contributes to the oil signal measured by the spin-echo method. Therefore, if the moisture content is greater than the normal hydration content of commercial seeds (approximately 10% for rapeseed and sunflower, 14% for soy), the weight excess must be removed in a drying oven (one hour at 80°C) before oil determination.

Advantage of the pulsed spin-echo method in low-resolution NMR. The pulsed NMR spin-echo technique permits the simultaneous determination of the oil and moisture content of seeds. The oil content of the seeds can be measured separately from the moisture without drying the seeds to constant weight.

In contrast, the CW NMR method measures only a signal equivalent to S1, proportional to water and oil contents of the sample and relies on either drying the seeds to constant weight (low residual moisture) or incorporation of an offset correction to measure an oil-only signal.

The spin-echo method, described in the Norm ISO DIS/10565 (1) is the best for routine measurements. However, the specified repeatability and reproducibility of the measurements can only be obtained with an instrument which has long-term magnetic field stability, good magnetic and RF field homogeneity over the sample volume, high-quality 90° and 180° RF pulses, and a high-quality stable NMR signal receiver.

The spin-echo method has the advantage of limiting the effects of magnetic field inhomogeneity. This method is also used in T2 experiments to measure accurate T2 values for various liquid components directly from the oilseed.

T2 measurements. T2 measurements of the different water and oil components of seeds can be easily achieved by means of the Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence and optional Minispec Exp-Sup (Bruker) software.

Figure 3 shows the envelope of 100 echoes between 1.5 and 150 ms. The duration between two 180° pulses is 0.75 ms. Analysis of the decay curve with a monoexponential

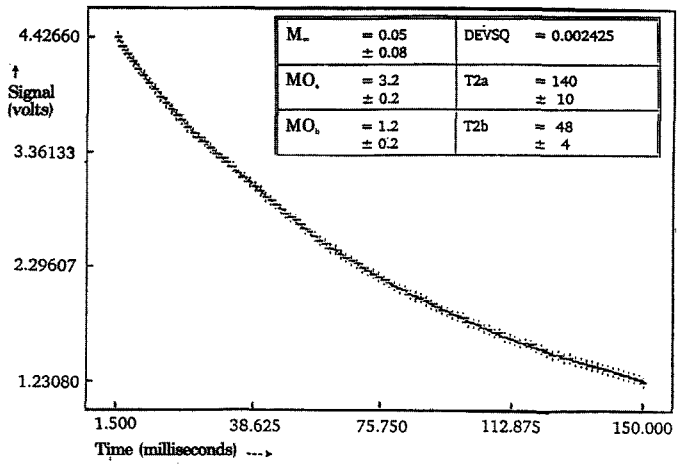

FIG. 3. Calculation of the T2 relaxation time constants for sunflower seeds. The fit results box lists signal magnitude (in volts) at time infinity (M_∞), of component a at time zero (Mo_a), or component b at time zero (Mo_b), and the T2 values of components a and b (T2a, T2b). DEVSQ represents the fit accuracy calculated as a quadratic sum of deviations between measured and fitted points.

(Mono) fit allows calculation of a best-fit relaxation time constant (T2 Mono). If a Marquardt fit is done on the curve, two components with different relaxation time constants are calculated (a long one, referred to as T2a; and a short one, T2b). Results of T2 analysis of two samples of normal sunflower seeds, TA and TB (composition C18:1 = 22%, C18:2 = 65%,) and two samples of oleic sunflower seeds, TOA and TOB (composition C18:1 = 83–88%, C18:2 = 2–7%), are summarized in Table 1.

These measurements can be used to differentiate different kinds of sunflower seeds, but studies of several samples from different origins are necessary to determine the selection criteria. For example, a sunflower seed sample is normal (not oleic) if T2 Mono is greater than 75 ms.

Apparatus. The analyzed seed sample must be representative of the laboratory sample, and a minimum weight of 10 g is necessary. We have chosen a sample volume of 40 mL, which corresponds to the best compromise between maximum sample volume and pulsed NMR requirements to obtain accurate and reproducible results.

For commercial transactions that require oil, moisture or seed quality verification, the Minispec PC 110/125/40RTA (Bruker) with sample tube diameter of 40 mm is recommended and was used in the collaborative studies for ISO DIS/10565 (1). The filling height (corresponding to 34 ± 6 mL) is 30 ± 5 mm with no precautions or instrument adjustments required. This is an important advantage for fast sample preparation. The corresponding sample weights are, approximately, as follows: rapeseed, 20–25 g; sunflower, 12–19 g; soy, 19–27 g; and linseed, 17–26 g.

A filling height of 50 mm (equivalent to a sample volume of 56 mL) is also possible, but then the magnetic field resonance tuning and the 90 and 180° RF pulse widths must be carefully adjusted, and the filling height must be constant at 50 ± 1 mm.

In some situations (e.g., for seed growers), only a limited quantity of seeds is available for analysis. Then, the Minispec PC 110/100/30RTA with sample tubes of 30 mm diameter or the Minispec PC 110/100/25RTA (Bruker) with sample tubes of 25 mm diameter can be used. The filling height is also 30 ± 5 mm for these systems. An electronic balance with 10- or 1-mg accuracy must be used to weigh each sample. The balance can be interfaced directly to the Minispec *via* an RS232 interface. Errors due to incorrectly entered weights can be avoided by using an interfaced balance.

TABLE 1

Relaxation Time (T2) Measurements of Sunflower Seeds

Sample[a]	T2 Mono[b] (ms.)	T2a (ms.)	T2b (ms.)
TA	90.5 ± 0.2	140 ± 10	48 ± 4
TB	81.3 ± 0.2	96 ± 6	29 ± 6
TOA	71.9 ± 0.2	83 ± 4	25 ± 4
TOB	66.8 ± 0.2	72 ± 2	16 ± 3

[a]TA, TB, two samples of normal sunflower seeds (C18:1 = 22%, C18:2 = 65%); TOA, TOB, two samples of oleic sunflower seeds (C18:1 = 83–88%, C18:2 = 2–7%).
[b]Mono, monoexponential.

Calibration procedures. Calibration samples must always be homogeneous with a low impurity content.

Calibration with pure oil. This method seems to be the easiest and the most accurate way to calibrate the instrument and is used in the CW NMR Norm ISO 5511 (2). However, the oil must be obtained through Soxhlet extraction (4) from the same kind of seeds as the seeds to be analyzed (e.g., rapeseed with high or low erucic content, sunflower, etc.).

The NMR signal is influenced by the hydrogen content of the oil (number of hydrogen atoms per unit mass of oil), which varies from one oil to another because of their differing fatty acid compositions, as previously described in the literature (9–11). Srinivasan *et al.* (11) have shown that the erucic acid (C22:1) hydrogen content in one variety of mustard seed varies between 105.55–106.48 (based on a water hydrogen content of 100). For this case, the NMR estimation of oil content in the first seed sample whose "true" oil content is 40% would give a theoretical value of 40.35%.

A calibration curve can be obtained with a minimum of three samples. The samples can be prepared by using three aliquots of oil, 5, 9 and 12 g, weighed to 1 mg accuracy (i.e., the 12-g sample will give a signal approximately equal to a 25-g rapeseed sample that contains 50% oil). To disperse the oil through the sampling area, each tube is filled to a height of 30 mm with dry absorbent paper (e.g., Kimwipes), the oil is droppered onto the paper, and the calibration tubes are allowed to stand for 10 min to allow for uniform distribution before measuring. Typically, a correlation of 99.99% can be obtained.

Calibration with seeds. Calibration with pure oil requires the use of freshly extracted oil because the oil is not stable over time. Many control laboratories in harvest offices have no chemical laboratory to do extractions and no opportunity to buy freshly extracted oil. Therefore, the BCR in Brussels was asked in 1987 to organize the sale of certified rapeseed samples as calibration standards. Three samples can be purchased from BCR with the following oil content values in percent, certified by an international collaborative study: A, 39.49 ± 0.14%; B, 42.00 ± 0.15%; C, 45.43 ± 0.18%. The water content in percent by weight is: A, 7; B, 7.4; and C, 7.7.

For sunflower seeds there is some solid wax in the external husk which is not liquid at the measurement temperature (20–30°C) and therefore is not present in the liquid NMR signal (pulsed NMR or CW NMR). It is, however, extracted through the reference method. By using pure oil as a calibration sample, the NMR results are approximately 0.8% lower for sunflower seeds than those obtained through the reference method. Therefore, to obtain an excellent correlation between NMR and the reference method, calibration with the same kind of oilseeds is necessary.

Calibration with one sample called "weight variation." Instead of using pure oil, an oilseed with a known percent of oil content is used as a calibrant. The quantity of seed in the probehead is varied to simulate seed samples of varying oil content. The NMR signal measured was found to be linearly related to the quantity of oilseed in the probe (Fig. 4). For this procedure to be successful, the percent oil of the seed used for calibration must be accurately known, and the homogeneity of the magnetic and RF field must be good over the entire sample volume. The resulting calibration line should be linear and pass through the origin.

Calibration with a minimum of three samples. The complete method is detailed in the text of ISO DIS/10565 (1). The BCR standard rapeseed sample weighs about 150 g, enough to prepare four or five sample tubes.

The calibration curve (Fig. 5), obtained from four replicate samples of oilseed A, oilseed B and oilseed C, indicates some inhomogeneity in the samples. Therefore, it is statistically better to measure twelve (4 × 3) samples rather than three (1 × 3). An empty tube is measured to generate a zero percent fat point on the calibration line.

Calibration for water. It is difficult to find samples with a known water content that are stable over several days and cover a large range (minimum between 4.5 and 8.5% for rapeseed and sunflower, respectively). In most cases, their water content reaches an equilibrium value (about 7 or 8% for rapeseed). Several calibration methods are possible.

Calibration with a minimum of three different samples. This procedure is similar to the method described previously for calibrating oil. The water content of one part of the samples must be controlled on the same day with the ISO Reference Method.

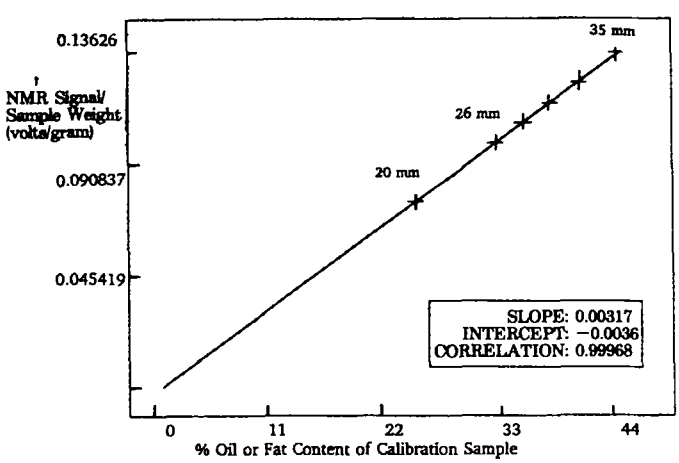

FIG. 4. Oil NMR signal calibration by weight variation of a rapeseed sample between 20–35-mm filling height. NMR, nuclear magnetic resonance.

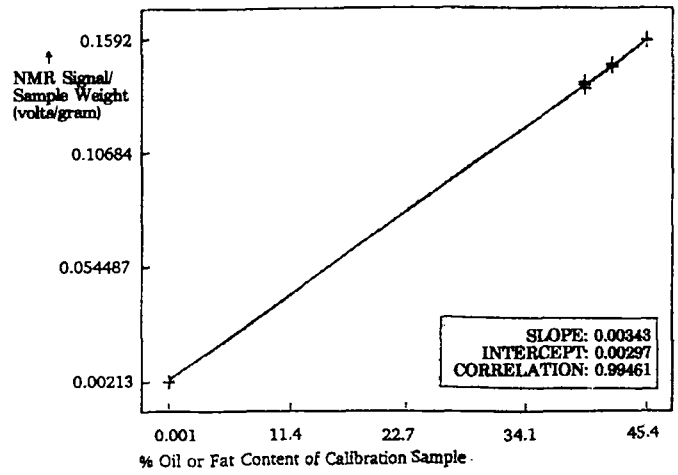

FIG. 5. Oil NMR signal calibration with three rapeseed samples and an empty tube. NMR, nuclear magnetic resonance.

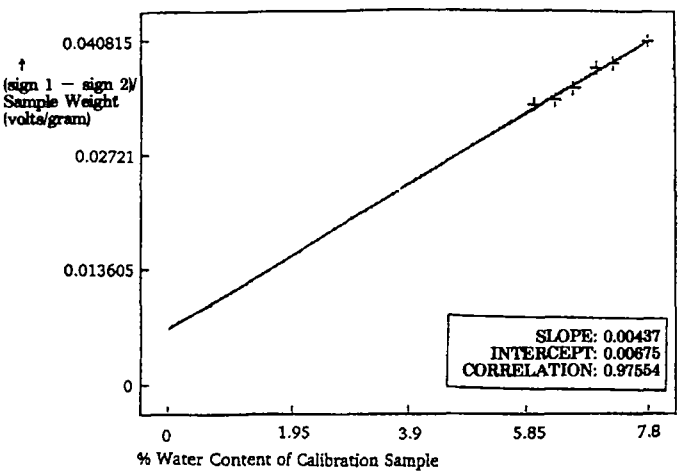

FIG. 6. Water nuclear magnetic resonance signal calibration by weight variation of three rapeseed samples.

Calibration with weight variation. The calibration line in Figure 6 was obtained by two different filling heights (35 and 30 mm) of the three rapeseeds reference samples (A, B and C). The water content is calculated in grams, then converted to percent water as in the weight variation method to determine percent oil.

Seeds that have been dried to constant weight (to a very low residual water content) should not be used as a 0% standard for the NMR because some rehydration could occur after the sample is removed from the drying oven.

Measurement of samples: temperature effects upon the results. NMR signal amplitudes depend on temperature and exhibit "Curie-Weiss"-type behavior. The amplitude S1 decreases as the temperature increases from A to B and follows (approximately) the relationship S1A × TA = S2B × TB. Amplitude S2 varies with the relaxation time constant T2 but not by a simple linear relationship.

For a variation of 1°C, the variation of the measured oil content of rapeseed that actually contains 40% oil is approximately 0.14%. The temperature also has an effect on the liquid oil phase variation for seeds containing wax.

Samples should be thermally equilibrated to a standard

temperature prior to measurement, or separate calibration curves can be set up for a variety of temperatures at which samples may be measured. For example, if room temperature varies depending on the time of day, a good solution is to record calibration lines at 20, 22, 24, 26, 28 and 30°C, so that the appropriate calibration lines for the lab temperature can be selected when unknown samples are measured.

Instrument repeatability. To test the "electronic" repeatability of the measurement, a rapeseed sample tube was inserted into the magnet for a measurement and withdrawn. This process was repeated 15 times with a wait period of at least 5 min between measurements. The magnet is regulated at a constant temperature of 40°C, which makes the temperature of the samples increase during measurement. The sample must be allowed to equilibrate to room temperature between measurements. The standard deviation was ±0.04 for the percent oil and ±0.05 for the percent water measurements.

Measurement repeatability can be influenced in some cases by poor seed homogeneity in the RF coil. If this is a problem, the seeds in the sample tube (30-mm filling height) are agitated between measurements. Typical standard deviations obtained under these conditions with 7 measurements every 10 min are: rapeseeds, oil, ±0.08; water, ±0.03%; sunflower seeds, oil, ±0.06; water, ±0.13%; soybean, oil, ±0.04; water, ±0.07%; and linseed, oil, ±0.09; water, ±0.05%.

Batch homogeneity. The previous results must be compared with the homogeneity of the samples taken from the bulk oilseed batch. Analyses of ten samples taken from two bulk oilseed batches of sunflower, soy and linseed, gave the following results: sunflower (in Batch 1 or Batch 2): oil, ± 0.58 or ± 0.32, water, ± 0.06 or ± 0.06; soy (in Batch 1 or Batch 2): oil, ± 0.12 or ± 0.18, water, ± 0.10 or ± 0.47; linseed (in Batch 1 or 2): oil, ± 0.11 or ± 0.08, water, ± 0.10 or ± 0.14.

Table 2 shows the results of the analysis of two batches of sunflower seeds during the collaborative study. Statistical analyses of the results will determine if two, three or five determinations are necessary. The same sample has to be analyzed by both methods to compare NMR measurements with hexane extraction.

Rapeseed results. The collaborative study was organized by AFNOR (Association Française de Normalisation, Paris, France), the French Secretariat of Subcommittee SC2 of Technical Committee ISO/TC34 in 1990, and the results were presented in document N403F at the ISO meeting in Winnipeg, Canada, in July 1991. Thirteen laboratories participated, including three from Germany, one from The Netherlands, eight from France and one from the United Kingdom. The Draft ISO DIS/10565 (1) was accepted in 1992 by a vote of more than 75% of the 13 international members and was published in 1993. It will be presented as a norm during the next session of the CEN (Comité Européen de Normalisation).

The measurements were calculated by following ISO 5725 (1), and the results are presented in Table 3. The

TABLE 2

Analysis of Two Batches of Sunflower Seeds

	Batch 1			Batch 2		
Sample tube	Weight	% Oil	% Water	Weight	% Oils	% Water
1	13.23	40.22	6.65	12.46	41.28	6.70
2	13.36	40.76	6.63	13.46	40.72	6.54
3	13.46	40.85	6.56	13.51	41.27	6.61
4	13.36	40.45	6.64	13.27	40.60	6.54
5	14.07	41.22	6.56	12.62	41.03	6.67
6	13.26	40.08	6.69	13.24	40.97	6.62
7	13.55	41.44	6.50	13.47	40.93	6.61
8	13.21	41.69	6.56	13.11	40.90	6.50
9	12.96	41.46	6.52	13.54	41.38	6.61
10				12.46	41.66	6.55
Mean	—	40.91	6.59	—	41.07	6.59
Standard deviation	—	0.58	0.06	—	0.32	0.06

TABLE 3

Rapeseed ISO Collaborative Study Results[a]

	Oil				Water			
	NMR		Reference		NMR		Reference	
	Batch 1	Batch 2	Batch 1	Batch 2	Batch 1	Batch 2	Batch 1	Batch 2
Number of laboratories	13	13	13	13	12	12	13	13
Number of accepted results	13	13	13	13	11	12	11	11
M (global mean)	40.83	41.38	41.00	41.47	7.01	6.07	6.7	5.6
sr (repeatability standard deviation)	0.25	0.15	0.20	0.20	0.04	0.03	0.0	0.3
r (repeatability 95%)	0.72	0.45	0.58	0.56	.011	0.09	0.1	0.1
CVr (coefficient of variation)	0.63	0.38	0.50	0.48	0.60	0.58	0.7	0.8
sR (reproducibility standard deviation)	0.55	0.55	0.53	0.57	0.24	0.36	0.1	0.1
R (reproducibility 95%)	1.56	1.56	1.50	1.62	0.67	1.02	0.5	0.5
CVR (coefficient of variation)	1.35	1.33	1.29	1.38	3.41	6.01	2.6	3.1

[a]ISO, International Standards Organization (Geneva, Switzerland). NMR, nuclear magnetic resonance.

results obtained from the two methods were compared by performing an analysis of variance on the data set of each method. There was no significant difference between the repeatability and the reproducibility of the pulsed NMR and the traditional methods of measurement for oil contents. Moisture measurements by pulsed NMR proved to be less reproducible than those by the traditional method. This is likely due to the fact that some of the calibration samples had moisture contents greater than 10%.

Sunflower, soy and linseed. A collaborative study was organized at the end of 1992 (by AFNOR, the French Secretariat of SC2 of ISO/TC34) for oil and water determination in the following samples: one normal sunflower for calibration, one for analysis, one oleic sunflower for calibration, one for analysis; one linseed sample for calibration, two for analysis; one soy sample for calibration, two for analysis. Twelve laboratories participated; 7 from France, 3 from Germany and the 2 from The Netherlands. The results have been discussed at the ISO meeting in Gembloux (Belgium) in June 1993. The pulsed NMR method results show good repeatability and reproducibility.

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