The Effect of Hydrogen Content on Estimation of Seed Oil by Pulsed Nuclear Magnetic Resonance

V.T. Srinivasan*[,a], B.B. Singh[a], P.K. Chidambareswaran[b] and V. Sundaram[b]

[a]Molecular Biology and Agriculture Division, Bhabha Atomic Research Center, Bombay, India, and [b]Cotton Technological Research Laboratory, Bombay, India

In the nondestructive estimation of seed oil by pulsed nuclear magnetic resonance (NMR), an assumption generally is made that the hydrogen content of the oil in the seed sample under investigation is the same as that of the oil standard or that of the oil in the standard seed sample. The hydrogen content is defined as the number of hydrogen atoms per unit mass of oil. The validity of this assumption has been investigated by: (i) calculating the Hydrogen contents of various oils on the basis of their reported fatty acid composition, and (ii) experimentally determining the hydrogen contents of cotton and mustard oils obtained from different varieties of seeds. The FID method was used to monitor the NMR signal intensities. Both calculated and experimental values show that the hydrogen contents can be different not only for different oils but also for the same oil extracted from different varieties of the seeds. This variation in hydrogen contents is shown to introduce an error in the oil content values as estimated by NMR methods. The magnitude of this error increases with oil content, and also with the difference in the hydrogen contents.

Various pulsed nuclear magnetic resonance (NMR) methods have been reported for the nondestructive estimation of seed oil. The free induction decay (FID) and extrapolation methods (1-3) involve the use of a standard oil and also necessitate the seed weight being known. Seed oil and seed moisture estimation also can be carried out without weighing the seed sample (4,5). Though good correlation has been shown to exist between the NMR values and those obtained by Soxhlet extraction (1,3,6), a close analysis of the data indicates significant differences in certain samples. Seed asymmetry and moisture associated with the seeds have been shown to affect seed oil estimation by NMR (2,3). Another factor which can influence the NMR results is the hydrogen content of the oil. It generally is assumed that the oil in the seeds and the reference oil have the same hydrogen content (number of hydrogen atoms per unit mass of oil). However, it has been reported that the hydrogen content varies from one oil to another because of differing fatty acid composition (7). The present study has been undertaken to investigate the effect of this hydrogen content variation on the oil content values as estimated by pulsed NMR methods.

MATERIALS AND METHODS

Pulsed NMR spectrometer. A pulsed NMR spectrometer fabricated at Jozef Stefan Institute, Ljubljana, Yugoslavia was operated at 32 MHz. The size of the radio frequency (r.f.) coil was 11 mm × 11 mm, but to reduce the effect of r.f. field inhomogeneity, only the central region, about 6 mm in height, was filled with the sample. The 90 pulse width was 13.2 μsec and the dead time of the receiver, 30 μsec. The free induction decay (FID) signal intensities were measured 50 μsec after the application of 90 pulse; phase sensitive detection was employed. Each signal intensity measured was an average of 30 repetitions. A sample of mustardseeds was used as a standard to monitor the sensitivity of the spectrometer. The same sample tube was used for all the oil samples.

Hydrogen content measurements. The FID signal intensities corresponding to different amounts of the sample were measured. For each sample, at least nine different weights were used, the ratio of the minimum to maximum amount being about 0.6. The overall range for all the samples extended from about 300 to 600 mg. The signal intensity of the standard seed sample was also measured periodically, and all the intensities were normalized to a fixed value of the standard sample.

The normalized signal intensities and the corresponding weights for each sample were fitted by the method of least squares. The linear regression coefficient thus obtained is proportional to the hydrogen content of the sample. For each set of oil samples, an aqueous solution of manganese chloride (0.18 mM) was also run and the regression coefficient calculated. Using this value, and taking 100 as the regression coefficient for water, all the other regression coefficients were normalized, i.e., the hydrogen content of water is taken as 100.

The oil samples used in the present studies were freshly obtained by Soxhlet extraction using petroleum ether (B.P.: 60-80 C) as the solvent. The extracted oil samples were kept overnight in an electric oven maintained at 85 C to remove the last traces of ether.

RESULTS AND DISCUSSION

Hydrogen contents of fatty acids and their acylglycerides, calculated on basis of molecular formulae. The hydrogen content of any compound can be calculated from its molecular weight and chemical formula. Table 1 shows the range of hydrogen contents of various

TABLE 1

Range of Hydrogen Contents[a] of Fatty Acids and Their Glycerides Calculated on Basis of Their Molecular Formulae

	Saturated 6:0 to 24:0	Unsaturated 16:1 to 22:2
Monoacylglycerides	85.23 to 109.87	91.99 to 104.78
Diacylglycerides	87.45 to 113.54	94.05 to 108.11
Triacylglycerides	88.56 to 114.96	94.89 to 109.42
Fatty acids	93.05 to 117.29	97.05 to 111.74

[a]Hydrogen content of water is taken as 100.

*To whom correspondence should be addressed at Molecular Biology and Agriculture Division, Modular Laboratories, Bhabha Atomic Research Center, Trombay, Bombay 400 085, India.

saturated and unsaturated fatty acids, as well as their mono-, di- and triacylglycerides. These values and all other hydrogen content values given in this communication have been normalized on the basis of 100 as the hydrogen content of water. (The absolute value for water is 0.1110 × N/g, N being the Avogadro's number.) For both saturated and unsaturated fatty acids, the hydrogen contents increase with the number of hydrogen atoms. For the same fatty acid, the values decrease in the order monoacylglyceride < diacylglyceride < triacylglyceride < fatty acid. In view of the wide variation in these hydrogen contents, it is reasonable to expect a similar variation among the different oils; this is discussed below.

Hydrogen contents of different oils, calculated on basis of reported fatty acid composition. Vegetable oils are known to consist of fatty acids in the form of triacylglycerides (8). The fatty acid composition of a large number of oils has been reported (9). The hydrogen content of any oil can be calculated on the basis of its fatty acid composition by use of the following relation

$$\text{Hydrogen content of oil} = \{\Sigma[(M_t/M_f)P_fD_t]\}/\{\Sigma[(M_t/M_f)P_f]\} \quad [1]$$

where M_f = molecular weight of the fatty acid,
M_t = molecular weight of the corresponding triacylglyceride,
P_f = fatty acid content (g/100g of fatty acids), and
D_t = hydrogen content of the triacylglyceride.

The hydrogen content values thus calculated vary from 99.73 (linseed oil) to 107.67 (neem oil). Pieter et al. have reported the standard deviations (an indication of both the sampling and analytical errors) associated with each fatty acid content measurement (10). The effect of these errors on the calculated value of the hydrogen content of the oil turns out to be less than 0.4%.

The variation in the hydrogen content values among the different oils can, therefore, be ascribed to the differences in the fatty acid composition. Generally, NMR analysis of different varieties of seeds belonging to a particular species is carried out using a reference oil extracted from one of the varieties belonging to the same species or a reference seed sample as a standard. However, the oils from different varieties of seeds belonging to the same species need not necessarily exhibit the same hydrogen content.

Hydrogen contents of oils from different varieties of seeds, calculated on basis of reported fatty acid composition. The fatty acid composition of oil extracted from different varieties of seeds belonging to the same species has been studied in detail for a wide range of species, and significant differences in certain fatty acid contents have been observed (11-14). The hydrogen contents of cotton, mustard and rape calculated on the basis of their fatty acid compositions are shown in Table 2.

The range, which is a measure of the variation in hydrogen content from one variety to another, is significantly larger than the s.d. expected on the basis of the errors associated with the fatty acid content measurements; it can be correlated with the amount of specific fatty acids present. Thus, for mustard 2 and rapeseeds, a positive correlation exists between the hydrogen contents and erucic acid (22:1) content, as can be seen in Figure 1. As the erucic acid content in mustard 1 varieties varies over a limited range (43.99 to 57.71%), the corresponding hydrogen contents also span over a smaller range (105.55 to 106.48). In cottonseed oil, no clearcut correlation could be established with any specific fatty acid.

TABLE 2

Hydrogen Contents of Different Oils Calculated on Basis of the Reported Fatty Acid Composition

Source of oil[b]	Hydrogen contents[a] mean	s.d.	range	Number of fatty acids analyzed	Ref. no.
Cotton (43)	104.07	0.20	0.80	6	11
Mustard 1 (20)	106.10	0.32	0.90	11	12
Mustard 2 (3)	103.08	1.13	2.04	5	13
Rape (7)	104.58	1.06	2.75	6	14

[a]Hydrogen content of water is taken as 100.
[b]Figures in parentheses denote the number of varieties studied.

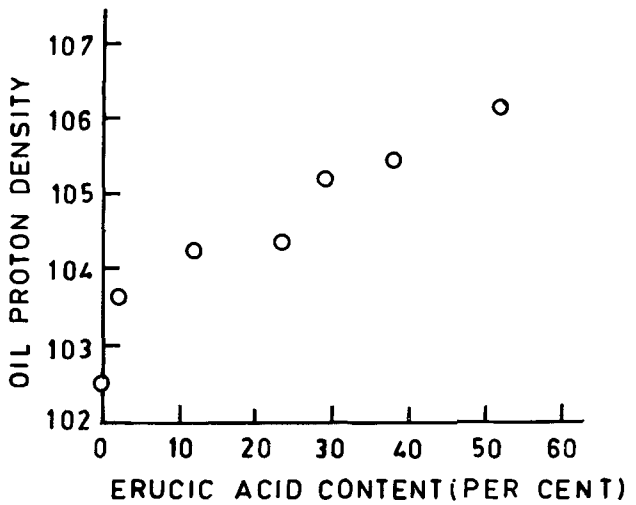

FIG. 1. Correlation between hydrogen content and erucic acid content for mustard and rapeseed oil.

Hydrogen contents of mustard and cottonseed oil as determined by NMR. The values of the hydrogen contents of oil extracted from different varieties of cotton and mustardseeds have been estimated from the linear regression coefficients obtained by a least square fit of the amount of sample and the corresponding signal intensity. The linearity of such plots is illustrated in Figure 2. The correlation coefficient for each sample was found to be better than 0.999, and the sample s.d. of the regression coefficient was less than 0.5% of the corresponding regression coefficient.

Table 3 shows the NMR values of hydrogen contents of four varieties each of cotton and mustard oil along with that of glycerol. The theoretical value for the ratio of the hydrogen contents of glycerol to water is 0.7825. The excellent agreement with the experimental value of 0.7828 justifies the validity of the present method used to estimate the hydrogen contents.

Statistical analysis of the data obtained indicates that the hydrogen content of cottonseed oil is different from

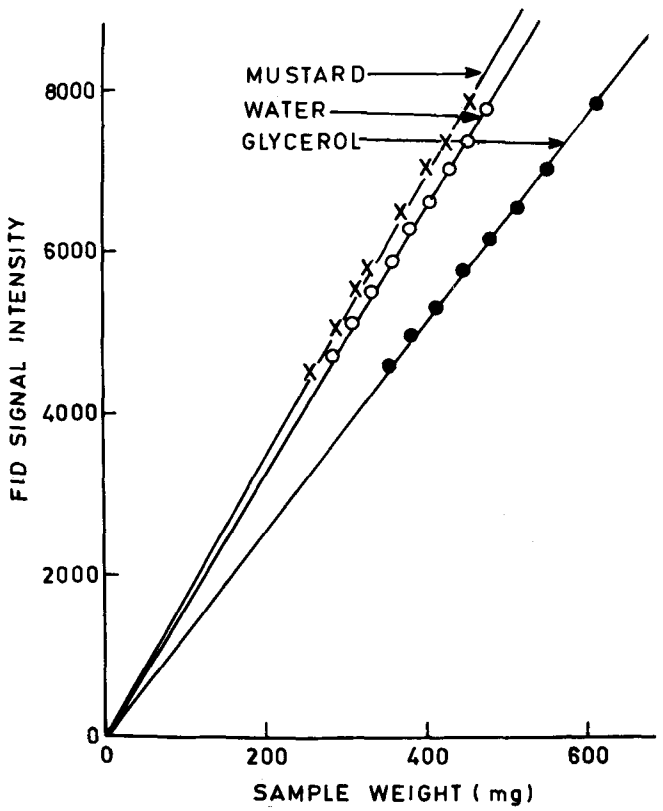

FIG. 2. Linearity between the amount of sample and NMR signal intensity as estimated by FID method.

TABLE 3

Hydrogen Contents of Glycerol, Cotton and Mustard Oil from Different Varieties of Seeds as Determined by NMR

Sample	Hydrogen contents[a]			
	1	2	3	mean
Glycerol	78.27	78.40	78.18	78.28
Cottonseed oil				
SRT 1	99.45	99.68	100.47	99.87
Lakshmi	99.70	99.71	100.32	99.91
Khandwa	102.32	102.40	102.75	102.49
Hybrid 4	99.95	99.84	100.65	100.15
Mustard oil				
TM4	94.82	95.03	95.23	95.03
Pusa	99.71	99.77	100.72	100.07
TM17	106.28	106.75	106.26	106.43
Varuna	106.41	106.37	106.70	106.49

[a]Hydrogen content of water is taken as 100.

that of mustard oil. Moreover, even within each oil, the hydrogen contents corresponding to different varieties are significantly different ($P < 0.005$). Robertson et al. also have shown that the NMR reading per g oil is different for different oils, and have pointed out the importance of linoleic acid content in the oil analysis of sunflower seeds (15).

Comparison between calculated and NMR values. As the varieties of mustard and cottonseeds used for the experimental determination of the hydrogen contents are not the same as those in Table 2, one should not expect to get identical values. In evaluating the calculated data, it must be borne in mind that various authors have not analyzed the same number of fatty acids. Moreover, it has been reported (16) that about 2 to 8% of the oil is composed of lipid compounds including unsaponifiable hydrocarbons, terpenes, sterols, tocopherols, glycolipids and phospholipids. Thus, the calculated values may not truly reflect the hydrogen content of the oil. The purpose of the calculated values is to get an idea of the likely variation in the hydrogen contents, which is found to be about 3%. On the other hand, the experimental values show a variation of 3% for cotton oil and 11% for mustard oil.

The very low value of the hydrogen content for the TM4 mustard variety may be due to the loss of some volatile compounds during the drying procedure, as this sample was found to be more viscous than the others. However, any change in viscosity is not expected to affect the measured hydrogen content, as glycerol, a viscous material, has been found to yield the correct value.

Effect of hydrogen content variation on seed oil estimation. Whatever may be the reasons for the difference between the calculated and NMR values, the hydrogen content of solvent extracted oil, as measured by NMR, ultimately will be a factor in determining the accuracy of oil content measurements. Even a conservative estimate of 3% for the variation between the hydrogen contents of two different oils can cause a 1% error in the estimated oil content, as shown below.

Let D_s and D_o = hydrogen contents, i.e., number of hydrogen atoms per unit mass of the seed oil and the reference oil,

S_s and S_o = NMR signal intensities per unit mass of the seed sample and the reference oil measured under identical instrumental conditions,

k = an instrumental parameter relating the signal intensity and the number of hydrogen atoms, a constant under identical instrumental conditions,

P_t = "true" value of the oil content (%) in the seed sample,

P_c = value of the oil content (%) estimated by the usual NMR method of comparing the signal intensities without taking into account the hydrogen contents.

$$P_c = 100(S_s/S_o) \quad [2]$$

As the signal intensities are proportional to the number of hydrogen atoms.

$$S_s = KD_sP_t/100 \quad [3]$$
$$S_o = KD_o \quad [4]$$

Substituting the values of S_s and S_o in equation 2.

$$P_c = (D_s/D_o)P_t \quad [5]$$

Thus, on the basis of the above relation, the oil content estimated by the NMR method will be equal to the actual value only when $D_s/D_o = 1$, i.e. when the hydrogen content of the seed oil is the same as that of the oil

standard. The NMR estimation of oil content in a seed sample whose "true" oil content is 40%, and whose oil hydrogen content differs from that of the reference oil by 3%, i.e., $D_s = 97$; $D_o = 100$, would yield a value of only 38.8%, an error of 1.2%. The extent of this error will increase with increasing oil content and also with increasing difference between the hydrogen contents.

Thus, before the pulsed NMR technique could be established as an analytical tool for the nondestructive estimation of oil content in seeds, it would be necessary to investigate the hydrogen contents of each oil from a large number of different varieties of seed samples to get an idea of the variation in the hydrogen contents that is likely to arise; this, in turn will decide the uncertainties associated with the NMR estimated values.

REFERENCES

1. Tiwari, P.N., P.N. Gambhir and T.S. Rajan, *J. Amer. Oil Chem. Soc. 51*:104 (1974).
2. Srinivasan, V.T., *Ibid. 56*:1000 (1979).
3. Srinivasan, V.T., B.B. Singh, P.K. Chidambareswaran and V. Sundaram, *Ibid. 62*:1021 (1985).
4. Tiwari, P.N., and W. Burk, *Ibid. 57*:119 (1980).
5. Gambhir, P.N., and A.K. Agarwala, *Ibid. 62*:103 (1985).
6. Sagare, B.N., and K.T. Naphade, *J. Maharashtra Agri. Univ. 8*:240 (1983).
7. Mansfield, P., *J. Amer. Oil Chem. Soc. 48*:4 (1971).
8. Cobb, W.Y., and B.R. Johnson, in *Physicochemical Properties of Peanuts—Culture and Use, A Symposium*, Stone Printing Co., Virginia, 19, p. 215.
9. Weast, Robert C., in *Chemical Rubber Company Handbook of Chemistry and Physics*, 63rd edition, CRC Press, 1982-83, p. D-225.
10. Pieter, J. van N., and A.E. C. Burger, *J. Amer. Oil Chem. Soc. 62*:531 (1985).
11. Pandey, S.N., and N. Thejappa, *J. Oil Technol. Assoc. India 13*:107 (1981).
12. Anand, I.J., *Ind. J. Gen. 35*:417 (1975),
13. Kirk, J.T.O., and R.N. Oram, *J. Aust. Inst. Agri. Sci. 47*:51 (1981).
14. Jonsson, R., *Hereditas 87*:205 (1977).
15. Robertson, J.A., and W.H. Morrison, *J. Amer. Oil Chem. Soc. 56*:961 (1979).
16. Downey, R.K., and D.I. McGregor, *Curr. Advan. Pl. Sci. 12*:151 (1975).

[Received December 2, 1985]

High Oleic Sunflower: Physical and Chemical Characteristics

Richard H. Purdy
Richard H. Purdy, Inc., 16 Josefa Ct., Novato, CA 94947

Treatment of normal varieties of sunflowerseed with chemical mutagens and development of their progenies have resulted in hybrids bearing oil with oleic acid contents in excess of 80% and linoleic acid contents less than 10%. Fatty acid compositions are unaffected by climatic conditions. Analyses of the seed, oil and meal produced from the first commercial U.S. production show values not to differ significantly from normal high linoleic sunflower, except where anticipated on the basis of fatty acid composition.

Sunflowerseed is one of the world's major oilseed crops. Although it originated in the United States, its utilization has been predominantly European. Introduction of high oil-bearing seed in the 1960s increased both production and utilization in the U.S. In recent years, sunflower has become an established crop in Minnesota, North and South Dakota, and Texas.

Numerous technical publications describing the composition and characteristics of sunflowerseed and oil are available in the literature (1-6). One of the more interesting characteristics is the effect of climatic conditions during seed formation on the fatty acid composition of the oil (7,8). Linoleic acid percentages may vary from the low 30s for seed grown in Texas to the mid 70s for Northern seed, with oleic acid values ranging from as high as 60 to less than 20%, respectively.

Soldatov and Kharachenko in Russia treated normal (Peredovik) planting seed with the mutagen dimethyl sulfate (9,10). Selected breeding resulted in progenies (Pervenets) with high oleic acid contents stable to climatic conditions. Fick, in the U.S., developed progenies from the Pervenet variety, demonstrating their environmental stability and incorporating the dominant genes into hybrids suitable for commercial production 9, 11).

In 1984, high oleic seed was grown commercially in the United States for the first time in North Dakota, California and Texas. This paper will report the composition and analytical characteristics of seed samples from the three growing areas; the crude oils and meals obtained from the commercial processing, and refined oil produced in the laboratory.

EXPERIMENTAL

Seed samples were drawn directly from trucks arriving at seed warehouses in Breckenridge, North Dakota; Fresno, California, and Plainview Texas, using state-approved procedures. The samples were split mechanically to a suitable size and hand-cleaned to remove foreign material.

Crude oil and meal samples were drawn from production storage tanks, but are not necessarily representative of the entire crushing operation at each location.

California production samples were identified as "CSE" (California Solvent Extracted) and "CPP" (California Pre-Pressed). Texas production included a limited quantity of North Dakota seed, and was