Measurement of Solids in Triglycerides Using Nuclear Magnetic Resonance Spectroscopy

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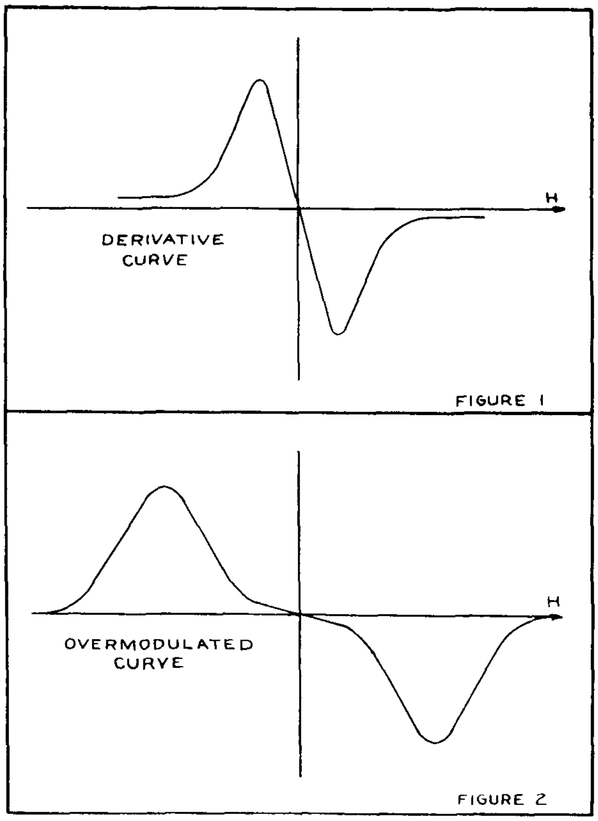
Abstract

A rapid and accurate method using nuclear magnetic resonance (NMR) spectroscopy is presented for determining the percentage of solids in fats and shortenings conditioned at selected temperatures or as received in the laboratory. This method provides more reliable information on the solids content of fatty materials than the empirical dilatometric solid fat index, and is applicable in the range of 50–100% solids which is beyond the limit of the official solid fat index method.

The relationship between instrument response and actual solids present was determined on known mixtures of liquid and solid triglycerides. NMR and solid fat index (SFI) measurements were made on a series of commercial margarine oils of varying composition and consistency. Comparisons are presented giving the precision of the two techniques and the relationship between percentage of solids by the NMR technique and the solids fat index.

Introduction

THE CONSISTENCY of shortening and margarine is closely related to the liquid and solid glyceride content. An index of solid glyceride content obtained by using the AOCS Official Method for Solid Fat Index (3) is frequently used to predict the consistency properties of blends of hard and soft margarine oils and shortenings. The Official SFI method requires 4–5 hr for completion and is not applicable to samples which have an SFI above 50. These two factors limit its use for control and research purposes. A more rapid method and broader coverage of solids content is desirable for both research and control.



FIGS. 1 and 2. Normal derivative curve and an over-modulated curve.

NMR of low resolution has been shown by Chapman et al. (1) to measure the liquid/solid ratio in samples of known composition. The NMR measurements indicate the liquid/solid fat ratio and the amount of liquid and solid glycerides actually present in the sample.

Experimental

Conditions for Operating NMR Spectrometer. A low resolution model 104 NMR Spectrometer with a model 1042 Integrator by Schlumberger Well Surveying Corp. was used. The instrument parameters were:

Sweep time	1 min
Sweep amplitude	1 gauss
Modulation amplitude	0.5 gauss

The derivative curve of the NMR absorption curve was over-modulated to compensate for field inhomogeneity and temperature effects on the spin-spin relaxation time. This was done by increasing the peak-to-peak amplitude of the modulation to several times the normal line width of the resonance absorption line. After this change the normal derivative curve no longer resembles Figure 1, but becomes distorted as in Figure 2. This was to allow the use of the integrator for determining the area under the curve, Figure 2. This is a more accurate measurement in these experiments than measuring the peak-to-peak height as in Figure 1.

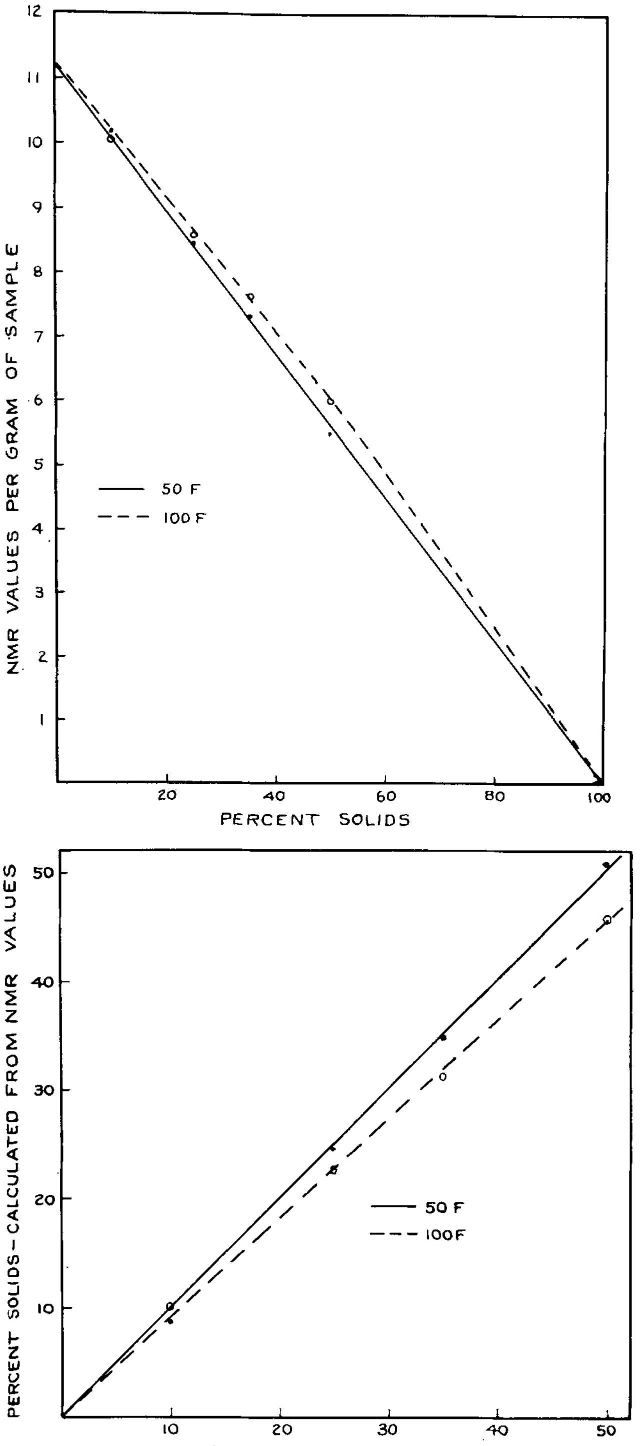
Thermal Treatment of Samples. Five NMR tubes containing ca. 4–7 ± 0.01 g of sample were prepared for each sample. The samples in the NMR tubes were then melted at 140F and placed in a 32F bath for 10 min. Then one tube was placed in each of the five SFI baths at 50,70,80,92 and 100F for 30 min.

NMR Measurements. Each sample tube was placed in the RF coil of an electrically resonant circuit in the gap of the magnet. The RF coil was tuned to give maximum RF level and the sweep cycle was started. At the completion of the cycle the integrator automatically records, directly in millivolts on the strip-chart recorder, the area under the recorded curve. All NMR spectrometer readings reported in this paper are the areas under the respective curves

TABLE I

NMR Values (mv/g of sample) on samples of known composition

°F	% Solids added					
	0	10	25	35	50	100
50	11.16	10.17	8.41	7.32	5.54	0.03
70	11.25	10.12	8.28	7.48	5.63	0.02
80	11.02	10.07	8.37	7.50	5.80	0.24
92	10.86	10.05	8.42	7.60	5.83	0.04
100	10.85	10.02	8.51	7.66	6.07	0.40

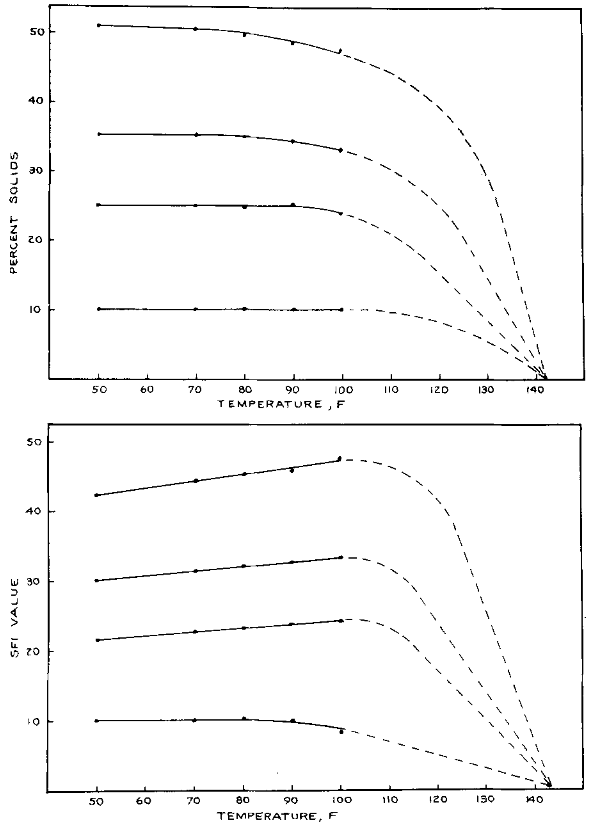


FIGS. 3 and 4. The relationship between NMR values at 50 and 100F and solids content by weight and between solids calculated from NMR values at 50 and 100F and solids content by weight.

calculated to a per gram basis at a setting of x 100 sensitivity. This signal, mv/g of sample on a x 100 sensitivity, will be referred to hereafter as the NMR value.

Consistency Measurements. The consistency measurements were made on the samples after conditioning 18 hr at the selected temp using the shortening consistometer described by Clardy et al. (2).

Measurements on Known Mixtures. NMR spectrometer and SFI measurements were made on known mixtures of liquid (safflower oil) and solid (62–64 titer hardened soybean oil) triglycerides at 50,70,80,



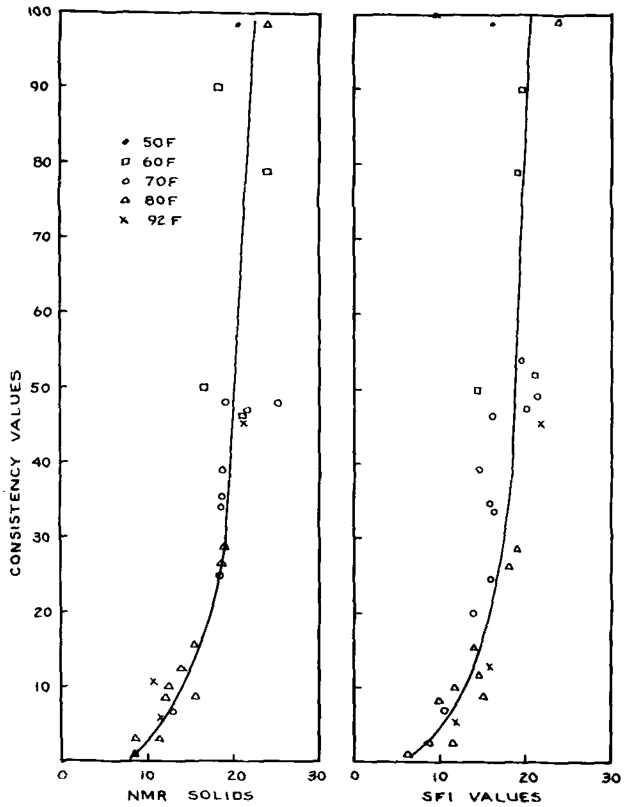
FIGS. 5 and 6. Solids content of blends of liquid and solid glycerides measured by NMR and SFI at temp between 50 and 100F.

92 and 100F. The precision of the NMR spectrometer measurements as expressed by the standard deviation was 0.1 which is equivalent to 0.9% solids. The NMR values show in Table I. If a linear relationship is assumed it will be noted that the measurements at 50F agree with this assumption, but those at 100F indicate a deviation from linearity increasing to a maximum at 50% solids by weight (Fig. 3). The solids content at 50F and 100F of the known samples predicted from the NMR spectrometer readings (Table I) using the linear relationship are shown graphically in Figure 4. The lower solids content obtained with increasing temp is assumed to be solution of a portion of the solid glycerides in the liquid glycerides. If the temp were increased to 144F the system would be completely liquid. The change of solids with temp including the probable change between 100 and 144F is shown graphically in Figure 5.

The data collected on known blends of solid and liquid glycerides indicate that the best way to express NMR spectrometer measurements is in terms of percentage of solids as read from the linear relationship in Figure 3. Subsequent NMR spectrometer measurements on fats, oils, and shortenings will be reported as percentage of solids on this basis.

The known blends of solid and liquid glycerides were tested by the Official AOCS Solid Fat Index, Method Cd 10-57 (3).

The change of SFI with temp including the probable change between 100 and 144F is shown in Figure 6. These data indicate that for a given blend of solid and liquid glycerides, 25–50% solids, the SFI value increases with increased temp up to 100F. This apparent increase in solid glycerides is not likely and is due, no doubt, to the solid specific volume increasing more slowly with temp than the liquid specific volume.



FIGS. 7 and 8. Relationship between consistency and % solids measured by NMR and between consistency and SFI values.

In comparing the NMR spectrometer measurements on known blends of liquid and solid glycerides with those by the SFI method, it appears that the NMR spectrometer will give a truer indication of the solid fat content and is applicable over the entire range of blends, whereas the SFI is limited to samples with an SFI value of 50 or less (ca. 50% solids). The precision of these two methods as expressed by the SD is 0.9% solids absolute by the NMR technique and 0.2 SFI value at a level of 6 and 0.9 SFI value at a level of 30. The SFI appears more precise at the lower levels but at higher levels the precision appears similar. The NMR procedure is preferable for investigational purposes because of its wider range and greater accuracy in indicating the actual solids content.

Measurements on Commercial Products. Eleven different blends of oils for margarine were tested for solids content at selected temp by the NMR and SFI methods. The margarines made from these blends were evaluated for consistency at 50,60,70,80 and 92F. These data show in Table II.

One of the main purposes of estimating the solids content of fats, processed oils, and blends is to predict the consistency of the finished product. It is recognized that the consistency of the finished product can, in some cases, be varied widely by processing and tempering; but if the conditions leading up to the consistency measurements are constant, the solids content of the product is a determining factor in the consistency of the product or sample. The percentage of solids determined by NMR and SFI values were plotted against the consistency of the margarine, determined at the same temp, Figures 7,8. These graphs clearly show the marked effects of small changes in solids content on consistency of the finished product. It also indicates that the consistency may be estimated at selected temp from the percentage of solids determined by NMR (less precisely from SFI) if standard procedures are followed in conditioning the samples for the NMR test and for measuring consistency. Once the relationship has been established the consistency of a sample could be determined more easily and in much less time from an NMR solids determination than by the usual methods for consistency. This procedure is rapid enough to be used for control at certain points in the process where a period of 40–60 min can be allowed for the test.

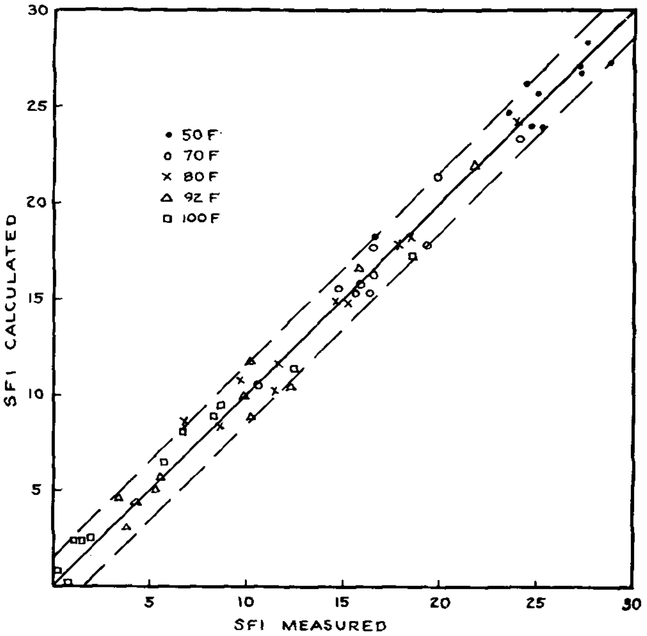


FIG. 9. SFI measured versus SFI calculated from the individual regression lines for each temperature.

The relationship between % solids by NMR and SFI values has been calculated for each temp at which measurements were made: 50,70,80,92 and 100F, and for the over-all relationship. The regression equations follow:

TABLE II

Sample	Margarine Oil						Margarine Oil						Margarine				
	NMR % Solids at °F						SFI Value at °F						Consistency at °F				
	50	60 [a]	70	80	92	100	50	60 [a]	70	80	92	100	50	60	70	80	92
1	33.5	25.8	18.2	11.9	5.6	3.2	27.3	21.5	15.7	11.8	5.2	1.2	205	129	35	10	[c]
2	33.8	25.9	18.3	8.7	3.8	0.0	27.2	21.7	16.2	8.7	3.4	0.8	192	147	25	3	[c]
3	29.2	23.3	17.4	11.5	5.4	1.7	24.6	19.3	14.0	9.8	3.2	0.0	130	79	20	0	[c]
4	20.5	16.5	12.6	8.6	5.0	3.3	16.6	13.5	10.4	6.8	4.0	1.0	99	50	7	1	[c]
5	35.0	31.1	27.3	24.2	20.8	17.6	27.8	25.9	24.0	23.7	21.7	18.6	[b]	[b]	146	99	46
6	29.2	23.6	18.0	11.0	6.0	3.2	25.4	20.0	14.7	11.2	5.3	1.9	[b]	90	39	3	[c]
7	37.5	31.2	25.0	18.2	10.5	9.6	29.0	24.4	19.8	17.7	12.2	8.2	[b]	138	48	27	11
8	28.5	23.3	18.2	13.2	9.0	7.1	26.9	21.3	15.8	14.2	9.2	5.6	[b]	130	34	12	[c]
9	31.6	26.2	20.8	15.8	10.0	7.7	25.2	20.8	16.4	14.6	10.0	6.6	[b]	52	47	9	[c]
10	32.4	26.7	21.1	18.4	15.8	11.8	24.2	21.7	19.2	18.2	15.8	12.4	[b]	110	54	29	13
11	30.5	24.8	19.2	15.2	11.5	10.5	23.5	19.8	16.2	15.2	12.2	8.8	182	123	48	16	6

[a] Calculated from avg of 50 and 70F values.
[b] Too firm to test.
[c] Too soft to test.

SFI at 50F = 72.69–6.143 NMR value
SFI at 70F = 87.27–7.880 NMR value
SFI at 80F = 104.03–9.431 NMR value
SFI at 92F = 111.09–10.079 NMR value
SFI at 100F = 104.35–9.463 NMR value

The common (over-all) regression equation is: SFI = 83.05–7.365 NMR value.

The relationship between SFI and NMR values was excellent. Using the regression equations or a graph for each temp, the SFI value can be estimated within ± 1.5 when the SFI value is less than 30 and within ± 3 if the common (over-all) regression equation is used. The data obtained using the regression equations for each temp are shown in Figure 9.

Discussion

The low *resolution* NMR spectrometer has been shown to determine the solids content of fats accurately over the entire range. The solids content of the sample can be determined as received or after any conditioning or storage sequence or period. This would be of particular advantage in studying degree of crystallization occurring under different tempering and storage conditions and the amount of solids at any given time. The determination of the solids content of a sample as received in the laboratory is not possible by conventional techniques, so NMR opens new potentials to the analyst as well as taking less time.

The limited data collected on consistency indicate that the % solids in a sample can be related to a given consistency test, and that once the relationship is established the % solids can be used to predict consistency.

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The Oxidized-Metallic and Grassy Flavor Components of Autoxidized Milk Fat[1]

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Abstract

Oct-1-en-3-one accounts for the metallic flavor of autoxidized milk fat. In combination with small amounts of aldehydes it gives an oxidized flavor to milk. The grassy flavor component observed in autoxidized milk fat is shown to be *trans-cis*-2,6-nonadienal. Mechanisms are proposed for the production of oct-1-en-3-one from linoleate and 2,6-nonadienal from linolenate.

Introduction

STUDIES ON THE volatile components of autoxidized milk fat, using gas chromatography, indicated that a single fraction could reproduce the oxidized flavor found in dairy products during the early stages of autoxidation (7). This fraction migrated close to octanal during gas chromatography on both polar and non-polar columns. Other experiments indicated that the tallowy flavor found in the later stages of autoxidation in dairy products was a blend of the oxidized component with 2,4-decadienal and a grassy-flavored component (8). The oxidized component and 2,4-decadienal were found among autoxidation products of methyl linoleate.

Forss et al. have reported a number of studies on the flavors of autoxidized dairy products (9,10,11,13, 14,22). Although they concluded that the oxidized flavor of dairy products was caused by a series of 2-enals and 2,4-dienals, they found a single compound which caused the metallic flavor sometimes found in autoxidized dairy products. This compound was eventually found to be the same component that we had called oxidized (8).

Recently Stark and Forss succeeded in identifying the metallic flavored compound as oct-1-en-3-one (vinyl amyl ketone) by using mass spectrometry in conjunction with gas chromatography (24). They found that previous attempts to isolate this compound as the 2,4-dinitrophenylhydrazone (2,4-DNPH) were vitiated by the poor yields obtained when vinyl ketones are reacted with the usual 2,4-DNPH reagents. They were able to isolate the vinyl amyl ketone-2,4-DNPH from autoxidized butter oil and safflower oil by suitably altered procedures.

The grassy flavored component which we had previously found to be an important flavor constituent in autoxidized milk fat was not produced during the autoxidation of oil which contained no linolenic acid, nor was this flavor found among the autoxidation products of highly purified methyl linoleate (8). When highly purified methyl linolenate was autoxidized at room temperature, it had a strong beany flavor. This flavor was isolated and fractionated by gas chromatography. The beany flavor was found to be a blend of short chain compounds with the same grassy component found in autoxidized milk fat. This grassy flavor had gas chromatographic properties similar to that of a cucumber-flavored component found in autoxidized milk fat by Forss et al. (9,10, 11). They concluded that this flavor was caused by 2-nonenal.

This paper contains a report of efforts to identify the oxidized-metallic and grassy compounds. The properties of vinyl amyl ketone were found to account for observations on the oxidized-metallic compound isolated from an autoxidized trilinolein concentrate, thus confirming the discovery of Stark and Forss. The grassy compound was found to be *trans-cis*-2,6-nonadienal rather than 2-nonenal.

Procedures

Methyl linoleate was prepared from safflower oil (20), and transesterified with triacetin under reduced pressure with a sodium methoxide catalyst. Methyl

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