⁂Comparison of the Direct and Indirect Wide-Line Nuclear Magnetic Resonance Methods for Determining Solid Fat Content

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ABSTRACT

A direct method of measuring the solid fat content by wide-line nuclear magnetic resonance (NMR) was compared with the conventional indirect wide-line NMR procedure. The direct method is based on the use of variable gate widths available on the Newport Analyser Mk IIIA. The results obtained for four different fats using the direct method did not differ significantly from those of the indirect method. As the direct method required additional measurements, was somewhat more complex theoretically and gave more variable results because of the weaker signal obtained at the wide gate, no real advantage could be found for its use.

INTRODUCTION

The determination of the solids content in fats is essential for process control and formulation in the fats and oils industry. Dilatometry is the official AOCS method for determining the solids content of fats (1) and although it is tedious and time-consuming, it does give reproducible results and works well considering its empirical nature. A number of alternative methods, e.g., differential scanning calorimetry (2), ultracentrifugation, density meter (3) and nuclear magnetic resonance (NMR) have been considered, but of these, only NMR has been implemented widely.

Many authors (4-9) have reported on the application of wide-line NMR for the determination of the solid fat content of fats. Wide-line NMR theoretically provides more accurate information on the solid-liquid ratio of fats than the empirical Solid Fat Index method. NMR is also applicable to fats which contain from 50 to 100% solids, which is beyond the limit of the official dilatometric procedure. A more recent development in applied NMR instrumentation has been the introduction of relatively inexpensive and compact pulsed NMR instruments specifically designed for solid fat determinations (10). In contrast to wide-line NMR, where a continuous, relatively small radio frequency (rf) field is applied, pulsed NMR sends out a very short and strong rf pulse to the sample. Since irradiation by means of pulses is considered to be a more efficient mode of proton excitation than continuous irradiation, the determination of solid fat content by pulsed NMR has been gaining ground. However, to date, the Instrumental Techniques Committee of the AOCS has not changed its 1972 recommendation (11) that the testing of solid fat content by wide-line NMR continue, and until a definitive statement is made, wide-line NMR appears to be favored for official status.

In 1979, Newport Instruments Ltd. introduced a wide-line NMR analyzer, the Mk IIIA, equipped with variable gate widths making the instrument more versatile. According to company literature (12), this instrument is capable of determining solid fat content in a manner similar to direct pulsed NMR. This direct method is based on the concepts of Van Putte and Van den Enden (13) who developed the direct method for pulsed NMR. As far as we are aware, no published reports have assessed the Mk IIIA Analyser for direct solid fat measurements. Therefore, the aim of this study was to evaluate the direct wide-line NMR method relative to the conventional indirect method.

THEORY

Conventional indirect NMR involves the use of a single narrow gate (1.5 gauss) which admits only proton resonances from the liquid portion of the sample. Based on the signal from the liquid portion, Haighton (6) developed a formula which enables the solid fat content to be determined indirectly by comparing the integrated signal from the fat to the signal of a reference oil held at the same temperature. The solids content can then be obtained from the following relation:

$$SFC = 100 - 100[(Rt_s)(R60_r)/(R60_s)(Rt_r)], \quad [I]$$

where: SFC = solid fat content; Rt = the reading at temperature t; R60 = the reading at 60 C; s = sample; and r = reference.

The use of a standard reference oil eliminates a number of variables and allows for interlaboratory comparisons. Olive oil (14) has often been used as a reference oil but several researchers (6,7,9) have suggested that triolein may be a more useful, universal standard for NMR work.

The direct wide-line NMR method uses the signal obtained from the narrow gate (1.5 gauss) as well as the signal from a wide (10 gauss) gate. The narrow gate admits the signal from the liquid portion while the wide gate admits the signal from the solid portion along with the signal from the liquid portion of the fat. Only a fraction of the total signal from the solid is measured at the wide gate. However, with the use of a correction factor, it is possible to estimate the solids content directly in a way which resembles the approach used in direct pulsed NMR.

The signal at the narrow gate has the following relationship to the liquid content of the sample:

$$Sn = PL, \quad [II]$$

where: Sn = the signal at the narrow gate; P = an instrument constant; and L = the liquid content.

The signal at the wide gate is defined as:

$$Sw = Q(L + S/f), \quad [III]$$

where: Sw = the signal at the wide gate; Q = an instrument constant; S = the solid content; and f = a factor which corrects for the fraction of the solid signal measured at the wide gate.

Solving for L and equating II and III,

$$Sn/P = Sw/Q - S/f \quad [IV]$$

Factoring out P and solving for S gives:

$$S = f[Sw(P/Q) - Sn]/P \quad [V]$$

Substituting in L = Sn/P and rearranging:

$$S + L = 1/P(f[Sw(P/Q) - Sn] + Sn) \quad [VI]$$

The ratio of solid (S) to the total (S + L) can be ob-

tained from the combination of Equations V and VI:

$$S/(S + L) = (f[Sw(P/Q) - Sn])/(f[Sw(P/Q) - Sn] + Sn) \quad [VII]$$

Since only the solid portion is required, Equation VII reduces to:

$$S = f[Sw(P/Q) - Sn] \quad [VIII]$$

Equation VIII is the fundamental relation upon which the direct wide-line NMR method is based.

The 'f' factor is temperature-dependent and has a value other than 1.0 when there are solids in the fat sample. The 'f' factor may be determined by measuring the oil equivalent (Oe) signal of the sample fat at the narrow and wide gates for each type of fat.

$$f = (1 - Oe_n)/(Oe_w - Oe_n), \quad [IX]$$

where:

$$Oe_n = (Sn/W)/(Sn_o/W_o) \times HR, \quad [X]$$

and

$$Oe_w = (Sw/W)/(Sw_o/W_o) \times HR, \quad [XI]$$

where: Sn = the signal of the fat sample at 1.5 gauss at t C; Sw = the signal of the fat sample at 10 gauss at t C; Sn_o = the signal of the reference oil at 1.5 gauss at t C; Sw_o = the signal of the reference oil at 10 gauss at t C; W = the weight of the sample (g); W_o = the weight of the reference oil (g); HR = the hydrogen ratio, i.e., the ratio of the weight percent of hydrogen in the reference oil to the weight percent hydrogen in the fat.

HR, the hydrogen ratio, is dependent on the chemistry of the fat and can be determined by comparing the signal of the sample per unit weight to the reference oil at a temperature at which both are completely melted.

$$HR = (Sn_r\ 60)/(Sn_s\ 60), \quad [XII]$$

where: Sn_r 60 = signal/g at the narrow gate for the reference oil; Sn_s 60 = signal/g at the narrow gate for the sample.

For most normal margarine blends, shortenings and hydrogenated oils, the hydrogen content varies between 11.5 and 12.0% with an average value of 11.76% having been reported (5). If triolein, with a hydrogen content of 11.8% is used as a reference oil, the HR term reduces to a value close to 1.0 when constant weights are used.

P and Q are instrument constants which can be estimated from the ratio of the two gate-widths or measured by finding the ratio of the signal from the reference oil at the narrow gate to the signal from that same reference oil at the wide gate.

$$P/Q = Sn_o/Sw_o \quad [XIII]$$

When using a reference oil, such as triolein which is liquid over the temperature range of interest, the P/Q value will be ca. 6.66 for a standard 1.7-g sample.

MATERIALS AND METHODS

The instrument used in this study was the Newport Analyser Mk IIIA equipped with a liquid CO_2 sample temperature controller (Type WR Mk II) for the magnet and sample compartment. For sample temperature equilibration, special aluminum blocks were used to keep the sample tubes dry and maximize heat transfer in eight preset constant temperature baths.

NMR measurements were made on four types of fat at eight selected temperatures (0, 5, 10, 15, 20, 25, 40 and 60 C). Triplicate samples of deodorized lard, vegetable shortening, margarine oil and a hydrogenated soya oil were analyzed. The experimental design (15) consisted of different complete factorial arrangements of the following factors: method of determining solid fat content, type of fat and temperature. All tests of statistical significance were made at the 5% level.

Prior to the analyses, the rf current for ideal resonant behavior was determined at the selected temperatures using relaxed water (0.012 M $MnCl_2$) and the reference oil triolein. 1.7 g of completely molten sample or triolein was accurately weighed into a previously tared 2-mL glass sample tube, the tube stoppered and the samples then put through the following temperature regimen: (a) holding at 0 C for 15 min; (b) tempering at 25 C for 30 min followed by holding at 0 C for 15 min; (c) stepwise transfer of the sample tubes to constant temperature baths and holding the samples for a minimum of 30 min to allow for temperature equilibrium before taking readings.

This regimen, which includes tempering, was based on the experience and suggestions of previous workers (7,10, 14).

After temperature equilibration, the sample tubes were wiped dry and readings were taken immediately. Three consecutive measurements were made for each sample and averaged. After each determination, the sample tube was returned to the same water bath until all the samples had been measured, and then transferred as a group to the next temperature bath. The solids content was calculated via Equation VIII, using values of 1.0 and 6.66 for HR and P/Q, respectively.

RESULTS AND DISCUSSION

The mean solid fat contents obtained by the direct and indirect methods for the four fats investigated are tabulated in Table I. For margarine oil, an anomalous increase in solids content was observed at 10 C which may have been caused by too short of a preconditioning period at 0 C (16).

Analysis of variance (ANOVA) was performed on the accumulated data to determine whether the solid fat con-

TABLE I

Mean[a] Solid Fat Contents Obtained via the Direct and Indirect Methods

Sample	Method	Solids (%)					
		5°	10°	15°	20°	25°	40°
Margarine	Direct	13.80	15.15	12.16	9.64	7.02	0.41
	Indirect	14.02	15.33	12.38	9.86	7.80	0.31
Shortening	Direct	29.07	27.83	22.19	19.82	18.41	9.03
	Indirect	29.23	27.99	22.36	19.99	18.62	9.29
Soya	Direct	53.97	48.01	35.91	26.92	19.95	0.00
	Indirect	54.13	48.17	36.09	27.09	20.17	0.00
Lard	Direct	35.11	33.30	27.54	24.06	19.08	1.12
	Indirect	35.27	33.46	27.70	24.23	19.28	1.10

[a]Three replicates. Standard error: direct method, 0.69; indirect method, 0.51.

tent values obtained by the two methods differed significantly. The ANOVA indicated no significant difference between the results of the two methods. Slightly better precision was obtained using the indirect method which had a standard error of 0.51 whereas the standard error for the direct method was 0.69.

The direct wide-line NMR method is analogous to the direct pulsed method described by Van Putte and Van den Enden (13) in that it measures a signal from the hydrogen in the solid fraction of the fat as well as a signal from the hydrogen in the liquid portion. The 'f' factor serves differing purposes in each method. In the case of pulsed NMR, it compensates for the decay of the solid signal during the dead time of the NMR receiver, whereas in the case of wide-line NMR, it compensates for the fact that only a fraction of the broad 30-40-gauss solid resonances pass through the 10-gauss gate. Furthermore, it is not possible to ignore the temperature dependence of the 'f' factor in wide-line NMR, whereas in pulsed NMR it can be considered a constant (13). This dependence is due to the change in total width of the resonance range of the solid fraction, which changes with temperature. A further difference between pulsed and wide-line direct methods is that with the Mk IIIA Analyser, the instrument's sensitivity is inversely proportional to the gate width used. This is compensated for by including the P/Q factor in the direct method calculations, but the change in sensitivity affects the precision with which the signal at the wide gate can be measured. For these reasons, any method which involves the use of the 10-gauss gate is inherently less accurate, and even though additional information is being gathered, the overall precision is reduced.

The objective of Newport Instruments Ltd in developing and suggesting the direct method was to demonstrate that the Mk IIIA Analyser was capable of making measurements analagous to the direct pulsed NMR method. The results obtained in this study indicate that the direct and indirect wide-line methods do not differ significantly, but that the direct method is somewhat less precise than the indirect method. The effect on the results of the less precise signal from the wide gate did not seem as important as anticipated, although this could be due to the relatively ideal operating conditions used in this study. The direct method was also found to produce reasonable, albeit more variable, results when no constants were used in the calculations (i.e., P/Q and HR were calculated from the instrument signals). A programmable calculator would be required to calculate the solids content in this manner on a routine basis.

Overall, the use of the direct method did not lead to an increase in accuracy nor did it speed up the method. Wide-line NMR can be used to obtain the solid fat content by the direct method, although there seems to be little practical reason for doing so.

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REFERENCES

1. "Official and Tentative Methods of the American Oil Chemists' Society," 3rd Edition, AOCS, Champaign, IL, 1973, Method Cd 10-57.
2. Walker, R.C., and W.A. Bosin, JAOCS 48:50 (1971).
3. Mills, B.L., and F.R. van de Voort, Ibid. (in press).
4. Ferren, W.P., and R.E. Morse, Food Technol. 8:112 (1963).
5. Haighton, A.J., L.F. Vermaas and C. den Hollander, JAOCS 48:7 (1971).
6. Haighton, A.J., K. Van Putte and L.F. Vermaas, Ibid. 49:153 (1972).
7. Mansfield, P.B., Ibid. 48:4 (1971).
8. Wettstrom, R.B., Ibid. 48:15 (1971).
9. Swindells, C.E., and P.A. Ferguson, Can. Inst. Food Sci. Technol. J. 5:82 (1972).
10. Madison, B.L., and R.C. Hill, JAOCS 48:4 (1978).
11. "Report of the Instrumental Techniques Committee," American Oil Chemists' Society, Champaign, IL, Ibid. 49:431A (1972).
12. "The Direct Measurement of Solids Content of Fats Using Continuous Wave Nuclear Magnetic Resonance," Newport Instruments Ltd., Buckinghamshire, England, 1979.
13. Van Putte, K., and J. Van den Enden, JAOCS 51:316 (1974).
14. Mertens, W.G., and J.M. de Man, Can. Inst. Food Sci. Technol. J. 5:77 (1972).
15. Steel, R.G., and J.H. Torrie, "Principles and Procedures of Statistics," McGraw-Hill Co., New York, NY, 1960.
16. Pohle, W.D., and R.L. Gregory, JAOCS 44:397 (1967).

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