Nuclear Magnetic Resonance for Determining Oil Content of Seeds

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Abstract

Twelve seed oils containing a variety of functional groups were examined with a commercial broad-band nuclear magnetic resonance (NMR) spectrometer. Instrument response (integrator readout) was directly related to hydrogen content of the oil ($r = 0.999$), regardless of the structures present.

NMR techniques were applied to the determination of oil in intact, partially dried seeds from 18 plant species containing 1.5–53% oil having a variety of structures. The correlation between integrator readout (calculated to a uniform 25-g sample weight) and oil content (determined by extraction with petroleum ether) is excellent ($r = 0.993$). If the readout is further modified by a correction for the variation in hydrogen content of the oils, the correlation becomes 0.996.

Introduction

FOLLOWING DEVELOPMENT by Shaw and co-workers (8) of broad-band nuclear magnetic resonance (NMR) procedures for the determination of moisture in vegetables, the technique has been used effectively for moisture in products obtained from the commercial wet milling of corn (3). The early suggestion (1) that NMR might provide a rapid method for determining oil content of plant materials has been proved by an extensive study of corn, corn germ, and germ process samples (4).

This paper reports on the feasibility of using NMR to determine oil in intact seeds containing oils of diverse types.

Principle. The NMR technique measures total hydrogen associated with the fat in seeds independent of the hydrogen associated with the nonoil matrix. Determination of total hydrogen in low- and high-molecular-weight compounds in solution (7,9) has been reported previously. This type of measurement is extended to oil in carbon tetrachloride and in dry seeds in the present study using a Schlumberger Model 104 NMR analyzer. This instrument is capable of total hydrogen measurements and of distinguishing between signals from the hydrogen in oil and those from "bound" water, carbohydrates, or proteins in solids.

NMR spectral characteristics depend on the molecular motion within the sample. Oil in seeds behaves like a liquid; it exhibits narrow, intense signals, and resonance is observed over a very narrow range of field strengths. In solids such as those in the nonoil matrix, hydrogen nuclei associated with bound water, carbohydrates, or proteins are fixed rather rigidly with respect to their neighbors and are capable of only restricted movement. Consequently, any given nucleus may be in an effective magnetic environment substantially lower or higher than the applied field. Therefore, signals from the matrix hydrogen are weak and very broad, because hydrogen resonance is limited to only a few nuclei at a given time. By measuring the derivative of the primary NMR absorption signal, mobile hydrogen signals (hydrogen in fat) can be separated instrumentally from hindered hydrogen signals (hydrogen in nonoil matrix) since the latter are virtually constant over a narrow range of the magnetic field.

Instrumentation. The Schlumberger analyzer (6) consists of three major components: The magnet unit housing the permanent magnet and associated coils; the console containing a portion of the operational control panels, recorder, and electronic circuitry; and the Model 104-3 integrator. The operation of the instrument is comparatively simple. A weighed sample is transferred to a cell and placed in the sample cavity in the magnet unit. After selection of instrument parameters, the "Start" switch is energized, and the NMR derivative curve is obtained as the instrument automatically "sweeps" through the resonance condition. Concurrently, the integrator stores information on the amount of energy absorbed by the sample. At the end of the cycle, the integrator automatically prints a record that is proportional to the total energy absorption and, therefore, to the amount of mobile hydrogen or oil.

Experimental

Materials and Preparation. Of the oils tested, isano, tung, oiticica, and castor were commercial products; the others were prepared in the laboratory by extraction with petroleum ether.

Seed samples were selected to give a range in oil contents and a variety of oil types. Foreign matter and damaged or obviously immature seeds were removed. Two samples of *Crambe abyssinica* Hochst. seed were tested; one in the pod, and one after removal of the pod. The sample of *Dimorphotheca sinuata* DC. seed included pericarp. Portions of each sample were dried in an oven under about 27 in. of vacuum at 50C for 16 to 60 hr until the rate of moisture loss became very small. Portions of three samples, *Ricinus communis* L., *Sesbania macrocarpa* Raf., and *Brassica juncea* (L.) Coss., were dried in a forced draft oven at 130C for 3 hr. Portions of two samples, *B. juncea* and *Crambe abyssinica* (in pod), were tested without drying.

Hydrogen contents of the oils were calculated from compositions based either on our analyses or on "typical" analyses reported in the literature. The calculation of oiticica oil was made on the basis of 76% of licanic acid, approximately the mean of the reported range. Isano oil was assumed to contain 45% of isanic acid, 45% isanolic acid, 5% linolenic acid, and 5% saturated acids in the mixed acids. This assumption oversimplifies the composition, but there is literature evidence for the high proportion of the two acetylenic acids in the oil (5).

TABLE I
Instrument Parameters and Conditions

	Carbon tetrachloride solution	Intact seed
Analyzer: Schlumberger Model 104		
Time constant: sec	2	2
Radio-frequency attenuator: decibel	32	32
Sweep time: min	2	2
Sweep amplitude: gauss	1	1
Sensitivity	50	200
Modulation amplitude: gauss	0.5	0.5
Integrator: Model 104-3		
Weight setting	3000	2400
Readout multiplier	10	20
Threshold: millivolt	0.1	Off
Signal multiplier	5	5

Sample temperature: 25–27C
Modulation frequency: 33.1 cycles per second (square wave)
Field strength: 1,717 gauss

For all lots of seed, except two, figures for oil content were taken from analyses previously made as part of an extensive investigation of the composition of plant seeds. Oil was determined by extracting the ground sample with petroleum ether for 6 hr in the Butt apparatus. Moisture results determined by heating a 2-g sample in a forced-draft oven for 2 hr at 130C were used to calculate oil results to the moisture-free basis. Previous analyses of *Ricinus communis* and *Simmondsia chinensis* (Link) Schneid. were not available for the present study. Accordingly, the subsamples used for NMR were later analyzed for oil by dispersing them in carbon tetrachloride, using a Virtis disintegrator, and by completing the extraction with carbon tetrachloride in a Soxhlet extractor. Moisture was not determined on these two samples.

Measurement. The NMR response of 12 different oils was determined by examining a 1-g sample in 25 ml carbon tetrachloride solution under the instrument parameters and conditions specified in Table I.

In the measurement of oil content of intact seeds by NMR, sufficient seed to occupy approximately 35 ml was weighed, transferred to the sample cell, and examined instrumentally under the conditions specified in Table I. The only exceptions were for *Dimorphotheca sinuata,* which was examined with a tenfold increase in sensitivity (Position 20), and for one *Ricinus communis* sample which was examined with a radio-frequency (r-f) attenuation of 36 decibels. Actual sample weights ranged between 2.24 and 29.35 g with only *Dimorphotheca* and *Crambe* (seed and pod) being below 12 g. Integrator readout was corrected to a uniform 25-g sample basis.

Discussion

Instrument response to oils in solution as recorded by the integrator was related to the total hydrogen content. The lower readings were obtained with highly unsaturated or oxygenated oils, and the higher readings with more nearly saturated oils. A correlation coefficient of 0.999 was calculated for the relationship between calculated hydrogen content and instrument response. Deviations of the calculated hydrogen contents from the regression line are shown in Table II.

These data give some idea of the accuracy attainable if tests are restricted to solutions of oils of similar hydrogen contents or oils of known composition. Obviously, lack of knowledge concerning hydrogen content limits the accuracy in determining the concentration of an unknown oil in solution or in seeds. If a hydrogen content of 11.10% midway between the extremes (9.35 and 12.80% H) encountered in this study were taken as a basis for calculating amounts of unknown oils from the NMR readout, the result of any oil containing either extreme of hydrogen content would be in error by about 17%. If *Simmondsia chinensis* oil (wax), and oils from *Ongokea gore* (Hua) Pierre, and *Licania rigida* Benth. are omitted, the range in hydrogen content is from 10.81 to 11.90%, and the error from selecting the midpoint as a basis for calculation is reduced to less than 5% for the extremes of the limited group. Deviations of this magnitude in the determination of oil content in seeds can be accepted in a screening program, which must balance accuracy against the time required for the analysis and the probability of overlooking a sample containing a useful percentage of oil. Even the 17% deviation would not eliminate a sample from further consideration unless the oil content of the sample approximated the lower limit set for materials warranting further study. For example, a report of 14% oil might mean abandonment of the sample, whereas a report of the true oil content of 16% might mean continued investigation.

The response of NMR to oil in seeds is apparently as uniform as that to oils in solution. The correlation ($r = 0.993$) between integrator reading and oil content of the 19 vacuum-dried seed samples tested is excellent (Fig. 1, Table III) for this brief study of feasibility. This correlation could probably be improved if the work were repeated with certain modifications. Differences in sample preparation and in sampling could be avoided by applying the extraction method to the portion used for the NMR test, rather than to a separate subsample prepared at a different time. Moisture content of the intact seeds at the time of testing by NMR could be obtained. One source of error not easily eliminated lies in the fact that optimum methods of sample preparation and analysis have not been established for most of the materials in this test. The methods used for grinding and extracting can hardly be equally suitable for all of the species tested.

Of the samples run, only three deviate from the

TABLE II
Comparison of Hydrogen Content Calculated from Oil Composition with That Read from the Regression Line

Oil source	Common name	Characteristic acid or structure	Hydrogen content calc., %	Deviation from regression line, % H
Ongokea gore (Hua) Pierre	Isano	Conj. acetylene; hydroxyl	9.35	+0.03
Licania rigida Benth.	Oiticica	Licanic acid	10.07	+0.03
Aleurites sp.	Tung	Eleostearic acid	10.81	0
Vernonia anthelmintica (L.) Willd.	Ironweed	Epoxyoleic acid	10.88	+0.04
Dimorphotheca sinuata DC.	Cape marigold	Conj. diene; hydroxyl	11.04	+0.03
Thalictrum polycarpum S. Wats.	Sierra meadowrue	*trans* Double bond	11.13	−0.06
Linum usitatissimum L.	Linseed	Linolenic acid	11.17	−0.10
Ricinus communis L.	Castor	Ricinoleic acid	11.31	+0.02
Glycine max (L.) Merr.	Soybean	Linoleic acid	11.53	−0.07
Zea mays L.	Corn	Linoleic acid	11.57	−0.11
Crambe abyssinica Hochst.	.....	Erucic acid	11.90	+0.05
Simmondsia chinensis (Link) Schneid.	Jojoba	C_{20} and C_{22} acids and alcohols	12.80	+0.02

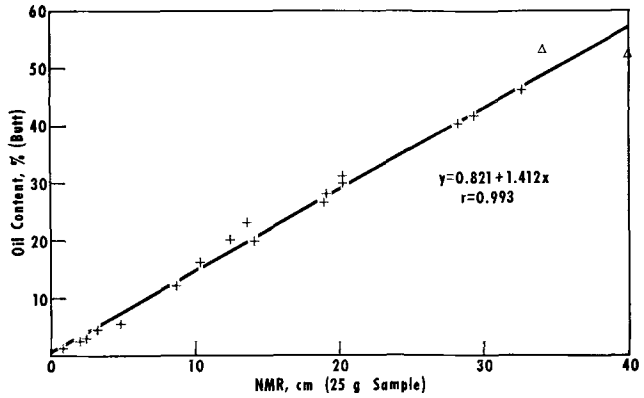

FIG. 1. Relationship between oil content of seed samples and NMR response calculated to a 25-g sample basis. (△, samples extracted with CCl_4).

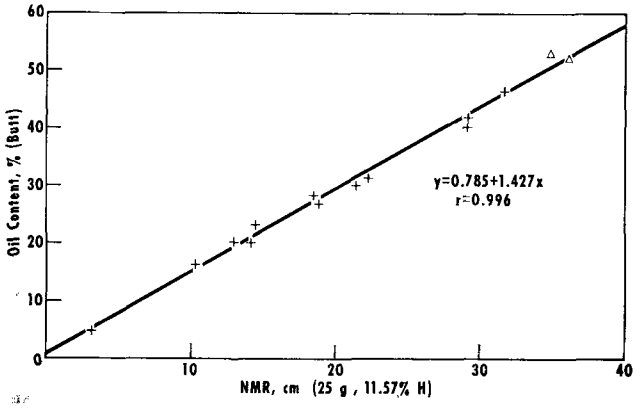

FIG. 2. Relationship between oil content of seed samples and NMR response for sample weight and hydrogen content of the oil. (△, samples extracted with CCl_4).

calculated line by as much as 3% oil: *Vernonia anthelmintica* (L.) Willd., 3.3% at the 20% level; *Ricinus communis,* 4.1% at the 50% level; and *Simmondsia chinensis,* 5.1% at the 50% level. Part of the deviation can be attributed to the differences in hydrogen contents of the oils. If corrections are made for the hydrogen content of the oil in the 14 species containing oil of known composition (corn oil, 11.57% hydrogen, arbitrarily taken as reference), the correlation coefficient is raised to 0.996, and no oil deviates from the regression line shown in Figure 2 by as much as 3% oil. Such a calculation has no applicability in screening plant species producing oils of unknown composition, but it indicates the accuracy and precision one might expect if tests are restricted to seeds with oils of similar or known hydrogen contents. Hydrogen contents of seed oils of unknown fatty acid composition might be determined by solvent extraction and NMR examination.

The effect of sample drying temperature on NMR response was studied briefly from the standpoint of possible "binding" of the oil to the nonoil matrix. Experience with corn germ indicates that under moderate drying temperatures the extractability of the oil from the germ is affected. Under severe drying conditions it is reduced substantially. NMR instrument parameters can be adjusted to detect the binding or to readout independently of this effect except under the most severe conditions. Castor beans dried at 130C gave an integrator reading of 34.57 cm (25-g basis) compared with 34.17 cm from the sample dried at 50C. This difference, representing about 0.6% oil, could well be within the error caused by sampling or by different moisture levels. Similarly *Sesbania macrocarpa* showed a difference equivalent to 0.2% oil and *Brassica juncea,* 0.8%. A sample of *B. juncea* not dried, differed from that dried at 50C by 0.2% oil, and from that dried at 130C by 1.0%. These small differences indicate that the heat applied in drying intact seeds caused no significant binding of the oil.

The effect of moisture in the seeds on instrument response was not investigated beyond the limited work mentioned above, but experience with corn and corn germ indicates that moisture levels up to 5% do not interfere. The reason is that such moisture in these materials is bound tightly to the nonoil matrix.

The determination of fat in intact seeds at higher moisture levels (5–15%) using an NMR "r-f saturation" approach has not been tried. It may apply if sufficient differences exist between the spin-relaxation times for the hydrogen nuclei associated with the oil and water. This approach, employing NMR differen-

TABLE III
NMR Response to Oil in Intact Seeds

Source	Common name	Hydrogen content of oil, calc., %	Sample weight, g	Integrator readout, cm		Oil content, %		
				Corr. to 25-g weight	Corr. to 25-g weight 11.57% H	Calc. from equation		By extraction, dry basis
						Fig. 1	Fig. 2	
Dried in vacuo at 50C								
Ricinus communis L.	Castor bean	11.31	18.860	34.1	34.8	49.0	50.4	53.1[a]
Simmondsia chinensis (Link) Schneid.	Jojoba	12.80	16.200	40.0	36.1	57.3	52.3	52.2[a]
Crambe abyssinica Hochst. (seed)	.....	11.90	19.697	32.6	31.7	46.8	46.0	46.4
Crambe abyssinica Hochst. (seed and pod)	.....	11.90	8.530	19.0	18.5	27.6	27.2	28.2
Brassica juncea (L.) Coss.	Mustard	11.65	21.869	29.3	29.1	42.2	42.3	41.8
Linum usitatissimum L.	Linseed	11.17	21.382	28.2	29.2	40.6	42.5	40.4
Satureja hortensis L.	Summer savory	10.51	15.680	20.2	22.3	29.3	32.6	31.4
Rudbeckia bicolor Nutt.	Cone flower	10.96	13.896	20.3	21.5	29.5	31.5	30.0
Lesquerella grandiflora S. Wats.	.....	11.58	16.920	18.9	18.9	27.5	27.8	26.8
Vernonia anthelmintica (L.) Willd.	Ironweed	10.88	12.100	13.6	14.5	20.0	21.5	23.3
Dimorphotheca sinuata DC.	Cape marigold	11.04	2.240	12.4	13.0	18.3	19.3	20.3
Glycine max (L.) Merr.	Soybean	11.53	22.230	14.1	14.1	20.7	20.9	20.1
Lavatera trimestris L.	Herb treemallow	11.61	18.130	10.5	10.4	15.6	15.6	16.4
Clitoria ternatea L.	Butterfly pea	.....	23.782	8.7	.....	13.1	.....	12.3
Sesbania macrocarpa Raf.	.....	.....	26.598	4.8	.....	7.6	.....	5.6
Zea mays L.	Corn	11.59	24.154	3.2	3.2	5.3	5.4	4.8
Cyamopsis tetragonoloba (L.) Taub.	Guar	.....	26.328	2.4	.....	4.2	.....	3.2
Crotalaria intermedia Kotschy.	.....	.....	29.349	2.0	.....	3.6	.....	2.7
Indigofera hirsuta L.	Hairy indigo	.....	28.941	0.9	.....	2.1	.....	1.5
Dried in air oven at 130C.								
Ricinus communis L.	Castor bean	11.31	18.350	34.5	35.3	49.5	51.2	53.1[a]
Ricinus communis L.	Castor bean	11.31	18.350	35.0	35.8	50.2	51.9	53.1[a]
Brassica juncea (L.) Coss.	Mustard	11.65	20.593	29.9	29.6	43.0	43.0	41.8
Sesbania macrocarpa Raf.	.....	.....	25.881	4.9	.....	7.7	.....	5.6
Not dried								
Brassica juncea (L.) Coss.	Mustard	11.65	20.432	29.1	28.9	41.9	42.0	41.8
Crambe abyssinica Hochst. (seed and pod)	.....	11.90	8.650	18.0	17.5	26.2	25.8	28.2

[a] As analyzed basis. Extracted with carbon tetrachloride.

tial integral signals, has been used successfully to measure fat (1–6%) in spent corn germ flake containing 4–11% moisture (2).

The results of the present investigation indicate that NMR procedures can be used to determine oil in plant seeds at the rate of one sample in about 3 min, with an accuracy comparable to present extraction procedures. We believe the instrumental method may be more accurate in certain applications, e.g., oil-rich samples difficult to grind for extraction, "oil-free" meals, and processed meals containing oil bound by excessive heating.

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Hydrogen Peroxide Oxidation of Tertiary Amines[1]

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Abstract

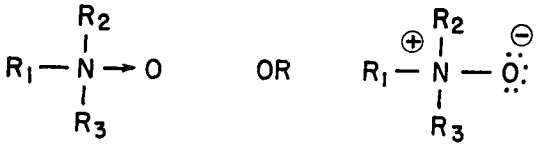
Oxides of tertiary amines, such as dimethyldodecylamine oxide, are known to be useful as detergents and foam stabilizers, and are now in commercial use. The results of an investigation leading to the selection of optimum conditions for producing these compounds are reported. A variety of hydrogen peroxide-derived systems for amine oxidation were investigated. These included hydrogen peroxide in water and in nonaqueous solvents, and peroxy acids under various reaction conditions. Reductometric, acidimetric, and gas chromatographic procedures were used for analysis of reaction mixtures. The preferred reaction uses hydrogen peroxide as oxidant and water as the reaction medium. The product of this reaction is a 30–40% solution of the amine oxide. Other factors affecting the rate and extent of conversion, such as amine purity, are discussed.

Introduction

THE FIRST STUDIES on amine oxides were made in the last decade of the 19th century. Dunstan and Goulding (1), who carried out many studies in this field, called these compounds "oxamines." Recent interest in amine oxides stems from the finding that certain of these compounds are useful as detergents and foam stabilizers. The fatty amine oxides, which show good surface active properties, are in addition biodegradable (2). This is of interest in view of the current demand for "soft" detergents.

Since the turn of the century, few articles have been published on the oxidation of aliphatic amines, although there has been much interest in the oxidation of heterocyclic amines. Cope and co-workers (3) and Cram and co-workers (4) have been active in amine oxidation. Their primary interest, however, has been in stereospecific olefin formation by pyrolysis of amine oxides. A detailed review of amine oxide chemistry was published by Culvenor (5) in 1953. A bibliography of recent amine oxide literature is available (6).

Only tertiary amines form amine oxides. Their structure is as follows:



The oxygen atom is bonded to the nitrogen atom with a polar bond, the electron density being greater on the oxygen atom. The dipolar nature of this bond imparts salt-like properties to the molecule.

It is well known that oxidation of amines can be brought about by the action of a peroxyacid. Aliphatic tertiary amines substituted by C_1 to C_3 alkyl groups have also been oxidized with hydrogen peroxide (3). However, no data have been available on the oxidation of fatty-substituted amines. In order to determine the preferred reaction conditions for oxidizing these amines, we have carried out the studies reported here.

Experimental Procedures

Distillation of Commercial Dimethyldodecylamine. Commercial grade dimethyldodecylamine ("Armeen" DM12D, Armour Industrial Chemical Co.) was vacuum-distilled using conventional apparatus. The column length was 30 cm, and distillate boiling in the range 93–103C was collected at 1–2 mm Hg. The n_D^{25} of the distillate fractions ranged from 1.4347–1.4357. The n_D^{25} of the residue was 1.4415. The yield of distillate was 95.3%.

Apparatus for Oxidation Experiments. The reaction apparatus used in all oxidation experiments was a creased 500 ml three-necked flask fitted with a water condenser, stirrer, and dropping funnel. A thermometer was inserted through the bore of the condenser into the reaction mixture. The flask was placed in a bath equipped to heat or cool.

[1] Presented at the AOCS meeting in Toronto, Ontario, 1962.