Analysis of Oil Content of Sunflower Seed by Wide-Line NMR

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ABSTRACT

The wide-line nuclear magentic resonance (NMR) analyzer is routinely used to determine the oil content of sunflower seed by plant breeders. This technique is now under consideration as the official method for the domestic trading of sunflower seed. A study of the effect of depth (volume) of sunflower seed in the NMR 130 ml sample tube showed that between a depth of 30-75 mm (23.5-62.5 g seed) the NMR response was uniform, but beyond 75 mm, the response rapidly decreased. Oil analysis of 10 sunflower seed samples showed that coefficient of variation (C.V.) was lower with a 130 ml sample tube (C.V. 0.4%) than with a 34 ml tube (C.V. 0.8%). As the temperature of the sample was increased 1 C, the instrument response decreased by 0.4%. Analysis of sunflower seed with 31-71% linoleic acid contents analyzed 0.1% higher for each 1% decrease in linoleic acid. Data show that linoleic acid content of NMR sunflower seed standard is important in NMR total oil analysis. Results of this study showed that the sample of sunflower seed for total oil analysis by NMR should be contained at least within the bottom 70 mm of the 130 ml sample tube, and NMR response of the standard calibration seed and sample being analyzed should be read at the same temperature, and their fatty acid compositions should be similar.

INTRODUCTION

Wide-line NMR is a term used to describe low resolution nuclear magnetic resonance (NMR). The NMR technique measures total hydrogen associated with the oil and water in seed (the only liquid constituents) independent of the hydrogen associated with the nonoil matrix (1). If the measurement is made on dry seed, the response of the apparatus is directly proportional to the quantity of oil present in the seed (2).

In 1960 Conway (3) first used NMR to analyze whole seed for oil content. Since the process is nondestructive and feasible even on single seeds, geneticists and plant breeders have used the technique extensively (4-6). In all official oil methods, the oil content is determined by some type of extraction process which is slow and requires large volumes of solvent. On the other hand, NMR provides a rapid means of measuring oil content of oilseeds. Wolff et al. (2) reported that with NMR one person could analyze at least 60 samples a day. In addition, NMR oil analysis was found to be more reproducible and statistically more reliable than the AOCS and other extraction methods (2,5,7,8).

The effects of several variables on NMR and analysis of sunflowers have been investigated. Zimmerman (5) found no difference in the NMR response per gram of oil between sunflower hybrid and open pollinated varieties. He also found that the response per gram of oil was consistent for seed that varied in oil content from 39-50%. Wolff et al. (2) reported that variations in the composition of an oilseed and the environment of the instrument (temperature, magnetic field, humidity) must not be neglected in the determination of oil content by NMR.

There is growing interest in the sunflower industry for the replacement of extraction methods by wide-line NMR for domestic trading of sunflower. Thus, we designed this study to investigate the effect of variables such as sample, size, temperature and oil composition on NMR oil analysis.

MATERIALS AND METHODS

The wide-line nuclear magnetic resonance (NMR) instrument used for these studies was the Newport Analyzer Mk III eqipped with a temperature controller to maintain temperature ± 0.01C. The NMR was fitted with a 40 ml sample coil assembly for temperture, size of sample and composition studies, a 150 ml coil assembly for sample size studies and a 2 ml coil assembly for oil analysis. The instrument was operated as described in the Newport Users Handbook (9).

The NMR analyzer was standardized by use of a clean, high quality sunflower seed sample of known oil content. The oil content of this calibration seed standard was carefully determined on 10 replicated samples by the AOCS tentative Method Ai 3-75 (10). The oil content of each seed measurement was calculated with the following formulas:

$$\text{CONSTANT} = \frac{\text{NMR reading of calibration standard}}{\text{(weight of seed) x (oil content of standard, by extraction)}}$$

$$\%\ \text{OIL} = \frac{\text{NMR reading of seed}}{\text{(weight of dry sample) x (constant)}}$$

For temperature studies, we used the 40 ml sample assembly and took readings on a single sample of seed (15.4 g) at 1 C intervals between 15 and 32 C with an equilibration time of 7-10 min between readings.

For analysis of different oils, 1.6-1.75 g of liquid oil (1.0-1.1 g of pure triglycerides) was weighed into 2 ml sample tubes and analyzed by NMR at 23 C. Palm oil, which is a semisolid at 23 C, was liquified at 40 C, gradually cooled with the NMR temperature controller to 23 C, and readings were taken immediately before oil recrystallized.

For the analysis of sunflower seed of various linoleic

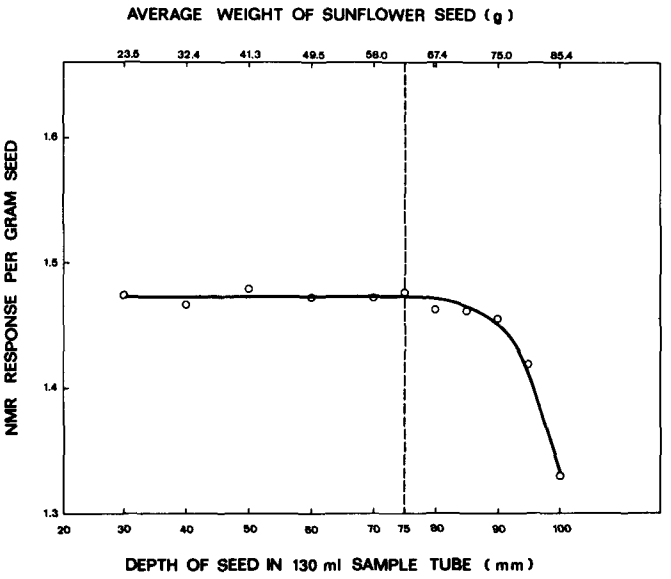

FIG. 1. Effect of depth of sunflower seed in 130 ml sample tube on NMR response (average of 4 analyses). NMR: r.f., 350 μA; gain, 500; integration, 128S.

TABLE I

Effect of Sample Size on NMR Total Oil Analysis of Hand Cleaned Sunflower Seed

	130 ml	Sample tube[a]	34 ml	Sample tube[b]
Sample	% Oil[c]	Std. dev.	% Oil[c]	Std. dev.
1	51.27	± 0.185	51.40	± 0.150
2	43.68	± 0.149	43.43	± 0.274
3	41.58	± 0.048	41.08	± 0.459
4	44.30	± 0.065	45.00	± 0.303
5	34.80	± 0.187	34.37	± 0.774
6	39.46	± 0.163	39.92	± 0.477
7	48.30	± 0.281	48.17	± 0.166
8	49.97	± 0.236	49.89	± 0.277
9	26.11	± 0.197	26.46	± 0.677
10	49.85	± 0.193	49.95	± 0.120
Mean	42.93	---	42.97	---
Av. std. dev.	---	± 0.170	---	± 0.368
Coef. var.	---	0.40%	---	0.86%

[a]Sample size, 42.4-62.8 g; NMR: r.f., 225 μA; gain, 500; integration, 128S.

[b]Sample size, 10.0-16.7 g; NMR: r.f., 225 μA; gain, 700; integration, 128S.

[c]Average of 4 analyses per sample.

TABLE II

Effect of Sample Size on NMR Oil Analysis of Mechanically Cleaned Sunflower Seed

	130 ml	Sample tube[a]	34 ml	Sample tube[b]
Sample	% Oil[c]	Std. dev.	% Oil[c]	Std. dev.
1	46.8	± 0.177	47.2	± 0.460
2	46.1	± 0.189	46.7	± 0.729
3	46.4	± 0.158	46.1	± 0.302
4	47.5	± 0.154	47.4	± 0.314
5	49.1	± 0.199	47.5	± 0.382
Mean	47.2	---	47.0	---
Av. std. dev.	---	± 0.175	---	± 0.437
Coef. var.	---	0.37%	---	0.93%

[a]Sample size, 52.4-61.4 g; NMR: r.f., 225 μA; gain, 500; integration, 128S.

[b]Sample size, 13.2-16.0 g; NMR: r.f., 225 μA; gain, 700; integration, 128S.

[c]Average of 5 analyses per sample.

acid contents, ca. 12-15 g dried seed (130 C for 3 hr) with linoleic acid contents from 34-71%, were analyzed with the NMR analyzer. Then, the seed analyzed by NMR was ground with a high speed grinder, quantitatively transferred to a Soxhlet thimble, extracted with petroleum ether (35-60 C, b.p) for 20 hr, solvent flash-evaporated under vacuum, and the oil weighed. The fatty acid composition of the extracted oil was determined by gas liquid chromatography (11).

RESULTS AND DISCUSSION

For accurate analysis of the oil of sunflower seed by NMR, all the seed to be analyzed should be contained within the volume of homogeneous r.f. field when inserted in the sample coil assembly. The effect of depth (volume) of sunflower seed in a 130 ml sample tube on NMR response is shown in Figure 1. The NMR response per gram of seed was uniform between 30-75 mm depth (23.5-62.5 g seed). The response decreased rapidly above 75 mm; and decreased slightly below 30 mm. The manufacturer refers to the coil assembly that holds the 130 ml tube as a "150 ml sample assembly." However, a 51 mm o.d. x 2 mm wall sample tube used in oil analysis has a volume of 130 ml at a depth of 75 mm.

The modern commercial wide-line NMR analyzer has a 40 ml sample assembly. Tests in our laboratory showed that the standard Nessler tube (32 mm o.d.) used with this assembly has a maximum usable volume of 34 ml (in the case of sunflower seed, 12-16 g maximum). Since sunflower seeds vary in size and oil content and a representative sampling for analysis is difficult, we though that a sample larger than 16 g would show more accuracy. Therefore, we compared the precision of oil analysis of sunflower seed between the 34 ml and the 130 ml sample tubes. The results are shown in Table I on 10 hand-cleaned samples ranging in oil content from 26.1-51.3%. Best results were obtained with the 130 ml sample tube with a coefficient of variation of 0.40% oil compared to 0.85% for the 34 ml tube. Table II shows similar results for five mechanically cleaned samples. Analyses would be slightly more expensive with the 130 ml than with the 34 ml sample tube, but accuracy and precision would be improved.

In general, accuracy of measurement is highest for the largest possible sample that can be contained within the length of the homogeneous r.f. field (9). For total oil analysis by NMR, therefore, the sample of sunflower seed should not extend above the bottom 70 mm of the 130 ml sample tube.

The effect of temperature on NMR response of sun-

TABLE III

Effect of Temperature on NMR Response of Sunflower Seed (14.5 g)

		Total oil[b]	
Temp. °C	NMR reading per g seed[a]	% Dry basis	Difference from room temperature
16	4.87	47.6	+1.4
18	4.83	47.2	+1.0
20	4.78	46.7	+0.5
22 (RT)	4.73	46.2	0.0
24	4.69	45.8	-0.4
26	4.64	45.3	-0.9
28	4.61	45.0	-1.2
30	4.57	44.6	-1.6
32	4.52	44.2	-2.0

[a]NMR: r.f., 225 μA; gain, 700: integration, 128S; 34 ml sample assembly.

[b]Total oil calculated at room temperature (22 C) with a sunflower seed standard of known oil content.

TABLE IV

Effect of Iodine Value of Liquid Oils on NMR Response[a]

Oils	Fatty acid comp (area %) 18:1	18:2	18:3	Calc. I.V.[b]	NMR reading per g oil[c]
Palm	37.6	11.0	---	51	80.3
Olive	75.7	8.1	0.7	82	79.1
Triolein[d]	100.0	---	---	86	77.9
Peanut	45.9	36.1	1.1	105	77.3
Corn	24.9	58.7	1.0	126	74.6
Sunflower	22.3	65.8	1.2[e]	136	73.6
Sunflower	14.6	73.5	0.2	140	73.3
Trilinolein[d]	---	100.0	---	173	65.3
Trilinolenin[d]	---	---	100.0	262	55.1

[a]Average of triplicate analysis.

[b]Iodine value calculated from GLC fatty acid composition.

[c]NMR: r.f., 100 μA; gain, 500; integration, 128S; 2 ml sample assembly.

[d]Single analysis.

[e]Sunflower oil was contaminated with soybean oil during processing.

flower seed is shown in Table III. As temperature increased from 15 to 32 C, the NMR response decreased 0.44% per 1 increase. Wolff et al. (2) reported that the response of their instrument varied 2.5% per 1 C. Possibly our variation in response was low because we had a newer model Newport NMR and had better temperature control. In calculated oil contents of sunflower seed, deviation was 0.2% oil for each 1 C change in temperature from that used to calibrate the instrument. Thus, NMR responses for the standard calibration seed and the sample must be read at the same temperature.

The effect of iodine value of various types of oils on NMR response is shown in Table IV. As the iodine value increased by 10 units, the NMR response decreased by ca. 1.2% (see Figure 2). The iodine value of northern- and southern-produced sunflower seed oil can differ by as much as 30 units and normally differs by 15-20 units. This difference in iodine value could represent a significant variation in sunflower seed oil content when determined by wide-line NMR. We, therefore, studied the effects of the unsaturated fatty acids of sunflower seed on NMR response and oil determination.

Sunflower seed ranging in linoleic acid content from ca. 31-71% were analyzed for oil content by NMR, extracted with petroleum ether (35-60 C) to recover the oil, and fatty acid composition was determined on the extracted oil. Figure 3 shows a linear regression fit between linoleic acid content and NMR response per gram of oil. Correlation coefficient between linoleic acid and NMR response was significant (r=0.87). For sunflower seed, the NMR response increased ca. 0.1% per 1% decrease in linoleic acid content. For sunflower seed containing 50% oil, a 10% difference in linoleic acid contents of the NMR seed standard and the sample would cause a difference of ca. 0.5% in oil content as determined by NMR.

Linear regression analysis showed that NMR response was significantly correlated (r = 0.88) with iodine values calculated from GLC fatty acid composition data. The NMR response of sunflower seed increased ca. 0.125% per 1 iodine value unit decrease. This agreed with the data in Table IV. Thus, a 10% change in linoleic acid is equivalent to an iodine value change of 8 units.

These results substantiate the findings of Karleskind et al. (12) on the oils of Primor and Major rape whose composition differed markedly (erucic acid content of 0.6% and 51%, respectively). The response of the NMR noticeably differed for equal weights of oil from the two rape varieties. Thus, they concluded that for correct NMR analysis of the

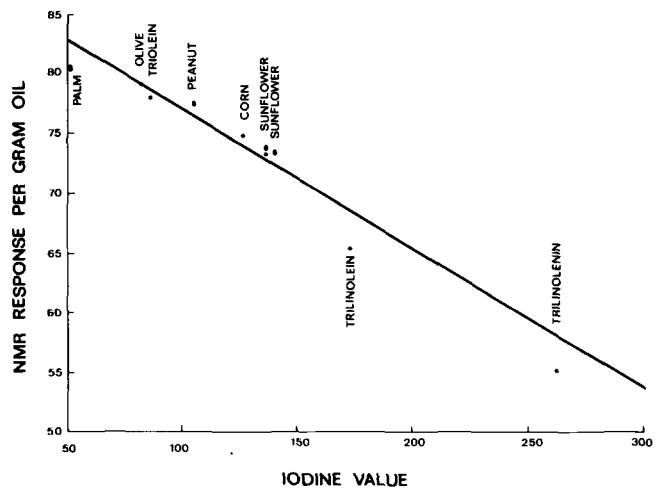

FIG. 2. Effect of Iodine Value of liquid oils on NMR response. NMR: r.f., 100 μA; gain, 500; integration, 128S; and 2 ml sample assembly.

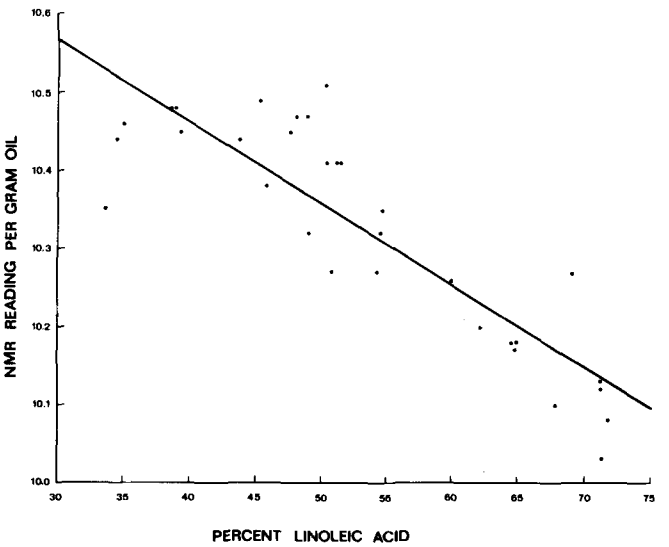

FIG. 3. Linear regression fit of the linoleic acid content vs. NMR response per gram of sunflower seed oil. NMR: r.f., 225 μA; gain, 700; integration, 128S; and 34 ml sample assembly.

oil content of seed, the instrument must be calibrated with seed of the same variety as the samples.

The data indicated that the linoleic acid content in the sunflower seed standard affected the accuracy of NMR analysis for total oil. Fatty acid composition of the standard and the sample should be similar. Thus, NMR seed standards for analysis of sunflower would differ between seed produced in the South and in the Red River Valley. Additional studies are being conducted at the Russell Research Center to determine the importance of these findings and the range in composition that could be allowed for a single standard.

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