Analysis of Oil Content of Groundnuts by Nuclear Magnetic Resonance Spectrometry

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One hundred groundnut germplasm accessions were analysed for their oil content by the standard Soxhlet extraction procedure using petroleum ether; the results were compared using functional analysis with those obtained with a commercial nuclear magnetic resonance (n.m.r.) spectrometer. On another 78 experimental samples, oil content was first obtained using the n.m.r. method and the Soxhlet values were predicted using the above functional relationship. On the 78 samples the predicted values were highly correlated ($r=0.972$) with those obtained by Soxhlet extraction. The n.m.r. method is rapid, easy, acurate and non-destructive.

Keywords: Groundnut; oil content; n.m.r. spectrometry.

1. Introduction

The physical principles of nuclear magnetic resonance (n.m.r.) spectrometry are well known.[1,2] N.m.r. provides accurate counts of hydrogen nuclei in oil even in a surrounding matrix of starch, protein etc. The nuclear count is then related to the oil mass which provides the same n.m.r. reading and the percentage of oil estimated from the mass of the sample.

The application of the wide line n.m.r. technique for the rapid non-destructive estimation of oil content in intact whole seeds has been successful in the case of other oil seeds,[3] maize,[4] soyabeans[5] and rapeseed.[6] The n.m.r. method of oil analysis, which is non-destructive, would help in the rapid analysis of oil content of groundnut cultivars in a breeding program. This study was undertaken to compare the n.m.r. and Soxhlet methods for determining the oil content of groundnuts.

2. Experimental

2.1. N.m.r. method

Groundnut seed samples were obtained from the Genetic Resources Unit of International Crops Research Institute for the Semi-Arid Tropics (ICRISAT). The experiments were carried out using a Newport Analyser Mark III (Newport Instruments Ltd, Milton Keynes, UK). A steady field value of 635×10^{-4} T and a radio frequency of 2.7 MHz was used for all analyses. The integration period was kept at 32 s, at a gatewidth of 1.5×10^{-4} T and a radio frequency (RF) value of 100 μa was employed for screening all samples. The amplitude frequency gain, although variable, was usually 300. The instrument was installed in a room where the temperature was maintained at $25 \pm 2°C$. N.m.r. tubes (Nessler glass tube) with an etched mark were used for all the estimations. About 26 g of groundnut kernels or 30 g of oil were needed to fill to the etched mark. Oil extracted from about 100 groundnut germplasm accessions was used as a reference oil.

The standard volume of groundnut seeds and reference oil was weighed and the n.m.r. reading obtained. N.m.r. oil equivalent in the groundnut sample representing oil plus moisture content in

the sample was obtained using the following relationship:

$$\text{N.m.r. oil equivalent} = \frac{\text{weight of oil}}{\text{n.m.r. reading of oil}} \times \frac{\text{n.m.r. reading of sample}}{\text{weight of sample}} \times 100$$

The moisture content of sample was determined by drying the sample at 110°C for 16 h and this value was subtracted from the n.m.r. oil equivalent to obtain the oil percentage in the groundnut seed sample. The effect of repacking the seeds on the n.m.r. reading was tested as follows: after taking the initial n.m.r. reading, seeds were removed and repacked in a random fashion. A reading was taken again and this procedure was repeated five times with each of 10 different cultivars. The effect of seed testa on the n.m.r. reading was evaluated by determining the oil content in 10 cultivars each with and without the testa.

2.2. Soxhlet method

Oil was determined by grinding about 10 g of groundnut seeds in a Krups, KM 75 (Robert Krups, 5650 Solingen 19, Postfach 190460, West Germany) blender and the groundnut meal was extracted with hexane (petroleum ether, 60°C b.p.) for 18 h in a Soxhlet apparatus. Hexane was evaporated on a hot sand bath and the oil residue was weighed to calculate the oil percentage in the sample. The precision of the method was checked by analysing five subsamples each of four cultivars.

One hundred germplasm accessions of groundnut were analysed by both the n.m.r. and Soxhlet extraction methods. After determining the moisture content of these samples, oil content for each sample was adjusted to 5% moisture level, in order to have comparative evaluation of oil content in groundnut cultivars and also because the mean moisture percentage in germplasm accessions was about 5%. The results were compared statistically using the functional analysis.[7] An additional 78 germplasm accessions were treated as unknown experimental samples and were analysed by the n.m.r. method. Their Soxhlet oil values were predicted using the functional relationship obtained with the original 100 samples. Later, Soxhlet values were obtained on these 78 samples and the predicted and actual Soxhlet values were compared.

## 3. Results and discussion

3.1. N.m.r. method

Varying the levels of reference oil in the n.m.r. tube (8.60–29.41 g) or the weight of seed in the n.m.r. tube (6.23–21.21 g) had little influence on the oil percentage values obtained in the seed sample. Therefore, it would appear to be possible to determine the oil content even with limited seed quantities using the n.m.r. method. The effect of repacking on n.m.r. readings was evaluated by taking five readings for each of the 10 cultivars. The means for these samples varied from 47.78 to 52.27 and the standard errors varied from 0.01 to 0.09 indicating that the orientation of samples in the magnetic field had little influence on n.m.r. readings. The effect of sub-sampling on n.m.r. readings was examined by analysing 10 sub-samples of one cultivar. Oil content in these samples as determined by the n.m.r. method varied between 40.16 and 41.13% with a mean value of 40.73% and a standard error of 0.09.

The testa content was determined on 10 groundnut cultivars which varied between 1.67 and 3.93% with a mean of 2.44%. When determined by the n.m.r. method, the oil percent in samples without testas ranged from 43.49 to 48.32% with a mean of 46.25% and in samples with the testa intact, oil percent ranged from 42.06 to 47.36% with a mean of 45.16%. Although the testas accounted for less than 2.5% of the seed, its presence reduced the oil content of seed by about one percentage point. A similar result was observed when the oil contents in these samples were determined by the Soxhlet method.

3.2. Soxhlet extraction method

The means and standard errors of four cultivars ranged from 39.93 to 44.67% and 0.21 to 0.31 respectively when sub-samples of a bulk ground meal of each cultivar were used to test the
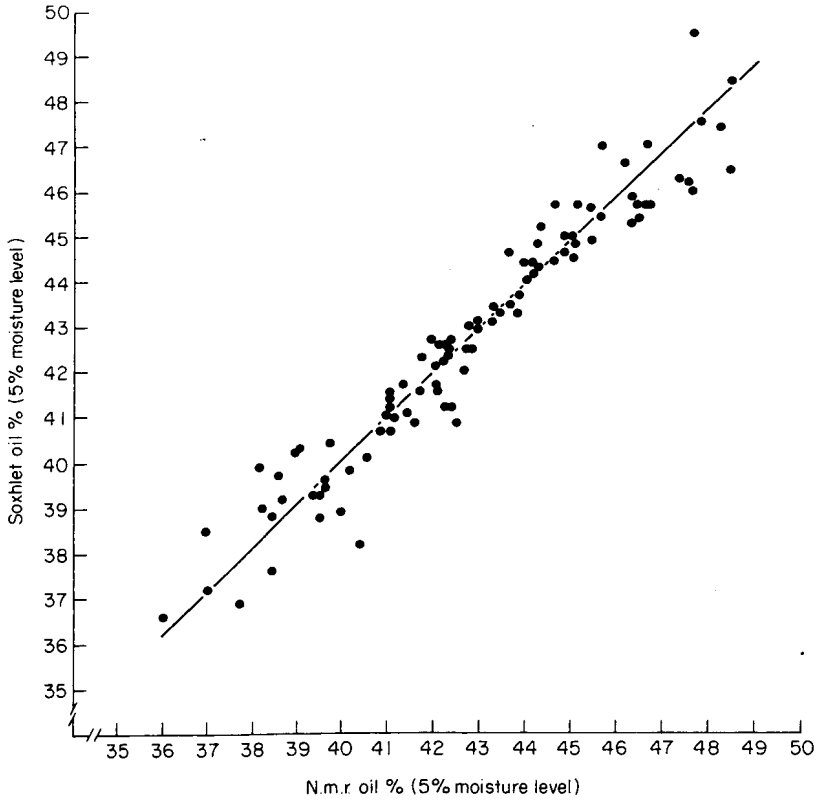
Figure 1. Functional relationship between n.m.r. and Soxhlet methods $y=1.337+0.968x$ $(n=100, \hat{\sigma}_{\xi}^2=0.2570)$.
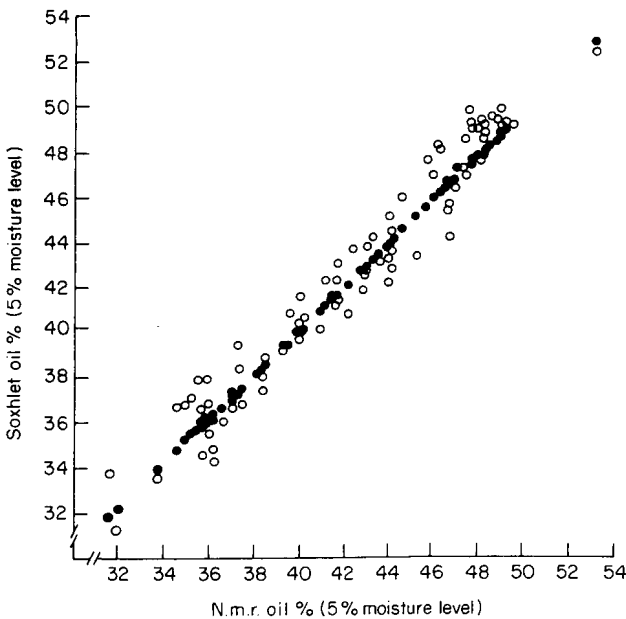
Figure 2. Agreement of n.m.r. oil values with predicted (●) and determined (○) Soxhlet values $(n=78)$.

precision of the Soxhlet method. When sub-samples of kernels of each cultivar were ground and extracted separately, the means ranged from 41.63 to 44.90% and standard errors of 0.42 to 0.65, indicating higher variability when sub-samples of kernels were used.

3.3. Comparison of results obtained by the n.m.r. and Soxhlet methods

One hundred germplasm accessions were analysed by the standardised n.m.r. method and Soxhlet extraction procedures and their moisture contents were determined. Oil content in these 100 accessions varied from 36.1 to 48.4% when determined by the n.m.r. method while Soxhlet extraction values ranged from 36.6 to 49.5%. The relationship between these two methods was examined using the functional analysis $Y = \alpha_0 + \alpha_1 X$, where α_0 was 1.337 with 95% confidence interval between -0.8045 and 3.478 and α_1 was 0.968 with 95% confidence interval between 0.9184 and 1.020. $\hat{\sigma}_{\varepsilon}^2$ had a value of 0.2570 (Figure 1). The computed values of α_0 and α_1 showed that these values were not significantly different ($P = 0.05$) from zero and unity. The percent variance in Soxhlet extraction method accounted for by the functional relationship in the measurement with the n.m.r. method was 93.4%. Therefore, the n.m.r. method can be recommended as it gave values which were similar to those given by the standard Soxhlet method (Figure 1).

Table 1. Agreement of n.m.r. values with Soxhlet values[a]

Number of samples	Soxhlet oil Range (%)	Soxhlet oil Mean (A) (%)	NMR oil Range (%)	NMR oil Mean (B) (%)	Error range between A & B (%)	Mean error between A & B (%)
3	31.00–33.99	32.86	31.45–33.66	32.30	−1.63 to +6.65	+1.70
11	34.00–36.99	35.94	34.58–37.29	35.95	−5.25 to +5.65	−0.02
11	37.00–39.99	38.28	35.24–39.87	37.43	−1.97 to +6.06	+2.22
15	40.00–42.99	41.58	39.35–44.10	41.55	−3.09 to +3.96	+0.07
13	43.00–45.99	44.27	42.29–46.74	44.45	−5.70 to +3.11	−0.41
11	46.00–48.99	47.71	45.66–48.16	47.02	−1.02 to +4.16	+1.45
13	49.00–51.99	49.25	47.47–49.39	48.35	−0.35 to +4.39	+1.83
1	52.00–55.00	52.15	53.13	53.13	—	−1.88

[a]All values reported at 5% moisture level.

Oil content was determined on another 78 groundnut samples by the n.m.r. method. Then, using the functional relation $Y = 1.337 + 0.968\,X$, Soxhlet values were predicted from the observed n.m.r. values, and the results obtained by these two procedures agreed well as shown in Figure 2. The validity of the above relationship was further confirmed by determining the oil content on all 78 samples by the Soxhlet method. Table 1 shows the results of analysis of 78 samples by both the n.m.r. method and Soxhlet procedures and the mean error percentage for the various groups. The correlation between the predicted Soxhlet values and determined Soxhlet values was 0.972 which explain for 94.5% of the variation by the Soxhlet method (Figure 2).

4. Conclusions

From the results obtained, it was concluded that the non-destructive method of determination of oil by n.m.r. gives results comparable to the standard Soxhlet procedure. An advantage of the n.m.r. method is the short time (5 min) needed to determine the oil in each sample, and it can be easily operated by semi-skilled personnel. Moreover, it is non-destructive, requires less space and does not require inflammable chemicals and expensive glasswares. The difficulty of grinding seeds with high oil contents is also avoided by the n.m.r. method. Therefore, the rapid, easy and accurate determination of oil content is possible using the n.m.r. method.

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