Determination of the Solid Contents of Fats by Wide-Line Nuclear Magnetic Resonance: The Signal of Liquid Oils

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ABSTRACT

In order to ascertain the sources of error in the determination of the solid fat content by wide-line nuclear magnetic resonance (NMR) an investigation has been made of the factors governing the amplitude of NMR signals of liquid oils measured with a Newport Quantity Analyzer. The varying saturation properties of the oils concerned were found to be of major importance. The spin-lattice relaxation times (T_1) of 16 oils were measured on a pulse spectrometer at 20, 40 and 60 C, and quantitatively related to the saturation phenomenon. The observed deviation from the theoretical linearity of the NMR signal with the inverse of temperature is explained by the strong dependence of T_1 on temperature and viscosity, and new relationships are derived for these parameters. Since the improvement of the signal to noise ratio of the NMR signal, obtained by increasing the RF level (H_1), increases the saturation as $(H_1)^2$, a compromise must be chosen between these two factors to obtain optimal measurement. The results are used to establish criteria for selecting two reference oils to be used in solid fat content determinations.

INTRODUCTION

It has been shown in a previous paper (1) that the reliability of solid-content determinations by wide-line nuclear magnetic resonance (NMR) depends on the reliability of the signal of the oil chosen as a reference (100% liquid). Various oils and oleins used did not, however, follow the expected linear relationship between signal and the inverse of the absolute temperature, mainly because long relaxation times cause saturation which produces a decrease in signal. Therefore these empirically found factors were introduced to overcome these deviations.

It was the objective of further research to take into account also the influence of saturation, having regard to the relaxation properties of the liquid triglycerides.

The normal equation for the NMR absorption (2) is:

$$S = C\, nH_0T^{-1}\gamma^2H_1T_2[1 + T_2^2(\omega - \omega_0)^2 + \gamma^2H_1^2T_2T_1]^{-1} \quad [1]$$

in which:

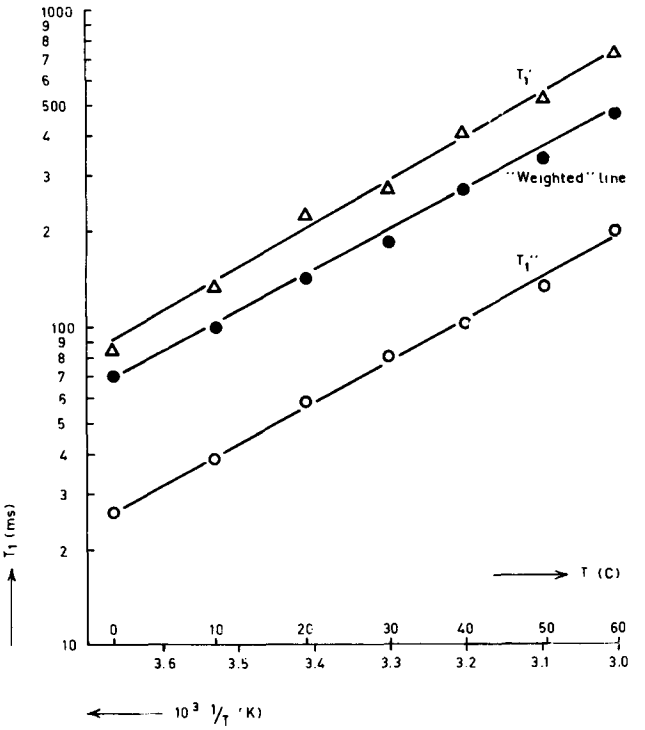

FIG. 1. Spin-lattice relaxation times T_1 for sunflowerseed oil (15 MHz) at different temperatures.

TABLE I

Effective or Weighted T_1, Iodine Value, Saponification Value and Percentage Hydrogen of the Samples

Oil or fat	Iodine value (IV)	Saponification value (SV)	Percentage hydrogen (%H)[a]	Effective T_1, ms		
				20C	40C	60C
Soybean oil	134	195	11.37	153	265	489
Medium chain triglycerides	0	281	11.38	---	219	401
Sunflower seed oil	132	193	11.41	145	176	476
Corn oil	122	193	11.48	117	237	406
Coconut oil	9	256	11.58	---	199	386
Hydrogenated soybean oil 28 C	101	193	11.62	---	203	350
Butterfat	38	232	11.64	---	196	361
Groundnut oil	89	191	11.71	107	206	337
Olive oil	83	193	11.73	109	196	333
Hydrogenated cottonseed oil 32 C	76	196	11.75	---	158	295
Rapeseed oil	104	176	11.76	118	205	336
Lard olein	73	195	11.78	---	188	335
Palm olein	64	199	11.80	---	176	327
Palm oil	51	203	11.85	---	186	299
Lard	63	195	11.85	---	178	329
Hydrogenated rapeseed oil 32 C	81	175	11.93	---	152	274

[a]Calculated according to %H = 14.265-0.0065 IV - 0.01017 SV (1).

TABLE II

Viscosity of Various Neutral and Deodorized Oils and Fats in the Liquid State Measured at Different Temperatures

Oil or fat	Viscosity,[a] cP			Constants[b]	
	20 C	40 C	60 C	a	b
Soybean oil	60	28	15	-0.073	46.6
Medium chain triglycerides	---	21	11	-0.306	50.1
Sunflower seed oil	63	29	16	-0.038	44.8
Corn oil	70	30	16	-0.142	49.9
Coconut oil	---	27	14	-0.242	51.0
Hydrogenated soybean oil 28 C	---	33	18	0.14g	51.1
Butterfat	---	34	17	-0.151	51.2
Groundnut oil	81	36	19	-0.080	50.5
Olive oil	82	35	17	-0.102	50.1
Hydrogenated cottonseed oil 32 C	---	45	23	-0.166	55.9
Rapeseed oil	93	41	21	-0.023	50.1
Lard olein	---	36	18	-0.151	51.9
Palm olein	---	37	19	-0.145	52.2
Palm oil	---	37	19	-0.192	53.8
Lard	---	36	19	-0.068	48.2
Hydrogenated rapeseed oil 32 C	---	49	24	-0.140	56.0

[a]Standard deviation of replicates 1%.

[b]Constants in the equation $\log \eta = a + 10^6 .b.T^{-3}$.

TABLE III

Influence of a Thickening Agent and Fat Crystals on the "Weighted" T_1 and the Viscosity of Sunflower Seed Oil and Margarine Oil at 20 C

Fat	"Weighted" T_1, ms	Viscosity, cP
Sunflower seed oil	145	66
Sunflower seed oil + 8% cab-o-sil	138	93.000
Olein from margarine fat	114	70
Olein from margarine fat + 20% crystals	115	plastic

TABLE IV

The Influence of Saturation on the Signal of Various Fats at 60 C

Fat	Influence[a] of saturation, %
Olive oil	9.6
Rapeseed oil	9.9
Corn oil	11.6
Lard	9.8
Butterfat	10.6
Hydrogenated cottonseed oil 32 C	8.9
Hydrogenated rapeseed oil 32 C	8.4
Hydrogenated soybean oil 28 C	10.3

[a]The term $100 - 100(1 + C_2A^2T_1)^{-½}$.

S = absorption signal as a function of ω(V)
C = constant
n = number of H-protons involved
H_o = field strength permanent magnetic field (gauss)
T = absolute temperature (K)
γ = gyromagnetic ratio (2.65×10^4 gauss s^{-1})
H_1 = radio frequency field (gauss)
ω = angular velocity of proton precession (s^{-1})
ω_o = angular velocity of H_1 (s^{-1})
T_1 = spin-lattice relaxation time (s)
T_2 = spin-spin relaxation time (s)

If a wide-line instrument does not give the absorption S, but the integrated curve, i.e., the total area I, Equation 1 must be integrated between $\omega = 0$ and $\omega = \infty$ giving:

$$I = C n H_1 T^{-1} [1 + \gamma^2 H_1^2 T_2 T_1]^{-½} \text{ (volt)} \qquad [2]$$

It is seen from this equation that the signal (I) is proportional to T^{-1} only when the saturation term $\gamma^2 H_1^2 T_2 T_1$ is negligible. It is the purpose of this paper to study the factors affecting this term.

EXPERIMENTAL PROCEDURES

The wide-line NMR measurements were performed on the Newport Quantity Analyzer working at approximately 2.7 MHz. The absorption signals were integrated for 22 sec and the results automatically printed out. In all cases the average of five readings was taken. The apparatus was working with high loss control and supplementary modulation. The 2 ml glass tubes containing a known weight of sample were placed in an air-thermostated sample holder located in the magnet. The temperature of the air could be adjusted between 0 and 60 C in steps of 5 C and was constant to within 0.2 C.

To study the RF saturation effects on the NMR signal from the Newport Quantity Analyzer it is important to take into account an electronic feedback in the RF oscillator and detector system which increases the signal amplification when the RF level (H_1) is decreased. The effect of this is to give an eccentric variation of signal with RF power instead of the linear increase demanded by Equation 2 when saturation is unimportant. This may be overcome by calibration measurements on a water sample containing 1.7 g $MnCl_2 \cdot 4 H_2O$ per liter (in which the paramagnetic ions lower the relaxation time). A sample tube is filled with this solution and sealed close to the water level to avoid evaporation and condensation outside the measuring range of the coil. (This sealed tube can also be used for tuning the instrument to achieve identical responses, thus eliminating day-to-day variations.) For each value of the RF an amplification correction factor may then be calculated which converts the plot of signal amplitude against RF level into an arbitrary straight line passing through the origin.

Initial T_1 measurements were carried out with a Bruker BKR-304 S pulse spectrometer (5) at 60, 15 and 4 MHz. When it was found that below 15 MHz the change of T_1 with frequency could be neglected, all further measurements were made at 15 MHz and directly applied to the results obtained with the Newport instrument working at 2.7 MHz.

RESULTS

Spin-Spin Relaxation Time (T_2)

In a homogeneous field (H_o) T_2 determines the line

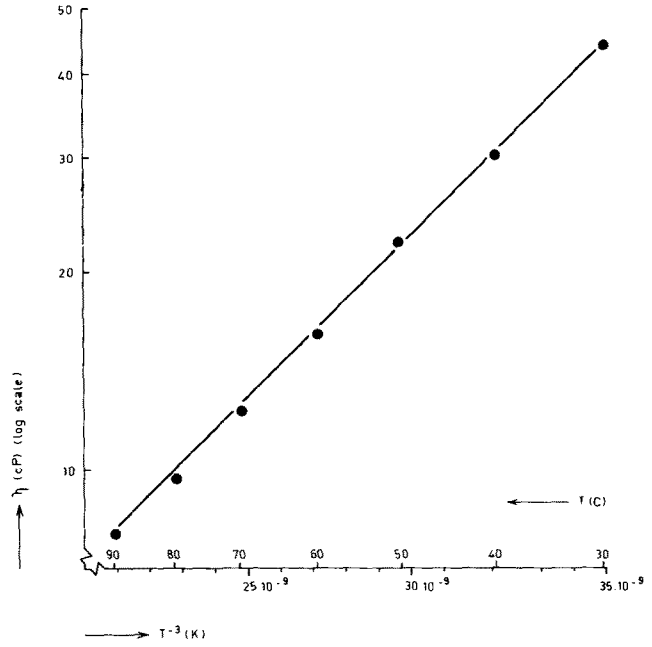

FIG. 2. Influence of the temperature (T) on the viscosity of corn oil: $\log \eta = -0.142 + 49.9.10^{-6}.T^{-3}$ cP.

width of the absorption curve. For liquids T_2 is usually approximately equal to T_1. In our case, however, the line width is strongly influenced by the inhomogeneity of the permanent field H_o and the presence of wiggles, caused by the rapid passages through resonance. Therefore it is better to use the symbol T_2*, defined as a time derived from the line width incorporating all sources of peak broadening. The absorption curves for different materials (oils, water, glycerol, propanol and the like) showed an almost constant peak width at half height at different temperatures. Hence the peak width parameter T_2* is also considered to be almost constant for all the oils measured.

Spin-Lattice Relaxation Time (T_1)

Little is known from the literature regarding the spin-lattice relaxation time for oils and fats. Ferren and Morse (4) give a value of 126 ms for unhydrogenated soybean oil without details about temperature or frequency. It appears that the longitudinal magnetization decay in liquid triglycerides is nonexponential. The decay could be fairly described by two relaxation times (T_1' and T_1''), one of them often being three to four times longer than the other. The occurrence of a number of relaxation mechanisms is probably due to differences in mobility of the protons in the molecule. The results for sunflowerseed oil at different temperatures are shown in Figure 1. At 60 C about 53% of the H-protons had the longer and 47% the shorter relaxation time. In Figure 1a "weighted" line is also shown representing the effective T_1 to be used in Equations 1 and 2. This effective or weighted T_1 is the sum of the relative contributions of each time. For Figure 1 (at 60 C): $T_1 = 0.53T_1' + 0.47T_1''$. The results for a number of oils, fats and oleins are shown in Table I ranked in order of decreasing % H.

Some conclusions can be drawn from Table I: (a) T_1 changes considerably with temperature; (b) an increase in hydrogen percentage causes, in general, a decrease in T_1; (c) partly hydrogenated fats follow the same pattern as unhydrogenated fats.

Relationship between T_1 and Viscosity

The relationship between the viscosity and T_1 was studied, not only to find equations for their mutual

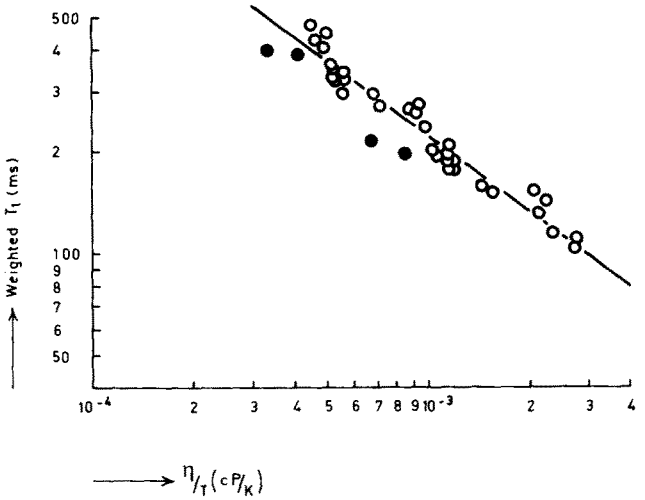

FIG. 3. The ratio of viscosity (η) to absolute temperature (T) against the "weighted" T_1 for different oils and fats at several temperatures: $\log T_1 = 0.123 - 0.743.\log \eta/T$.

conversion, but also to obtain an impression of the effect on the NMR signal of the strong viscosity increase due to fat crystallization. The dynamic viscosity values for the fats and oils mentioned in Table I were measured with a Couette-type viscosimeter at different shear rates (all samples behaved Newtonian). Only those temperatures were chosen at which the samples were completely melted. The results are given in Table II. Viscosity is often related to T^{-1} but it was found that in the case of triglycerides a relationship with T^{-3} gives far better results (Fig. 2), the equation being:

$$\log \eta = a + b\, T^{-3} \text{ (cP)} \qquad [3]$$

The a and b values found for each sample are also given in Table II.

Bloembergen et al. (6) derived equations for liquids consisting of one kind of small spherical molecules. Accordingly the plot of $\log T_1$ versus $\log \eta/T$ should be a straight line with a slope of -1. The values given in Tables I and II were plotted in the same way (Fig. 3). By regression calculcation a straight line was obtained for all glycerides with a mean carbon number above 14. The line is given by the equation:

$$\log T_1 = \alpha + \beta \log \eta/T$$
$$\text{in which } \alpha = 0.123 \text{ and } \beta = -0.743 \qquad [4]$$

The slope of this line is different from the value expected by Bloembergen. The relationship also seems dependent on the chain length because coconut oil and the medium chain triglycerides lie significantly outside the 95% confidence limits. Equation 4 may be used to calculate T_1 from a viscosity value with a standard deviation of about 9%.

In this respect it is important to note that viscosity is a "bulk property," whereas NMR gives information of motions on a molecular scale. The presence of small amounts of solid materials, e.g., 8% cab-o-sil (very finely divided SiO_2, particle size ($<1\mu$) in sunflower seed oil or 20% crystals in margarine oil gives a remarkable increase in viscosity but has no measurable influence on the spin-lattice relaxation time T_1. Table III illustrates these effects. An important conclusion is that the T_1 of a melted fat does not change during the crystallization process from liquid to plastic, whereas the viscosity of the system can vary considerably.

PRACTICAL APPLICATION

Calculation of NMR Signal

If a known weight of sample is measured the amplifica-

tion corrected signal I can be given in volts per gram, I^1, which enables n in Equation 2 to be replaced by the percentage by weight of hydrogen in the sample, N (1). It is not necessary to know the absolute value of the RF field and on the Newport instrument a micro ampère meter gives a reading A which is linearly proportional to H_1. (The linearity between A and H_1 was checked by field measurements. For the 2 ml sample tube the relationship $H_1 = 6.7 \times 10^{-5}$ A [gauss] was found to be constant between 0 and 60 C.) Consequently it is possible to write Equation 2 in the form:

$$I^1 = C_1 N T^{-1} A [1 + C_2 A^2 T_1]^{-½} \quad [5]$$

in which C_1 and C_2 are constants depending upon the instrument and settings used. These values may readily be calculated from measurements of I^1 and T_1 for an oil at different temperatures, but constant RF level (for our instrument C_1 was found to be 0.360 ± 2% and C_2 0.20×10^{-3}). Since the response of the instrument is sensitive to the magnetic properties of the sample, various values of C_1 were found for different groups of substances. However, for triglycerides a constant value can be used.

With measured T_1's the NMR signal can then be calculated for oils of different hydrogen content at varying temperatures and RF levels. These values were compared with experimental measurements and for 13 oils the standard deviation was not more than 1%. In Table IV the absolute value of the influence of saturation is seen to be about 10% but the variation between different oils is less than 3% and, therefore, the use of a reference oil (olive oil) does not introduce large errors.

Determination of Solid Contents

In the calculation of the per cent solids the "oil line" must be extrapolated from the measured signal at 60 C (completely melted sample) to lower temperatures (solid-liquid mixture). In a previous paper (1) empirical values (C_t) were given in order to apply corrections for deviations caused by saturation. The exact value can now be derived from Equation 5.

$$C_t = {}^{333}/_{273+t} \cdot (1 + C_2 A^2 T_{1.60})^{½} (1 + C_2 A^2 T_1 \cdot t)^{-½} \quad [6]$$

C_t = factor by which the 60 C signal should be multiplied to obtain the liquid signal at tC.

$T_{1.60}$ = T_1 at 60 C for melted fat(s)

$T_{1.t}$ = T_1 at tC for liquid portion of fat(s)

Instead of using such calculation signals, however, it is more practicable to use a reference oil. For the calculation of solid fat content (SFC) the fat signal is then compared with the signal of this oil at the same temperature (7). The SFC is then given by:

$$SFC = 100 - 100\left[\frac{\text{signal sample at tC}}{\text{signal sample at 60 C}} \times \frac{\text{signal reference at 60 C}}{\text{signal reference at tC}}\right]$$

The reference must have about the same saturation properties as the liquid part of the fat. For most hydrogenated fats, palm oil, lard, tallow and butter, reference oils with an IV of about 80-90 are suitable (tri-olein, olive oil), i.e., errors are lower than about 2% rel. For blends with a high linoleic acid content, sunflowerseed oil or corn oil are better alternatives.

It is clear that although it is advisable to minimize the difference in saturation effects between the sample and reference oil by working at a low RF level of the instrument, a compromise must be chosen to avoid the increased inaccuracy resulting from the correspondingly lower signal to noise ratio.

Additional information on relaxation times as measured by pulse-NMR can be found in (8).

ACKNOWLEDGMENT

J.C. Van der Enden, C. den Hollander and W. Dijkshoorn gave experimental assistance.

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[Received June 14, 1971]