Analysis of Oil Content of Soybeans by Wide-Line NMR

F. I. COLLINS, D. E. ALEXANDER, R. C. RODGERS and L. SILVELA S.[2]
Illinois Agricultural Experiment Station, Urbana, Illinois

Abstract

Samples of soybeans, ranging from single seed to 25 g, were scanned by NMR and then gravimetrically analyzed for oil content. High positive correlations of NMR and oil for single seed ($r = 0.998$) and for 25-g samples ($r = 0.999$) were found. Single 30-sec NMR scans gave accurate estimates of the oil content of soybean seeds which had been dried to less than 4% moisture content. Fifteen samples of known percentage composition, created by mixing calculated weights of soybean oil with oven dry soybean meal (made lipid-free by petroleum ether and by carbon tetrachloride extraction), were scanned by NMR. These 15 samples, starting at 2% oil and increasing by increments of 2% oil up to 30%, had a linear relationship of NMR readouts with these known percentages of oil. These results indicate that wide-line nuclear magnetic resonance spectroscopy is an accurate, rapid and nondestructive tool for determining the oil content of soybean seeds. Since NMR scanning of seed does not alter its composition or destroy its viability, this method of oil analysis could accelerate the development of new soybean strains.

Introduction

IT WOULD BE ADVANTAGEOUS to plant breeders to have methods of analysis for chemical compositions of seeds which were rapid, accurate, and nondestructive of the living seed. NMR analysis for oil does not destroy germination in the seed, and thus provides a means of selecting the single seed or seeds of plants which most likely have the genetic capacity to produce progeny seed of desirable chemical composition. Furthermore, because of the negative correlation between the oil and protein content of soybeans, NMR analysis for oil may provide, indirectly, an estimate of the protein content of the sample.

Literature Review

Nuclear resonance has been used for more than 15 years with great success in solving problems of physics and of physical organic chemistry (1,2). Conway (3) and his associates at Corn Products Company, Argo, Ill., were the first to apply the method to oil analysis of whole seeds. Conway used NMR readout to determine oil content of bulk samples of corn, as well as single kernels. As the NMR readout for oil determination is based on resonance of hydrogen nuclei in liquids, high seed moisture could result in error. He reported, however, that when moisture in corn was below 5% it did not contribute significantly to the hydrogen resonance produced by the liquid oil in the seed. The method he used for drying corn, 5 days at 65C, did not reduce its germination. Conway and Earle (4) reported on the use of NMR for oil analysis on whole seeds of 18 species, as well as extracted oil of 12 species. The correlation of NMR readout and extracted oil content for 25-g seed samples was $r = 0.993$. Brim et al. (5) used NMR analysis for oil in soybeans as a tool for selecting living seed with desirable chemical compositions. Belikov and Sazenenko (6) reported successful use of NMR to measure the oil content of single soybean seeds. Alexander et al. (8) studied extensively the effects of sweep time, moisture, multiple scans, and sample size (single seed to 25-g samples) on NMR analyses of corn, soybeans, sunflowers, safflowers, and oats. They found the linear relationships of NMR readouts and percentages of oil by gravimetric analyses of these seeds provided an accurate estimate of their oil contents. Very high correlations ($r = 0.99$) were obtained. Fehr et al. (7) compared analyses of soybean seeds by NMR, along with seed density, specific gravity, and chemical methods of analysis for their effectiveness in selecting soybeans with desirable chemical attributes. They found NMR analysis for oil was more reproducible and statistically more reliable than AOCS oil analysis for use in making selections for oil content of soybeans.

The experimental data presented herein are the details of the study on soybean seed and are a part of the results from a cooperative and parallel study on the use of NMR analysis for oil in corn, soybeans, and several other oil seeds, by Alexander, Silvela, Collins, and Rodgers.

Materials and Methods

The nuclear magnetic resonance instrument used for these experiments was a Varian PA-7 unit equipped with an integrator and two probes, one for 20 to 25 g of soybeans and the other one suitable for 1 to 3 soybean seeds. For soybeans, moisture contents of the seed should be 4% or lower to obtain satisfactory estimates of oil content by NMR analysis. Moistures in soybeans can be lowered to 4% without damage to living seed by drying the samples for 5 days at 52–55C in a forced draft oven. However, if there is no need to maintain viability in the seed, sufficiently dry seed for NMR analysis can be obtained in 1–3 hr in a forced draft oven at 130C. For use in experimental testing of the validity of NMR oil tests, the following samples of soybeans were prepared. Bulk samples of 400–500 g each of viable soybean seed were selected by AOCS analysis to obtain 7 samples with a range in oil of 14.2% to 24.5%. These samples were dried 5 days at 52–55C to reduce their moisture contents to 3–4%. Whole seed moistures in each sample (3.4–3.6%) were determined by AOCS official method. Samples of 25 g each were made into 7 pairs of matching samples by repeated scanning with NMR. Both 1-min and 30-sec scans were used. Thirty-second scans by NMR were found to be entirely reliable and as reproducible as 1-min scans, as was similarly reported for corn by Alexander et al. (8). One sample of each matched pair was flame sealed in a glass NMR cell to make a total of 7 permanent standards, and the other 7 were subjected to gravimetric analysis for oil content.

Similarly, 7 matched pairs of single soybean seed with a wide range in oil content were found by repeated scanning of many seeds from these same bulk

[1] Contribution of the US Regional Soybean Laboratory and the Illinois Agricultural Experiment Station, Urbana, Ill. Publication No. 490 of the US Regional Soybean Laboratory, Urbana, Ill.
[2] ARS, USDA, Plant Genetics Department, Urbana, Ill.

samples. One seed of each of the 7 pairs was flame sealed in a glass NMR cell. The other 7 single seeds were each analyzed gravimetrically for total oil content.

In order to test the linearity of NMR readout with a wide range of known percentage compositional samples, 15 samples ranging from 2%, 4%, 6%, etc., to 30% oil were prepared as follows. Calculated weights of soybean oil plus soybean meal (meal which was oil-free by NMR tests) were added to each NMR cell to equal 400 mg for each percentage composition of 2% to 30% oil. The oil plus the added meal in each cell was intimately mixed within a few minutes by capillary action and ultrasonic vibration of these NMR cells.

AOCS official methods of analysis were used on the samples of 25 g each except that prolonged extraction of residual oil was done with carbon tetrachloride in special Butt-type equipment with all glass joints. Analysis of single seed was done in a shaker-type ball mill (Spex mixer/mill) followed by 16 hr of extraction with petroleum ether of the residual oil in all glass Butt extraction equipment to obtain oil-free meal by NMR tests.

Results and Discussion

Seven samples of soybeans of 25 g each, one from each matched pair by NMR readout, were analyzed by AOCS methods for oil and whole seed moisture content. It was necessary to know the moisture content of standard samples which were flame-sealed within NMR cells in order to calculate accurate oil contents of soybeans of unknown compositions. Five grams from each sample were tested for whole soybean moisture content by the AOCS method (9). The remaining 20 g of each were ground in a Bauer mill (9) and 3 samples of 2 g each were used for AOCS oil analyses (9). Immediately following oil analysis, the filter paper-wrapped samples of meal were dried 20 min at 105C to remove moisture and traces of solvent. Since the hydrogen in the cellulose of filter paper does not interfere with hydrogen resonance in the oil, these samples of meal, still wrapped in filter paper, were tested for residual oil by NMR scanning. NMR signals indicated as much as 1% of oil remained in many of these samples. To remove this residual oil, these samples were subjected to 16 hr of additional extraction with carbon tetrachloride in special Butt-type equipment with all glass joints. Following this additional extraction of oil, the NMR tests showed that the meal was oil-free. Fig. 1 shows the calculated total weight of oil in each 25 g of soybeans plotted against its corresponding NMR readout. NMR readouts were the average of a total of 18 scans of 1 min of sweep time. Correlation of total oil and NMR readouts was $r = 0.999$.

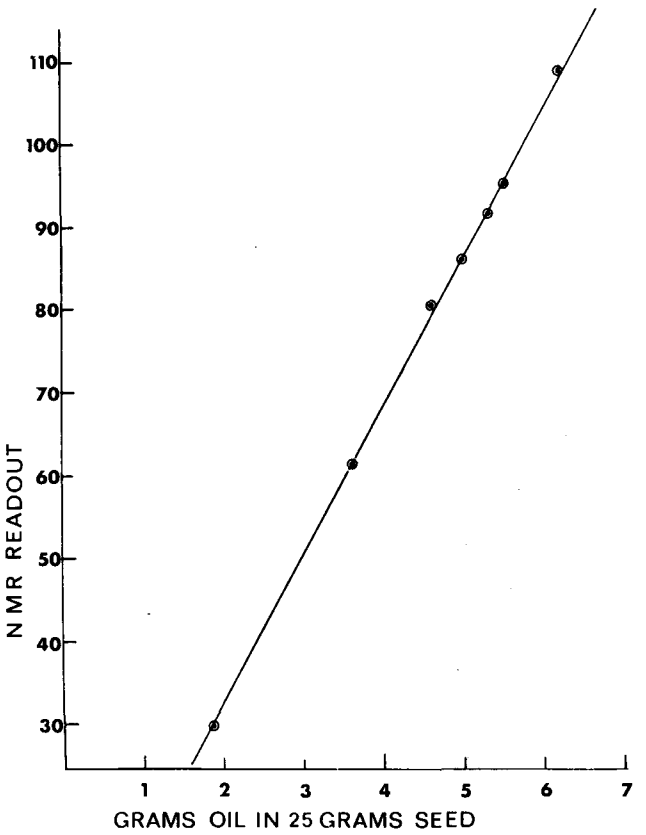

FIG. 1. Relationship of NMR readout and amount of oil in 25-g samples of soybeans as determined by gravimetric analysis. NMR readouts—mean of 18 one-minute scans. $r = 0.999$. NMR parameters; time constant 1, sweep time 1, sweep amplitude 0.5 gauss, sensitivity × 100, modulation amplitude 0.1 gauss, dial weight of the sample, threshold 0.1 miv, readout × 10, signal × 2. Radio-frequency setting 28.

Analysis of single soybean seed for each matched pair by NMR readouts required special methods of oil analysis. Extraction of the oil and grinding of seed at the same time was accomplished in a Spex mixer/mill. The cylinder, which houses the seed, solvent, and ball, was fitted with special Teflon gaskets, so that the solvent-oil-meal mixture could be sealed to prevent any leakage during the 15 min of the grinding extraction process. This shaker-type ball mill was effective in grinding one seed or 5 g of whole seed to flour-like fineness within 5 to 15 min without any loss of material. (The solvent may be either petroleum ether, [30–60C], or carbon tetrachloride.) The extraction cylinder was cooled in ice before being opened. Then the mixture of meal, oil, and solvent was transferred quantitatively to a filter paper. Immediately following filtering, the meal (which also contains some residual oil by NMR test) was wrapped in a second filter paper, and subjected to 16 hr of additional extraction in Butt-type all glass jointed equipment which produced oil-free meal to NMR tests. Thus the oil by ball mill extraction, plus the residual oil by prolonged extraction, accounted for all of the oil in the single seed. Fig. 2 shows the oil percentage obtained from 7 single seed with a wide range in oil content. NMR readouts are the averages of 10 scans of 1 min each. The correlation of oil and NMR readout was $r = 0.998$.

Single seed could not be analyzed successfully until this shaker-type mill became available. With this shaker-type ball mill fitted with Teflon gaskets, oil seed of all kinds can be ground in solvents without loss of material. A great deal of confidence in the analyses for oil is indicated by the linear relationship of NMR readout and oil. High oil content seeds such as sunflower, safflower, and corn germ, which lose a portion of their oil in attrition-type grinding mills, can be extracted to oil-free meal by NMR tests in this shaker ball mill by using either petroleum ether or carbon tetrachloride as the oil solvent followed by exhaustive extraction of residual oil.

Fig. 3 shows the linearity of NMR readout to percentages of oil in samples of soybean oil plus soybean meal of known composition. NMR readouts are based on the average of 6 scans. Correlation of NMR in this test was $r = 0.999$. The instrument is giving acceptable

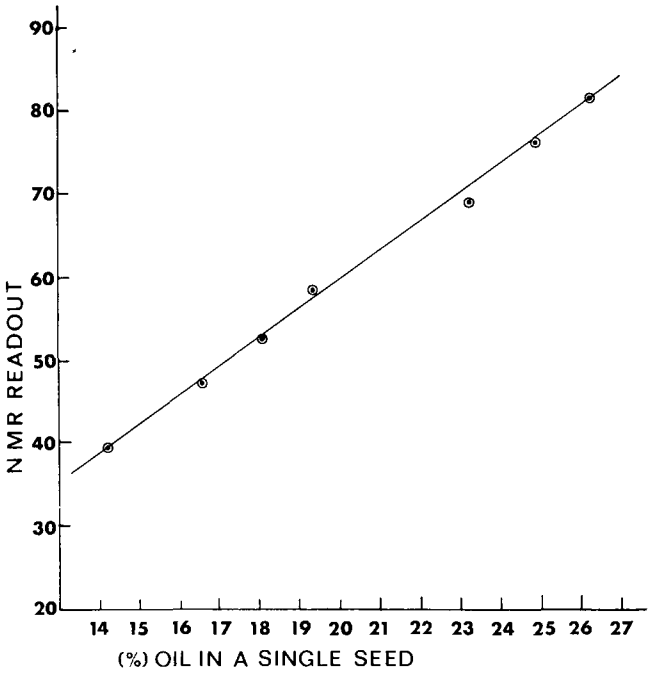

FIG. 2. Relationship of NMR readout and percentage of oil in a single soybean seed as determined by gravimetric analysis. NMR readout—mean of 3 one-minute scans. r = 0.998. NMR parameters; time constant 1, sweep time 1, sweep amplitude 0.1 gauss, sensitivity × 10, modulation amplitude 0.2 gauss, dial weight mg of sample, readout × 10, signal × 5. Radio-frequency setting 28.

NMR readouts when two successive readouts are within one unit of agreement. For example, 75 and 76 for the same sample which averages 75.5 are acceptable. The mean standard error of estimate for 25 g of corn scanned twice in succession in 30-sec sweep times was 14.7 mg of oil amounting to 0.059%. Similar "Standard error" would be anticipated for 25 g of soybeans although this test was not made on soybeans.

Experience has shown that NMR readouts shift downward gradually by 2 or 3 units over an extended time of several weeks, probably due to tube aging and unknown factors. This is not a serious problem from an operational standpoint since the linearity of NMR readout and the percentages of oil in the standards is checked daily and at regular intervals during the day to detect any shifts. Daily shifts in NMR readouts occur with small changes in room temperature even in an air-conditioned room. Several times within the past 2 years, replacement of aged tubes and reconditioning of the instrument have restored

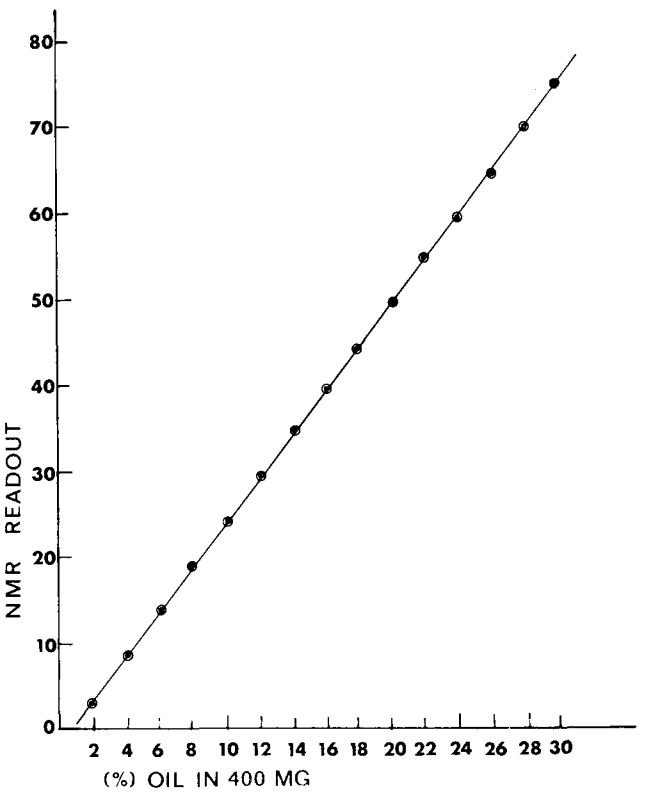

FIG. 3. Relationship of NMR readout and percentage of soybean oil in 400 mg samples of soybean meal with known composition. NMR readout—mean of 6 one-minute scans. r = 0.999. NMR parameters; time constant 1, sweep time 1, sweep amplitude 0.5 gauss, sensitivity × 50, modulation amplitude 0.1 gauss, dial 400 mg of sample, readout × 2, signal × 10. Radio-frequency setting 28.

the NMR readouts of our standard samples to values in close agreement with those obtained originally.

ACKNOWLEDGMENT

Research supported in part by NSF Grant GB 1732 and the Bear Hybrid Corn Company, Decatur, Ill. Technical assistance in preparation of samples by Vail E. Sedgwick.

REFERENCES

1. Block, F., W. W. Hanson and M. E. Packard, Phys. Rev. *70*, 127 (1946).
2. Purcell, E. K., R. C. Torrey and R. V. Pound, Ibid.
3. Conway, T. F., Proceedings of a Symposium on High Oil Corn, Department of Agronomy, University of Illinois, 29–32 (1960).
4. Conway, T. F., and F. R. Earle, JAOCS *40*, 265–268 (1963).
5. Brim, C. A., W. A. Schutz and F. I. Collins, Crop Sci. (in press).
6. Belikov, I. F., and M. K. Sazenenko, Dokl. Akad. Nauk. USSR Proc. Chemical Tech. Section *167*(1), 225–227 (1966).
7. Fehr, W. R., C. R. Weber and F. I. Collins, Crop Sci. (in press).
8. Alexander, D. E., L. Silvela S., F. I. Collins and R. C. Rodgers, JAOCS (in press).
9. AOCS Official and Tentative Methods (Revised to 1966).

[Received June 29, 1967]