A Nuclear Magnetic Resonance Study of the Liquid / Solid Content of Margarine Fat

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THE CONSISTENCY of margarine and other commercial fats depends to a large extent on the relative amounts of liquid and solid fats present. The usual method for determining these values is by dilatation measurements. Indeed the dilatometer is used fairly considerably for this work. There are however certain inherent errors in this type of measurement. Craig *et al.* (1) state that "the calculation of solid and liquid content from dilatometric data is impossible in complex mixtures of glycerides" while Bailey (2) stated that "the calculation of solid/liquid ratios from dilatometric curves of natural fats is not possible with any degree of accuracy." These statements arise from the fact that a prior knowledge of the glycerides present is required, and mathematical adjustment is needed to take care of the different melting dilatations. Such knowledge is clearly not always available.

Since this is the case, the question arises as to whether some other simple method would give better results. The low resolution nuclear magnetic resonance (n.m.r.) spectrometer appears to provide a solution to the problem.

The magnetic field strength at the center of a hydrogen resonance line always has the same value for a given frequency, but the shape of the line is influenced by the chemical and physical state of the sample. The width of the absorption line is related to the mobility of the hydrogen in the sample or the mobility of the compound containing the hydrogen and to the field homogeneity. The effective magnetic field strength at a nucleus is the sum of the applied magnetic field plus the field contributed by neighboring nuclei. In a solid where the nuclei are fixed rather rigidly in position, any given nucleus may find itself in a total magnetic field significantly lower or higher than the applied field. Since the nuclei will pass through magnetic resonance at widely different values of the applied field, the line width will be comparatively broad (of the order of several gauss). In a liquid in which all the molecules are in a state of violent thermal agitation, the fields contributed by hydrogen nuclei at any given point are averaged out very rapidly compared with the period of precession. As a result of this, the absorption line is very narrow, and the line width is determined principally by the inhomogeneity of the applied magnetic field. This inherent disparity in line widths between solids and liquids (Figure 1) appears to lend itself to a method for determining the liquid/solid content of fats. The derivative of the curve is shown in Figure 1b. A preliminary notice of the work has been published recently (3).

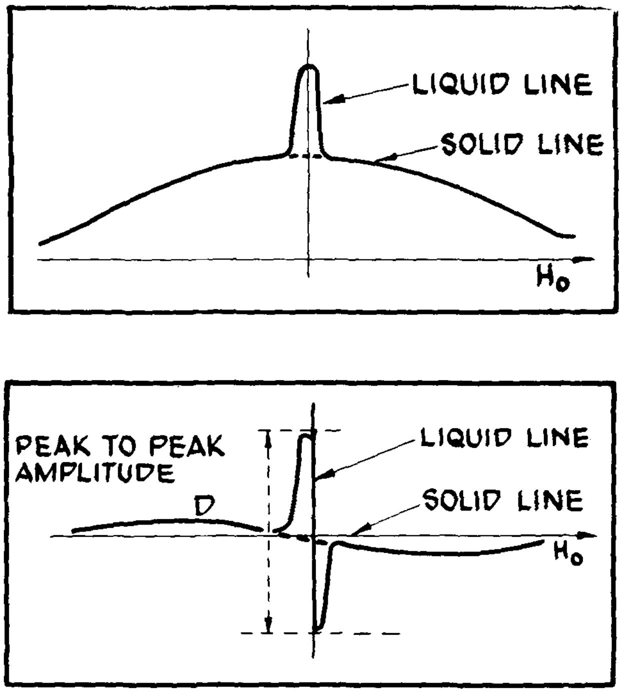

FIG. 1a. The absorption curve arising from liquid and solid materials; 1b, the derivative curve.

Experimental

A bridged-T n.m.r. spectrometer was used (4, 5). The glass sample tube is inserted into the R. F. coil, which is part of the resonant circuit. The magnetic field at the sample is swept linearly with time, across the nuclear resonance value, by means of a small current applied to the sweep coils by the sweep generator. The derivative curve presentation is obtained by using a magnetic field modulation much smaller than the line width. The temperature of the sample is varied by placing the holder in a Dewar vessel containing either ice/water or dry ice/acetone, the temperature of which is measured by a pentane thermometer.

The samples examined consisted of two samples of pure glycerides, tristearin (m.p. 72.7) and triolein (5.0°C.) and mixtures thereof, and a sample of margarine fat. The margarine fat had been previously examined by dilatometric measurements (2) after two different thermal treatments (by Messrs. Oakley and Lavery of the Unilever Research Laboratory, Port Sunlight) over a range of temperatures extending below the temperature of formation of 100% solid, through the intermediate temperatures, and over a range of temperatures above the temperature of formation of 100% liquid. The liquid/solid content was deduced from a graphical plot of these results. The liquid content of the fat is plotted in Figure 2.

The n.m.r. measurements were made on the margarine fat after identical thermal treatments.

Thermal Treatment

Stabilized Sample. The fat was melted at 60°C., and the liquid was mixed thoroughly. The liquid fat was cooled slowly to room temperature and kept at

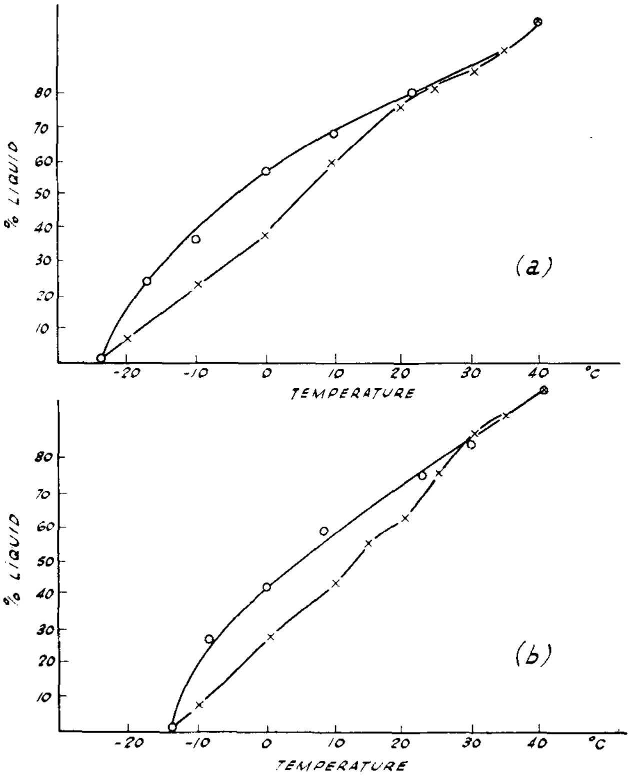

FIG. 2. The variation of the liquid/solid content of margarine with temperature by dilatometry and by n.m.r. spectroscopy, a) with stabilized fat and b) with unstabilized fat.

x—line values deduced by dilatometry.

o—line values deduced by n.m.r.

this temperature (about 20°C.) for three days. It was next kept at −5° for 7½ hrs. (using salt/ice), at −15°C. for 16 hrs. (using ice/$CaCl_2$), and at −50°C. for 2 hrs. (using dry ice and alcohol).

Unstabilized Sample. The fat was melted at 60°C., then chilled for 1½ hrs. in ice/water and for ¾ hr. at −50°C. (dry ice/alcohol).

Results and Discussion

Many experiments were carried out to determine the best conditions for determining the liquid content of the fat blend at various temperatures. The original idea of measuring the area of the broad band of the solid and the narrow line of the liquid simultaneously was found to be untenable although interesting information was obtained, which is given in the Appendix.

The magnetic field was modulated at a sinusoidal frequency of about 40 c/s with a magnitude of about 30 milligauss peak to peak. The line width was determined by the magnetic field inhomogeneity and was about 100 milligauss at half height. No signal due to the broad band of the solid is detectable, and a measurement of the peak-to-peak height D (Figure 1b) is a direct measure of the amount of liquid present.

Three samples of mixtures of pure tristearin and triolein were first examined. These contained prepared amounts of liquid/solid content. Published data (1) with other methods suggests that the tristearin does not form a solid solution in the triolein phase.

The method adopted was as follows. A sample of pure triolein was placed in the coil, and the curve was traced six times. Without alteration of any of the experimental conditions the tube was replaced by an identical tube containing each of the known mixtures. Six runs were made with this sample after the bridge had been rebalanced. The triolein was again inserted, and the procedure was repeated four or five times. The peak-to-peak heights of the liquid line were measured and averaged. By comparison of the peak height of the pure triolein with that of the mixtures the percentage of liquid in the mixture could be calculated. The results are shown in Table I; they were accurate to within 3%.

These conditions on the apparatus were used to investigate the liquid content of the margarine sample. A sample of the margarine fat was melted and placed in the apparatus. The length of the peak-to-peak height was determined. The stabilized margarine was next inserted, and the procedure was repeated. The liquid content at other temperatures was calculated from the alteration of the peak-to-peak height. The results are plotted in Figure 2a and can be compared with the dilatometric data obtained on the same fat. Determinations on other days agreed well with these results.

The unstabilized margarine was examined in an identical manner, and the results are plotted in Figure 2b and can be compared with the dilatometric data.

It is of particular interest to compare the dilatometric data with the n.m.r. determination. The results agree well at room temperature, also at the point of solidification. At the intermediate temperatures however differences of some 10 to 20% liquid content occur. Since the n.m.r. determination is independent of the composition and the polymorphic forms of the solid material present, the results by this method are regarded as more nearly correct. It is of interest to note that both methods indicate that the liquid content of the stabilized margarine is higher at any particular temperature than the unstabilized margarine. Further work is required to study the relationship between dilatation measurement and liquid/solid content, using mixtures of pure glycerides in order to clarify the difference observed.

Conclusions

1. The low resolution n.m.r. spectrometer provides a new technique for the determination of the liquid/solid content of fats.
2. It has many advantages over the usual dilatometric method. Fats may be examined in the state in which they are received, *i.e.*, without altering the structure by melting and crystallization as with the dilatometric method. The results obtained are independent of the composition of the fat; no prior knowledge of the glycerides present is required. The results obtained are independent of the polymorphic form or forms of the solid present. It requires little preliminary work, such as weighing, sample preparation, etc., and is rapid. Determination of tristearin/triolein mixtures gives good agreement with expected results. The n.m.r. results and dilatometric results on margarine fat show divergences at temperatures intermediate between the temperature near 100% solid and room temperature. Future work will be carried out on other simple mixed glyceride systems.

Appendix

The original idea of measuring and comparing the area of the narrow line of the liquid and the broad line of the solid was found to be not possible, and conditions on the apparatus were altered so as to determine the liquid content only. Interesting information was obtained however in these preliminary experiments. These are now described.

Stabilized Margarine. At room temperatures, using a modulation amplitude of about 0.13 gauss, a derivative spectrum was obtained with a fine structure (Figure 3a). When resonance was swept through more slowly, the fine structure was very marked even though the whole signal was very narrow. The line width (AB) of the broader line was only 0.9 gauss. The narrower line arises from the liquid. The broader component arises from molecules in the solid state. Since the line width AB is so narrow, the molecules must be undergoing various forms of motion quite freely. It is interesting that a comparable line was obtained from the α_L-polymorphic forms of 1-oleodistearin, 1-oleodipalmitin, and 2-oleodistearin (6). With these compounds the line width was about 0.8 gauss. It is reasonable therefore to attribute the broader component to the presence of glycerides in the margarine fat blend with an unsaturated chain and probably in the α_L-form.

Below −24°C. the curve corresponding to the liquid disappeared. On increasing the modulation amplitude (to about 0.5 or 0.6 gauss) and increasing the amplifier gain, a much broader curve was obtained, *e.g.,* at −28°C. with a modulation of 0.5 gauss it had the shape shown in Figure 3b. The line width of the broader line was 7.6 gauss. This value is intermediate between the line widths of the α_L-forms and of the higher melting forms of most of the glycerides studied at room temperature (6). At −35°C. the line width had increased to 8.2 gauss, and it remained at this value right down to −78°C. (the lowest temperature recorded with the dry ice/acetone coolant). The broad curve was still seen on raising the temperature above −28°C., but the gain had to be greatly increased and at about 8°C. it was just disappearing into the noise level. This broader curve must arise from molecules in the solid form which are not in such free motion as those giving the narrower curve, and the decrease in line width with increase in temperature indicates the gradual increase of motion of these molecules.

With decrease in temperature the narrow curve increased very slightly in area as would be expected as more molecules passed from the liquid state into this state. Also measurements below the solidification point showed that the line became broader, therefore having a greater second moment. At −29°C. the line width had increased to 1.0 gauss and at −47°C. to 1.1 gauss; at −57°C. it was 1.5 gauss. Further decrease in temperature caused the narrow line to disappear at −64°C., and below this temperature only the broad line was obtained. Thus decrease in temperature gradually caused more molecules to pass from a highly mobile state into a more restricted state, also the remaining molecules themselves in the mobile state gradually become more restricted, giving broader lines. The line of about the same width as that obtained with the α_L-form of 1-oleodistearin was still present at −20°C. (6).

At−78°C. six runs on the fat gave an average line width of 8.2 ± 0.3 gauss and a second moment at 17.6 ± 1.1 gauss^2. This may be compared with the runs carried out on 1-oleodistearin with the β'_L and α_L polymorphic forms at −78°C. The β'_L modification gave an average line width of 13.4 gauss and a second moment of 20.4 gauss^2 while the α_L-form gave a line width of 10.5 gauss and a second moment of 17.8 gauss^2. When the fat sample was cooled to 90°K by using liquid oxygen as the coolant, the average line width from six traces was 13.9 ± 0.3 gauss, and the second moment was 24.7 ± 0.7 gauss^2. Cooling to the lower temperature has therefore further restricted the motion of the molecules as was found with the pure glycerides. All the pure compounds (glycerides) studied at 90°K and 20°K gave second moments within the range 24.3–25.2 gauss^2 (6). The molecules are therefore probably effectively stationary at 90°K. This may be compared with results by Andrew (7) on pure long-chain hydrocarbons.

On one occasion only an interesting effect was noticed on cooling the sample. At −44°C. there was slight splitting of the narrow curve (Figure 3c). At

TABLE I

Known percentage of liquid	Average percentage found
75.1	74.0
50.2	51.2
25.1	25.4

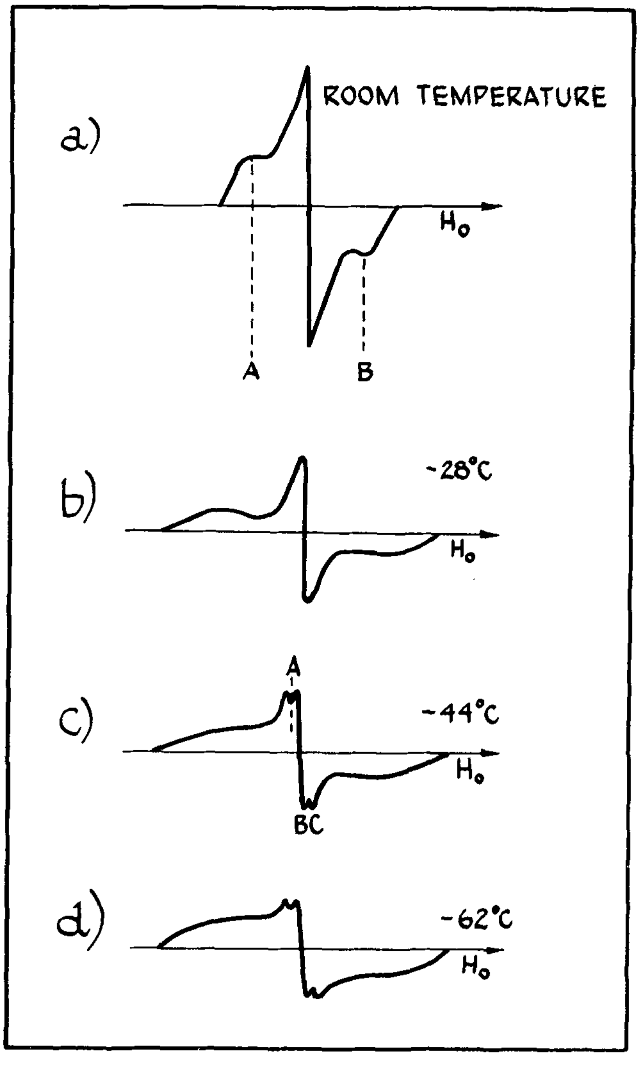

FIG. 3. The derivative spectra of margarine fat at different temperatures.

−50°C. this splitting was even more pronounced; AB was about 0.9 gauss and AC was about 1.6 gauss. At −62°C. the splitting was very sharply defined as in Figure 3d. Thus the sample has set so that some of the molecules which possessed a very free form of motion are now very slightly restricted.

Unstabilized Margarine Sample. This showed similar effects to the stabilized margarine except that from −78°C. upward the general molecular motion was not so marked. Thus at −78°C. the unstabilized sample gave a broader line. The second moment was 19.1 ± 1.4 gauss^2, and the line width was 8.4 ± 0.4 gauss. Also the narrow line disappeared at a higher temperature, *i.e.*, at about −55°C. At −49°C. its line width was 1.9 gauss, at −14°C. this had decreased to 1.2 gauss, and at 19.5°C. it was 1.0 gauss so that the line width was at all temperatures broader than with the stabilized sample. (The curve corresponding to the liquid disappeared at −14°C.)

The broad line showed about the same line width variations as with the stabilized sample. In agreement with the above conclusions of less molecular motion in the sample the line did not sink into the general noise level until temperatures above 11°C. were employed.

The measurements at 90°K removed any differentiation between the two samples since the second moment was 24.7 ± 0.4 gauss^2 and the line width was 14.2 ± 0.2 gauss.

Acknowledgment

We wish to thank H. B. Oakley and H. Lavery for the dilatometric measurements and the directors of Unilever Ltd. for permission to publish the information given in this paper.

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Report of the Smalley Committee, 1959-1960

HEREWITH is presented the 42nd annual report of the Smalley Committee. Again this season nine different types of samples were distributed by seven subcommittees. These were cottonseed, soybeans, peanuts, meal, vegetable oils, tallow and grease, glycerine, drying oils, and edible fats. In all, 4,408 samples were distributed to 506 collaborators, and about 15,000 results were tabulated. Table 1 shows the distribution and participation. There was a slight increase in participation of about 3% this season.

As of March 21, 1960, the Smalley account on the A.O.C.S. books showed $7,249.00 in receipts and $6,992.67 in expense, leaving a net of $256.33. This net will be decreased by at least $150 by charges yet to be received. A new mixer was purchased for preparation of the cottonseed samples at a cost of $382.50. A detailed accounting has been given to the Governing Board, and a final report to the collaborators, summarizing the work and listing the relative standing, based on our various grading systems.

It is fitting to express thanks to various subcommittee members for their contributions this past season:

K. H. Fink, Armour and Company, for tabulating and mailing the tallow and grease results and calculating the final grades; he also assisted with the edible fat tabulations

J. F. Anodide, Lever Brothers Company, for tabulating and mailing the soybean oil results

J. P. Hughes, Southern Regional Research Laboratory, for tabulating and mailing the cottonseed oil results

R. A. Decker, Armour and Company, for calculating the final grades on the vegetable oils

E. H. Tenent Jr., Woodson-Tenent Laboratories, for preparing and shipping the vegetable oils and tallow and grease samples

S. J. Rini, HumKo Company, for selecting the bulk vegetable oil

R. B. Jones, Darling and Company, for selecting and shipping the bulk tallow and grease samples

G. Conner Henry, Law and Company, for handling the preparation of the cottonseed, meal, and peanut samples

J. L. Hale, Swift and Company, for the preparation and distribution of the edible fat samples

Bart Teasdale, Canada Packers Ltd., for remailing the vegetable oil and the tallow and grease samples in Canada

George Reid, Spencer Kellogg and Sons, for the preparation and mailing of the drying oil samples.

TABLE 1

	Number of collaborators	Number of samples	No. of determinations per sample
Cottonseed	42	10	6
Soybeans	38	10	2
Peanuts	11	7	5
Meal	143	15	3
Vegetable oils	82	6	3
Tallow and grease	83	5	7
Glycerine	24	5	5-3
Drying oils	15	6	5
Edible fats	53	5	14

Reasonably sound grading systems have been established in all the series, and certificates of proficiency have been presented this year in all categories.

Cottonseed. With 42 chemists participating, first place was a tie between Ben C. White, Barrow-Agee Laboratories, Shreveport, La., and Paul D. Cretien, Texas Testing Laboratory, Dallas, Tex., with grades of 99.70%. Honorable mention was given to W. N. C. Kesler, Woodson-Tenent Laboratory, Little Rock, Ark., with 98.26%.

Soybean. First place among 38 chemists was a three-way tie, all with grades of 100%, for B. D. Brock, Barrow-Agee Laboratory, Greenwood, Miss.; W. D. Wadlington, Woodson-Tenent Laboratory, Chicago, Ill.; and J. G. Bowling, Woodson-Tenent Laboratory, Des Moines, Ia. Honorable mention was given to E. H. Tenent Jr., Ben C. White, Robert H. Hein, W. J. Johnson, T. C. Law, and R. A. Preckschat, with grades of 99.40%.

Peanut. Of the 11 chemists participating in this series first place was won by Mr. Cretien with 99.76%; second place by W. C. Dean, Dothan Oil Mill, Dothan, Ala., with 99.68%; and honorable mention by P. C. Whittier, Law and Company, Wilmington, N.C., with 98.92%.

Tallow and Grease. There were 83 collaborators, of whom Harry Gebel, Swift and Company, Hammond, Ind., won first place with a grade of 100%; D. S. Brake, General Testing Laboratory, Vancouver, B.C., second place with 99.36%; and E. R. Hahn, Hahn Laboratories, Columbia, S.C., honorable mention.

Vegetable Oils. With 82 collaborators participating, the first place certificate was given to P. L. Phillips, Barrow-