

Determination of the Solid-Liquid Ratio of Fats by Wide-Line Nuclear Magnetic Resonance¹

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ABSTRACT

The Newport Quantity Analyzer is suitable for the determination of the solid-liquid ratio in fats provided an accurate and rapidly reacting temperature control for the sample between the magnets is used. Hydrogen content and relaxation times determine the NMR signals. The hydrogen content can be calculated from the iodine value and the saponification value. In order to obtain a reliable "liquid line" for a given sample, the NMR read out is measured at 60 C (completely molten state) and this value is multiplied by fixed factors for each temperature. With the Newport instrument, these conversion factors are still slightly dependent on the hydrogen content. Determinations of the solids content of various margarine blends, shortenings and hydrogenated oils were made. The relation between dilatometry and NMR results is temperature dependent. The correlations were especially good at low temperature, but poor at > 30 C.

INTRODUCTION

There is a growing interest in industry for the replacement of dilatometric determinations by wide line nuclear magnetic resonance (NMR) since this method is expected to give better results with less work in a shorter time. Furthermore, NMR offers greater possibilities for automation. However, its widespread use has so far been hampered by the high costs and the highly specialized knowledge required. Recently, relatively cheap, tailor made and robust instruments have been developed and investigations which had been discontinued (1) were started again. One of these instruments is the English Newport Quantity Analyzer from Newport Instruments Ltd. The aim of this investigation was not only to test this instrument, but also to develop a practical procedure for the solids content determination.

METHODS

This development is divided into two parts: determination and calculation, and tempering. The first procedure offers possibilities for an exact approach. The second

is a matter of compromise and standardization and beyond the scope of this paper. All our samples were tempered in the same way. The molten samples (60 C) were placed in crushed ice for 90 min and subsequently kept for 30 min at each measuring temperature in a waterbath, starting at 10 C in steps of 5 C upwards. An essential point is the temperature control of the sample in the instrument (2). The equipment used for this purpose was made in our laboratory. It produces an air stream at temperatures ranging between 0 and 60 C and fixed at graduating levels of 5 C (0, 5, 10, 15 C, etc.) to facilitate future handling by unskilled personnel.

The air flows through a hole in the bottom of the sample holder and keeps the temperature in the sample holder constant to within 0.2 C. The time required for a steady air temperature after a change is only about 5 min.

In addition to the standard Newport instrument we used supplementary modulation, a digital voltmeter and a readout printer.

All measurements were made in 2 ml sample tubes at a radiofrequency setting recommended by the manufacturer (60 μ A).

The NMR Signal of Liquid Oils

The equation used to calculate the magnitude of the steady-state NMR peak height can be found in handbooks. Wettström (3) derived an equation for high saturation levels for constant instrumental conditions:

$$S = K_1(N/T)(1/T_1) \quad [1]$$

in which S is the resonance signal (V/g); K_1 , constant; N, number of hydrogen protons in liquid state; T, temperature (K); T_1 , spin-lattice relaxation time (sec).

The following equation applied for low saturation levels (Proctor, private communication):

$$S = K_2(N/T)(1 - K_3 T_1 T_2) T_2 \quad [2]$$

with K_2 , K_3 as constants; T_2 , spin-spin relaxation time (sec).

Several authors (2,4,5) used the equation:

$$S = K(N/T) \quad [3]$$

Because the Newport Instrument measures peak areas, Equation 1 and 2 cannot be used; equation 3 can be used only in the absence of saturation.

N is directly related to the amount of sample and its

¹One of 10 papers to be published from the Symposium "Wide Line Nuclear Magnetic Resonance" presented at the AOCS Meeting, Minneapolis, October 1969.

TABLE I
NMR Signal (S) (V/g) of 4 Oils at 20, 40 and 60 C

	Palm oil	Coconut oil	Soybean oil	Safflower oil
IV, SV, %H	46, 210, 11.83	9, 255, 11.61	135, 192, 11.43	144, 193, 11.37
S_{20}	PC ^a	PC ^a	5.535	5.484
S_{40}	5.290	5.100	4.927	4.928
S_{60}	4.825	4.576	4.400	4.378
K_{20}	---	---	141.9	141.3
K_{40}	140.0	137.5	134.9	135.7
K_{60}	135.8	131.2	128.2	128.2
$C_{20} = S_{20}/S_{60}$	---	---	1.258	1.253
$C_{40} = S_{40}/S_{60}$	1.096	1.115	1.120	1.126

^aPartly crystallized.

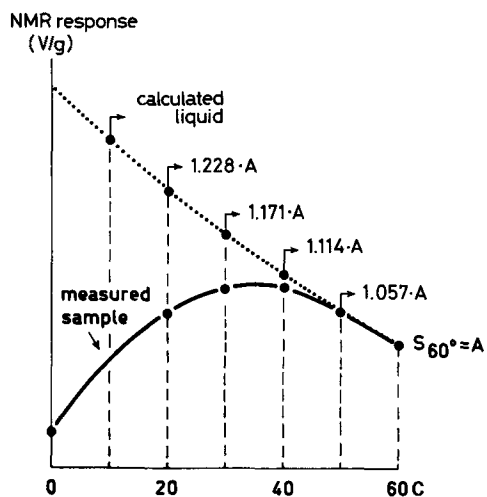


FIG. 1. Method for determination of solids content by NMR.

$$\% \text{ solid} = 100 - \left[\frac{\text{sample signal at } tC}{\text{calculated liquid signal at } tC} \right] 100$$

hydrogen content. When, therefore, the amount of sample is constant, N can be replaced by the hydrogen content of the sample. The latter can be calculated from the oil fatty acid composition (6). We used a simpler method. If the number of $-\text{CH}_2$ -groups in the acids is taken as p and the number of $\text{HC}=\text{CH}$ groups as q , the number of H-atoms per triglyceride molecule is $2p+2q+14$.

P can be calculated from the saponification value (SV), and q from the iodine value (IV). This gives:

$$\% \text{H} = 14.265 - 0.0065 \text{ IV} - 0.01017 \text{ SV} \quad [4]$$

This equation was checked by combustion analysis. Our method gave much better duplicates, but the results were about 0.10% H lower than those given by the combustion method.

Temperature Dependency of the Signal of Liquid Oils

The use of Equation 1 and 2 even if modified to apply to peak areas, is not practicable because, normally, values for the relaxation times will be missing. Equation 3 is more adequate. With slight saturation, however, deviations caused by changing relaxation times (brought about by changing viscosities), must be accepted and corrected as shown below.

According to Equation 3 the ratio of T_x/T_y (at temperatures x and y) should be used to calculate a signal at T_y if the signal at T_x is known. This, however, is only valid at constant K values which is not the case at changing T_1 and T_2 (Table I). The T_x/T_y ratio should be extended by a second factor, K_y/K_x

$$S(T_y) = S(T_x) \cdot (T_x/T_y) \cdot (K_y/K_x) \quad \text{or} \\ S(T_y) = S(T_x) \cdot C_y \quad [5]$$

The K values calculated by means of Equation 3, in which $N = \% \text{H}$, are given in Table I. Some C_y values calculated from the signals are also shown. To investigate the temperature dependency over a wider range, 25 oils and fats were tested. These included soybean, safflower, sunflower, corn, olive, cottonseed, peanut, coconut, palm kernel, palm and rapeseed and hydrogenated oils, such as whale (mp 37 C), sunflower (mp 34 C) and palm (mp 45 C). The last three were studied only in the liquid state and in mixtures with liquid oils to lower the melting point. Signals were measured at 5, 10, 15 C etc. up to 60 C.

Sunflower oil was used as reference for instrument tuning. With the potentiometer for the audio frequency amplifier, the readout was set at a given value. The Newport

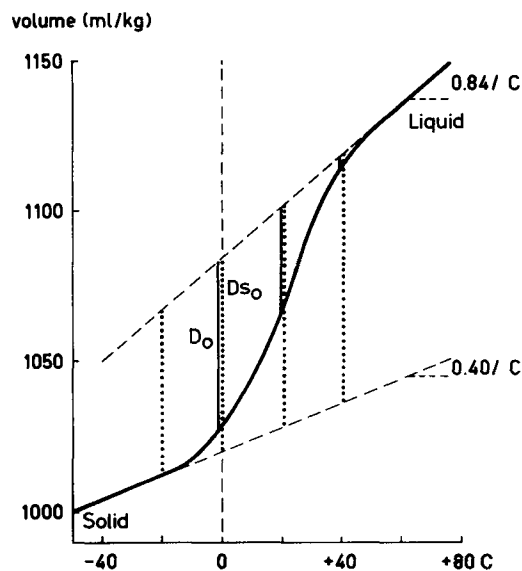


FIG. 2. Dilatometric curve showing change in volume with change in temperature.

instrument was so stable that tuning was only necessary at the first measuring temperature each morning. For each oil, the ratio between the readout at a given temperature and the readout at 60 C was calculated.

If we compare these ratios, a significant relation with the hydrogen content is obvious (also Table I). This may be expected from the dependence of relaxation time on viscosity which is here related to the proton content. This set of values was used for a computer multiple regression calculation which yielded:

$$C_t = 1 + (60 - t)(0.0308 - 0.00214\% \text{H}) \quad [6]$$

(C_t = factor by which S_{60} should be multiplied to obtain the signal at tC).

It should be borne in mind that Equation 6 depends on the magnetic conditions, e.g., saturation. Deviations from the $1/T$ law for oils have been reported earlier, but no exact correlations are given. However, the values published (1,5) relating to the Varian PA-7 point to deviations of the same magnitude.

Determination of Solids Content by NMR

A number of earlier procedures for determining the course of the liquid line recommended the use of reference oils, calibration curves, fats with known solid fat indexes, etc. We chose, however, the procedure illustrated by Figure 1, because of its simplicity and optimal precision.

The sample signal, measured at 60 C, when the fat is completely melted, is multiplied by fixed conversion factors (Equation 6) in order to obtain the liquid signals at other temperatures. The solids content is then calculated in the usual way. The advantage of this procedure is that differences in hydrogen content and viscosity are accounted for in the 60 C signal which is an individual value for each fat. Errors in sample weight are not serious, provided the same tube is used at all temperatures.

An important question is which H content should be substituted in Equation 6. For most normal margarine blends, shortenings and hydrogenated oils the H content varies between 11.5 and 12.0%. An average of 11.75% was found and used in further work (Figure 1). For soft blends with high oil contents a value of 11.50% would be better.

Apart from the liquid signal, some other sources of error could influence the results. On crystallizing, the IV of the remaining liquid will increase and hence the signal will be lower than calculated (Proctor, private communication). To

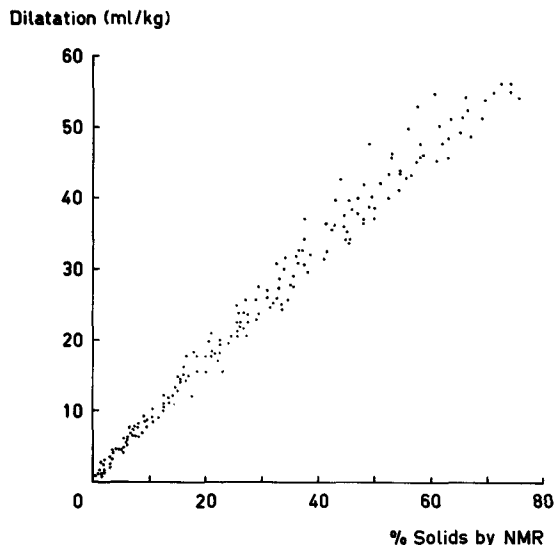


FIG. 3. Comparison of results from dilatometry and NMR for several fats and blends (all temperatures depicted).

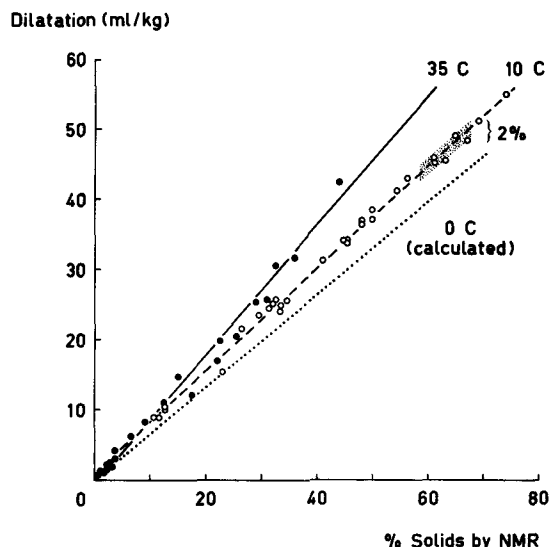


FIG. 4. Comparison of results from dilatometry and NMR for several fats and blends at 0, 10 and 35 C.

be better informed about the magnitude of this error we chose 12 different margarine blends. They were crystallized at 10 C and subsequently separated by detergent washings into the solid and liquid part. The average value for the hydrogen content of the original blends was 11.71%, whereas the oils obtained had a value of 11.66%. This drop of about 0.5% relative indicates that the liquid content obtained will also be about 0.5% too low. Depending on its amount at 10 C, the solids content will be found to be 0.2-0.5% (absolute) too high; e.g., for an American margarine at 10 C we found a value of 20.0% when using the extrapolated S_{60} blend signal, but 19.5%, when using the true oil signal.

Because of their rapid movements very small and imperfect crystals could contribute to the liquid signal. These crystals are formed by shock chilling (quenching) the fat from a high temperature to far below zero, e.g., by solid CO_2 . Occasionally we applied quick cooling and obtained lower values for the solids content and therefore abandoned this quenching technique.

The results with the NMR procedure developed and were found to be in good agreement with those obtained by the dye dilution method (Table II). This method (7), modified in our laboratory, is at the moment the most reliable procedure we have for determination of the solids content in fats.

Determination of the Solids Content by Dilatometry

The solids content can also be determined by dilatometry. The first part of a dilatation curve (Fig. 2) represents the expansion of the solids (slope 0.40 units per degree), the last part, the expansion of the liquid (slope 0.84). The drawn distances in Figure 2 are called the dilatation (D) and the dotted distances, the total melting dilatation (Ds). From the difference in slopes it can be seen that Ds will increase by about 0.44 ml/kg C. If the Ds at C

is taken as a reference we arrive at the relation:

$$\% \text{ solids (by dilatometry)} = 100 \text{ Dil} / (D_{s_0} + 0.44 t) \quad [7]$$

(Dil = dilatation at t C, t in degrees C)

For the D_{s_0} of margarine blends and shortenings the value of 65 is normally used here. Two serious errors can be made. The true melting dilatation may vary between 50 and 90, depending upon the nature of the triglycerides (blend melting point). Further, during melting, the melting point of the remaining solids will increase and hence Ds will increase more rapidly than indicated by the factor 0.44. NMR results which are not subject to these serious sources of error can be compared with those from Equation 7.

Comparison of NMR and Dilatometric Method

A number of shortenings, margarine blends and partially hydrogenated fats were studied by means of the dilatation method and that of NMR. The results are given in Figure 3 in the way they are normally presented. It is understandable that most authors feel unhappy with the poor correlation especially in the high solids region. If, however, according to Equation 7 the temperatures are also taken into account a much better picture appears (Fig. 4) for the same set of results up to 75% solids. In this figure, for the sake of clearness only, two temperatures have been chosen.

At low temperatures (≤ 30 C) good agreement is generally obtained. In the case of the 10 C line the scattering is not more than one unit on either side of the line. Less good agreement is observed at higher temperatures. The scattering around the 35 C line is about 2-3%. Especially at these temperatures, the NMR values will give a better impression of the true solids content, because the Ds from Equation 7 will become gradually less reliable at increasing temperatures.

The relation between both methods can be represented by:

$$\% \text{ solids (NMR)} = 100 \text{ Dil} / (65.9 + 0.69t) - 1 \quad [8]$$

This equation originates from a regression calculation. The fan of lines should pass through the origin, but the term -1 is too significant to neglect. Comparing Equation 7 with 8, it is clear that the Ds values of 65 and 65.9 are in good agreement, but not the factor 0.44.

TABLE II

Solids Content (%) of Margarine Fats Cooled From 60 C in Crushed Ice and Kept Overnight at 20 C by NMR and Dye Dilution Method at 20 C

Sample	NMR	Dye dilution
Dutch puff pastry	30.9	30.9
Belgian table margarine	20.2	20.4
French table margarine	8.4	7.4
German table margarine	12.1	12.9

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