Analysis of Oil Content of Maize by Wide-Line NMR

D. E. ALEXANDER, L. SILVELA S., F. I. COLLINS, and R. C. RODGERS,[1]
Illinois Agricultural Experiment Station and the United States Soybean Laboratory, Urbana, Illinois

Abstract

A series of experiments to define feasibility, accuracy, and precision of wide-line nuclear magnetic resonance spectroscopy as a nondestructive analytical tool for the oil content of living seeds is described. Corn samples, ranging from single seeds to 25 g, were scanned by NMR and gravimetrically analyzed. A high positive correlation ($r = 0.99^{+}$) was invariably encountered. Single, 30-sec NMR scans on 25-g corn samples gave estimates within ± 33 mg of the "true" oil content 95% of the time. Error associated with single, 30-sec scans of individual corn kernels amounted to ± 1.3 mg of oil at the same confidence level. Samples containing more than 4.5% moisture contribute to the NMR signal, and therefore oil content is overestimated.

A new dimension is added to the breeding and genetics of oil crops by the application of the NMR technique in that the process is non-destructive and feasible even for single seeds. The usefulness of the technique in studies involving the nature of water and its surrounding medium is suggested.

Introduction

THE RECENT APPLICATION of wide-line nuclear magnetic resonance spectroscopy, hereinafter designated "NMR," to nondestructive analysis of oil in living seeds opens up new opportunities for geneticists and plant breeders (1–5). This report will emphasize the usefulness of NMR spectroscopy, describe its precision and accuracy, as well as define machine parameters and their interactions with sample size, water content, and other characteristics of the seed.

Although extensive application of wide-line NMR has been made in physical-chemical studies prior to 1960, it remained for T. F. Conway and his associates of Corn Products Company, Argo, Ill., to apply the method to oil analysis in whole seeds. Conway (7) found that NMR readout and oil content were correlated in five samples of corn ranging from 1% to 12% oil. Furthermore he found that the method could be used in single-kernel analysis. The following year Conway and Smith (8) found that single-kernel corn analyses could be made with an error of $\pm 0.3\%$ in 95% of the scans and that the analytical method did not materially reduce germination. They also found evidence of some restriction of the response to NMR of hydrogen nucleii in liquid oil within dry corn germ. NMR analyses of solutions of corn oil, obtained by "wet milling" the samples of corn germs in carbon-tetrachloride, were more reproducible than analyses of the dry samples.

In a later more definitive paper Conway and Earle (6) reported on feasibility for NMR oil analysis on whole seeds of 18 species as well as extracted oils from 12 species. They found that the correlation between NMR readout and oil content of 25-g seed samples was excellent ($r = +0.993$).

[1] Professor of Plant Genetics, Fellow in Genetics, U.S. Department of Agriculture Chemist, and Crops Testing Technician respectively, Urbana, Ill.

Materials and Methods

Although physical principles of nuclear magnetic resonance are described fully elsewhere (8), a brief commentary is appropriate. Nuclear magnetic resonance is a form of radio frequency spectroscopy, and the basic principle of its application to oil analysis is that it provides accurate counts of hydrogen nuclei in liquid oils even in a surrounding matrix of starch, protein, etc. The hydrogen population density is then related to the mass of the sample, and the sample in turn may be analyzed for oil by classical gravimetric methods.

The unit used in these experiments was a Varian PA-7 unit equipped with an integrator and two probes, one to handle samples as large as 25 g and the other suitable for single kernels of corn. The unit was designed to resonate hydrogen nuclei, particularly those associated with liquids. Oil in seeds behaves like a liquid; it exhibits narrow, intense signals. Hydrogen associated with solids in the sample are detected but are disregarded by the instrument. Hydrogen in liquid water however has resonance qualities similar to that in oil. Accurate estimates of total hydrogen in the liquid oil component of a sample can be made only if water is reduced to approximately 4.5%.

In the gravimetric analyses used as a reference in these studies, samples were ground in Skelly F or carbon tetrachloride in a high-speed impact shaker mixer mill (Spex Industries Inc., Metuchen, N. J.). The mill is capable of reducing corn or soybean samples to floury-like fineness in 15 min, permitting virtually a complete recovery of the oil and meal. Immediately after grinding the mixture of oil and meal was transferred quantitatively to a filter paper. The solvent and oil were collected in a 100-ml extraction flask. The filter paper containing meal and residual oil was tightly wrapped in a second filter paper, and the packet was subjected to 24 hr of extraction with Skelly F or carbon tetrachloride in a special all-glass, jointed Butt-type apparatus. Excess solvent was evaporated from the flask on a steam bath. Final traces of solvent were removed in a forced draft oven at 105C for 15 min. Cooled flasks were weighed to determine the total weight of oil in the samples.

Residual meal from the gravimetric analyses was always dried to 2% moisture, or less, then scanned by NMR to detect fat. If a signal was encountered, the sample was extracted again for 16 to 24 hr. Secondary oil recoveries were then added to the original amounts of oil, and the original analysis was corrected.

In experiments described in this paper, samples were first dried to 4.5% moisture or less except in the special experiments involving effect of water on NMR signals.

Accuracy and Precision Experiments

In order to establish clearly the legitimacy of NMR readout as a measure of oil content, experiments designed to assess accuracy and precision were carried out. In this frame of reference, accuracy is defined as being the agreement between NMR values and the "true" amount of oil in the same sample as measured

gravimetrically. Precision is defined as being the degree of agreement between repeated NMR scans of the same sample.

In the accuracy experiment involving 25-g and single-kernel samples, NMR and gravimetric determinations were obtained for 17 and 14 corn samples ranging in oil content from 0.68% to 16.09%. Each sample was scanned two times in succession, then analyzed subsequently two more times, again with two scans per replication. The scanned samples were analyzed as unknowns by the gravimetric method described previously. Similar procedures were followed in the single-kernel experiments except that a special probe for small samples was used.

The four samples used in the 25-g and single-kernel precision experiment contained approximately 4% and 8% oil. These samples were scanned after elapsed times of 0, 6 min, 2 hr, 4 hr, and 8 hr with 1-min and 30-sec scans to determine the effects of environmental and instrument variables on the NMR readouts.

Coordination Experiment

To determine whether NMR oil estimates made on 25-g corn samples agreed with those made on individual kernels, six 25-g samples were first analyzed, and later each kernel within each sample was separately analyzed. Four of these samples involved segregating populations or heterogeneous mixtures created by mixing various strains to approximate a desired oil level. The other two were inbreds.

Moisture Experiments

A 25-g sample of each of the corn inbreds Wx B217 (8.8% oil), SD5 (4.9%), and Pa 35 (2.7%) were dried in a conventional forced air oven at 60C from 7.5% to 2.5% moisture. At 12-hr intervals during the drying period the samples were taken from the oven and cooled to room temperature. They were weighed, and repeated NMR scans were made on both standard, sealed-in-glass samples and the samples under study. At the end of the experiment the water content of the three samples was determined by oven drying and weighing (AOAC method for whole corn kernels). Water content of each sample for each scan period was then calculated with the assumption that weight loss was attributable to a decrease in water content only.

Exactly the same procedure was followed for 25-g samples and for single kernel samples except that the final water content was not determined for the individual kernels because of technical difficulties.

TABLE I

NMR Readout and mg Oil in the Same 25-g and Single-Kernel Corn Sample as Determined by Gravimetric Analysis

Variety	NMR Readout $\bar{x}$ of 3 scans	Mg oil ($\bar{x}$ of analysis)[1]	Variety	NMR readout $\bar{x}$ of 2 scans	Mg oil in sample
Low oil 1	4.9	170	Low oil	11.7	9.4
Low oil 2	7.6	237	ND408	28.7	15.2
R151	18.1	485	R127	29.4	14.9
ND408	28.3	732	R71	36.9	18.6
H45	37.2	967	R904A	81.9	31.4
R109B	47.1	1195	I.F. Purple	82.3	33.0
R904A	59.1	1512	(R78 × R84)	87.5	35.7
(B14 × C103)	68.9	1767	R78	87.6	32.7
(R78 × R84)	77.1	1940	Alexho	104.5	39.7
(R78 × R84)	90.2	2277	(B14 × C103)	164.3	59.4
(R78 × R84)	98.5	2465	(B14 × C103)	172.5	61.2
(R78 × R84)	111.7	2767	(B14 × C103)	190.7	68.8
(R78 × R84)	119.5	2985	High oil 1	217.5	76.2
(R78 × R84)	128.3	3210	High oil 2	256.4	90.5
(R78 × R84)	141.9	3510			
(R78 × R84)	149.6	3722			
Ill. high oil	162.9	4022			

[1] Calculated value based on mean of two analyses of 5 g each.

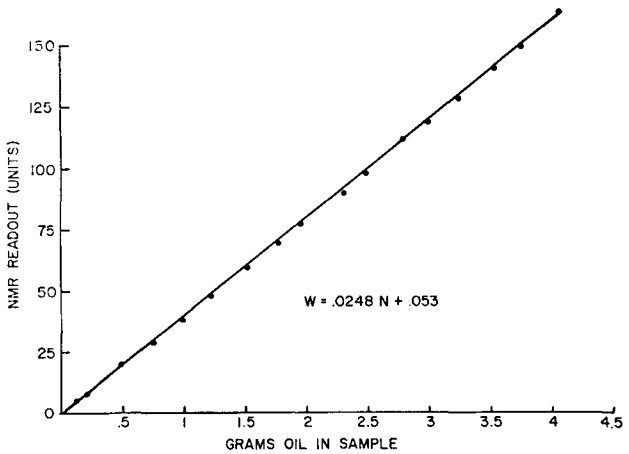

FIG. 1. Relationship of NMR readout and amount of oil in 25-g samples of corn as determined by gravimetric analysis. NMR readout—mean of 3, one-half minute scans.

Their initial water content was assumed to be the same as in the variety from which they were taken.

Results and Discussion

Accuracy Experiments

Data on NMR readout and gravimetric analyses for 25-g samples and individual kernels of corn are given in Table I, and plots of the same data are shown in Figures 1 and 2.

Agreement between the NMR signal and oil content is very high in both the 25-g and single-kernel samples ($r = +0.995$ and 0.999 for 25-g and single-kernel samples respectively). It should be pointed out that all error is assumed to be associated with NMR since the gravimetric method was taken as the final authority and was assumed to be absolute and without error. However estimated mean standard error for 25-g samples, scanned twice in succession at 30-sec sweep time, was 14.7 mg of oil or 0.059%. Estimated standard error for two 30-sec scans on single kernels was 0.61 mg of oil or, in terms of a 250-mg kernel, 0.244%.

Precision Experiments

Table II gives the estimated standard deviations of repeated scans of the same samples over different periods of time. Repeated scans of the same samples were carried out not only to establish the degree of precision of NMR but to estimate the magnitude of error that might occur over an extended time as a

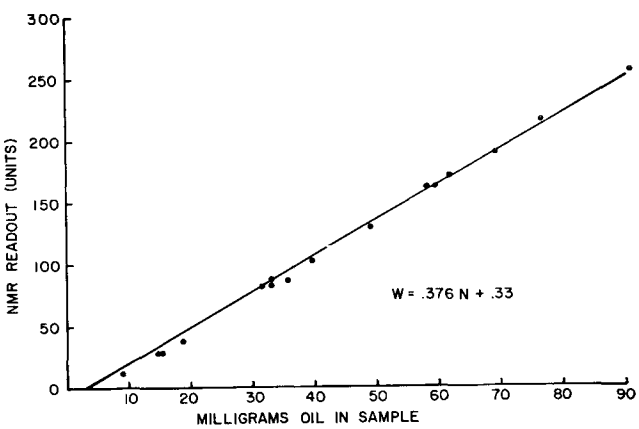

FIG. 2. Relationship of NMR readout and amount of oil in single kernels of corn as determined by gravimetric analysis. NMR readout—mean of 2, one-half minute scans.

TABLE II

Standard Error Estimates of Repeated Scans of Two 25-g and two Single-Kernel Corn Samples Over Time (mg Oil)

	Time between scans	60-sec scans				30-sec scans			
		Single obs.		$\bar{x}$ of 2 Consec. obs.		Single obs.		$\bar{x}$ of 2 Consec. obs.	
		R109B[a]	R84[b]	R109B	R84	R109B	R84	R109B	R84
25 g	6 min	7.5	10.0	5	10	10	10	5	7.5
	2 hr	10	12.5	5	10	10	12.5	7.5	10
	4 hr	10	12.5	5	12.5	15	15	7.5	12.5
	8 hr	12	12.5	10	25	25	25	12.5	22.5
Single kernel	6 min	0.12[c]	0.15[d]			0.18	0.21	0.09	0.12
	2 hr	0.15	0.18			0.24	0.24	0.21	0.15
	4 hr	0.18	0.18			0.24	0.27	0.21	0.15
	8 hr	0.18	0.18			0.24	0.27	0.21	0.15

[a] The 3.9% oil 25-g sample.
[b] The 8.3% oil 25-g sample.
[c] Kernel R109B weighed 267 mg, contained 10 mg oil, i.e., 3.7% oil.
[d] Kernel R84 weighed 226 mg, contained 18 mg oil, i.e., 7.9% oil.

consequence of machine malfunction, temperature change, or other unknown variables. Although error increases with time, it offers no serious problem in operations, particularly if standard samples are scanned at appropriate intervals to detect systematic shifts in the NMR signal and if repairs or compensating changes in instrument settings are made. The standard deviation in NMR units for any particular sample increased with time. This was usually caused by changes in temperature in the laboratory.

Experience has shown that remarkably accurate and precise estimates of oil content can be made with two 30-sec scans made in succession. If, by chance, two scans do not agree closely as a consequence of "nuisance" parameters, re-runs are normally made. If normal operating conditions are involved, single, 30-sec scans on 25-g samples give estimates within ±33 mg of oil 95% of the time, i.e., ±0.13%. The mean of two consecutive 30-sec scans reduces the error to 30 mg of oil and ±0.12% at the same confidence level. With single-kernel scans one 30-sec observation has an error of +1.3 mg of oil at the 95% level of confidence, i.e., ±0.52% oil for a 250-mg kernel. The mean of two consecutive scans reduces the error to ±1.2 mg or 0.48% for a kernel of the same size. The error reduction from one to two consecutive scans is not large. The main advantage of two consecutive scans is that gross errors because of sudden changes or malfunctions may be detected.

Coordination Experiments

Table III gives data obtained from NMR analyses of six 25-g samples and subsequent kernel-by-kernel analysis of the same samples, also by NMR.

A somewhat disconcerting discrepancy exists between oil content determined through bulk analysis and component single kernel analysis. Individual kernel analyses tend to overestimate the amount of oil in the whole sample if one assumes that the bulk analyses are correct. This is particularly true for lower oil samples. The data suggest that the original single-kernel "referee" samples, particularly the lower oil ones, are in error. Figure 2 shows that NMR tends to over-estimate the oil in single kernels of low oil content. As shown in Table III, this "overestimation" is positive and is multiplied by the number of kernels in each sample 80, 112, 108, 65, 99, and 108. At any rate, agreement between bulk and individual kernel estimates is quite good at those oil levels which are normally encountered in corn.

TABLE III

Comparison of NMR Bulk and Single-Kernel Oil Analyses

Variety	No. of kernels	Oil Content			
		By bulk analysis 25-g sample		By single-kernel analysis	
		mg	%	mg	%
Ill. low oil	80	275	1.1	409	1.6
R71	112	874	3.5	1043	4.2
Pa83	108	1349	5.4	1445	5.8
B14 × C103 ×	65	1753	7.0	1780	7.1
R78 × R84 ×	99	2302	9.2	2339	9.3
(R78 × R84) Ill. high oil	108	2948	11.8	2967	11.9

A subsequent experiment was carried out to assess further the accuracy of NMR oil determinations. Refined corn oil (40 mg) was diluted to different concentrations by the addition of either fat-free corn meal, soybean meal, or carbon tetrachloride. Multiple NMR scans were then made at each level of concentration. The results are plotted in Figure 3.

The regression of NMR readout on oil concentration is clearly different as a consequence of the medium in which the oil is held, that is, the net NMR signal per hydrogen nucleus is greater at high concentrations. Furthermore the net signal per nucleus is quite different in carbon tetrachloride as compared with corn or soybean meal, particularly at lower concentrations. It is obvious that the NMR readout is a function of the hydrogen population in the oil and its matrix.

To obtain entirely reliable analysis of oil seed by NMR it has been found that each different seed crop must be compared with standard samples of its own kind. Furthermore these standards must be similar in weight and moisture content, also within the same range of oil percentages as the samples of unknown composition.

Moisture Experiments

The effect of moisture level on NMR signal is shown graphically in Figure 4. As the samples became progressively drier, the total NMR signals decreased as expected since hydrogen nuclei were being lost through water evaporation. The reduction in signal

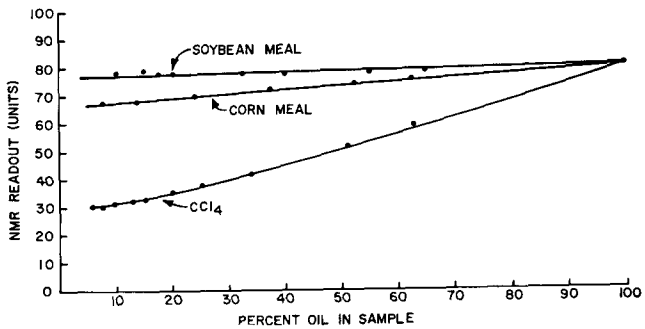

FIG. 3. Effect of oil concentration and carrier medium on NMR readout.

per unit of water removed is considerably less at levels below 4.5–5% than at levels above that point. From an analytical standpoint it appears unnecessary to dry corn samples below 4.5% in order to insure accurate estimates of oil content. It is obvious, of course, that accurate estimates of oil content will not be made if water content is high.

Regression coefficients were calculated for the data shown in Figure 4: a) curvilinear, whole curve, b) linear, c) linear segment below 5% moisture, d) linear segment above 5%. Each regression coefficient was highly significant statistically. The best fit involves two linear regressions which intersect approximately at 5% moisture.

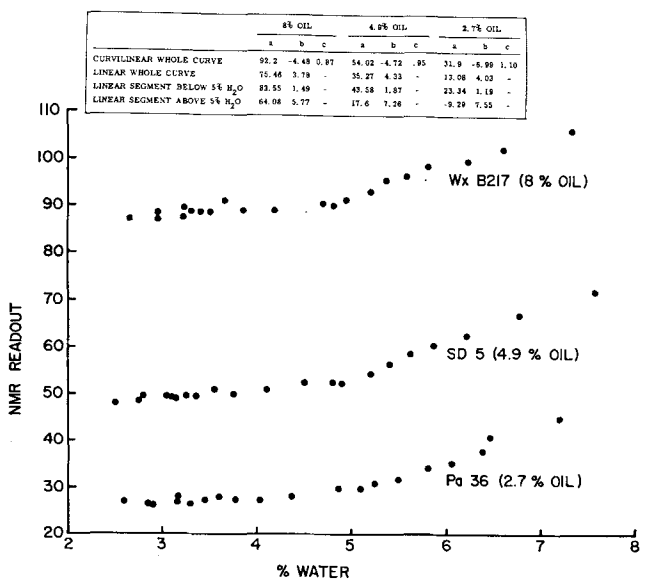

FIG. 4. Effect of water content of corn kernels on NMR signal (25-g samples).

Precisely the same kinds of experiments have been carried out with soybeans and oats with an outcome similar to that in corn. Excellent spectra are obtained from individual soybeans although resolution is not as good on individual oat groats as in single corn or soybean seeds, primarily because the mass is too low. Satisfactory results have been obtained with oats if the sample contains three to four groats or if the groat is large and is high in oil. Bulk samples as well as individual seeds of sunflower and safflower may be satisfactorily analyzed with errors comparable with those in corn.

Accurate NMR estimates of oil content of corn kernels, and of other seeds as well, depend largely on uniform moisture content of all samples. It seems quite likely that simultaneous determination of oil and water content will ultimately be possible through wide-line NMR. However, under current methods, water content should be less than 4.5–5% if accurate oil estimates are to be made.

ACKNOWLEDGMENTS

This research was supported in part by N.S.F. Grant GB 1732, Corn Industries Research Foundation, Washington, D. C., and the Bear Hybrid Corn Company, Decatur, Ill. The authors express appreciation for invaluable assistance and advice of T. F. Conway of Corn Products Company, Argo, Ill.

REFERENCES

1. Bauman, L. F., T. F. Conway and S. A. Watson, Science *139*, 498–499 (1963).
2. Silvela S., L., and D. E. Alexander, Crop Sci., in press.
3. Brown, C. M., D. E. Alexander and S. G. Carmer, Crop Sci. *6*, 190–191 (1966).
4. Lambert, R. J., D. E. Alexander and R. C. Rodgers, Crop. Sci., in press.
5. Brim, C. A., W. A. Schutz and F. I. Collins, Crop Sci., in press.
6. Conway, T. F., and F. R. Earle, J. Offic. Agric. Chemists *60*, 256–268 (1963).
7. Conway, T. F., Proc. of a Symposium on High-Oil Corn, Department of Agronomy, University of Illinois, pp. 29–32, 1960.
8. Ferraro, J. R., and J. S. Ziomek, eds., "Developments in Applied Spectroscopy," Vol. 2, Plenum Press, New York, 1963, pp. 115–127.

[Received March 14, 1967]