The Determination of the Solids Content of Fats and Oils by Nuclear Magnetic Resonance

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Abstract

A nuclear magnetic resonance (NMR) method was developed for the determination of the solids content of fats and oils at various temperatures. This method is more rapid, accurate, and universally applicable than the generally accepted solids fat index (SFI) by the dilatometric procedure. A standard deviation of 1.0% solids, unaffected by parameter interactions and attainable by inexperienced operators, indicates good reliability and reproducibility.

The utilization of an NMR variable-temperature accessory and a more precise mode of NMR tuning improves the precision and accuracy. The direct calculation of percentage of solids from the NMR signals eliminates the need for standards and compensates for the temperature variable. The results indicate that two separate laboratories, using somewhat different methods and modes of NMR operation, agree reasonably well.

Introduction

VARIOUS AUTHORS (2,3,5,6,8) have reported on the application of nuclear magnetic resonance (NMR) for the determination of the solid or liquid content of fats and oils. Only Pohle et al. (5) have made studies for the explicit purpose of defining an NMR method by which comparable results may be obtained by different laboratories. Since this laboratory was exploring the use of NMR for similar purposes, samples of varying liquid-solid ratios were obtained from Swift and Company together with their data for SFI by dilatometric and NMR procedures. The respective methods differed somewhat, mainly because the instrument was capable of controlling sample temperature within the instrument. Also the derivation of percentage of solids from the NMR signal differed.

By using the tempering technique described by Pohle et al. (5), a method is now presented which is considered more precise and accurate. Data are included to indicate that the results by each laboratory with its respective instruments and methods are reasonably comparable. In addition, a rapid method is suggested for control purposes which should have approximately the same precision and accuracy.

Experimental Procedures

Apparatus

A wide-line or low-resolution NMR spectrometer (Model PA-7 Process Analyzer, Varian Associates, Palo Alto, Calif.), equipped with an integrator and a variable-temperature accessory, was used in this study. The integrator provided for the dialing-in of a four-digit sample weight, and a recorded value of millivolts per unit mass was obtained. The temperature accessory was designed to supply and control sample temperatures ranging from −60 to +250C. Use of this accessory limited sample size to approximately a 2.5-ml volume. The instrument parameters employed were:

Time constant, seconds	0.5
Sweep time, minutes	'
Sweep amplitude, gauss	1.0
Sensitivity	variable (as required)
Modulation amplitude, gauss	0.5
RF attenuation	44
Integrator threshold, mv	normally 0.1 (or as required)
Integrator signal multiplier	normally ×5 (or as required)
Integrator read-out multiplier	normally ×10 (or as required)

Theory

The application of NMR for determining the solid or liquid content of fats and oils is based on the fact that NMR can distinguish between mobile hydrogen nuclei in liquids (like water and oil) and nonmobile hydrogen nuclei which are part of a crystallatine structure (like ice and solid fat). The theoretical aspects are discussed in various brochures of Varian Associates and by other publications (1,7).

The magnitude of the NMR signal from a particular sample can be expressed by Equation 1.

$$S = k\,\frac{N\,H\,\gamma\,I\,(I+1)}{T} \qquad [1]$$

where S = NMR signal strength (mv)
k = constant
N = number of hydrogen nuclei
H = magnetic field strength
γ = gyromagnetic ratio
I = spin of isotopic species
T = absolute temperature (273.16 + °C)

At a defined temperature all values except S and N are constant, and the signal becomes a direct measurement of N, the number of hydrogen nuclei present in the liquid portion of the sample. When a sample is completely liquid, the signal reflects the total hydrogen content of the sample. Therefore, if a series of measurements is made over different temperatures and these signals are corrected for the effect of temperature, the percentage at each temperature can be calculated, as indicated in Equation 2.

$$\%\ \text{Solids at a°C} = 100 - \left[\frac{\left(\begin{matrix}\text{Signal (mv/mass)}\\ \text{at a°C}\end{matrix}\right)(273.16 + \text{a°C})}{\left(\begin{matrix}\text{Signal (mv/mass)}\\ \text{at b°C where the}\\ \text{sample is liquid}\end{matrix}\right)(273.16 + \text{b°C})} \times 100\right] \qquad [2]$$

where a°C = the particular temperature of measurement
b°C = the nearest decile of temperature above the liquefying temperature of the sample, e.g., 50C for samples melting between 40 and 50C

A major advantage of this calculation is that the results are individual for each sample and are more accurate than those which are based on results from a series of standard samples. The NMR signals are not entirely a direct linear function of the inverse of the absolute temperatures (Equation 1). This is readily observed by examining a liquid corn oil over a large temperature range. This deviation is small however and within the precision of the NMR measurements over the temperature ranges which are normally employed. It therefore has no significant effect on the accuracy of the method.

Samples

Seventeen samples covering a large range of solids content were supplied by Swift and Company, to-

gether with data on the solid-liquid ratios by the standard SFI procedure (4) and by their NMR method.

It is known that the consistency and plastic properties of fats are greatly affected by the manner in which they are chilled and stored. These factors are likewise extremely important in the selection of a tempering technique for the measurement of solids where rapidity, accuracy, reproducibility, and sufficient latitude in technique are required without altering precision. Pohle et al. (5) studied conditioning variables, and their data emphasized the critical nature of this phase of the methodology. Subsequent studies showed that the initial temperature of the sample, prior to chilling in the dry-ice and acetone slurry, may be anywhere in the range of 50 to 70C, provided the sample was completely melted or was liquid at the temperature employed.

Method

The samples should be melted at approximately 70C and thoroughly mixed. A sufficient amount should be added to a previously tared 2.5-ml sample tube so that the bottom of the meniscus of the liquid fat will be at the 2.5-ml mark, and no higher. The sample should be weighed and determined to the third decimal place. The weight should approximate 2.2 g, $\pm$0.1 g. Each sample tube should be stoppered, and the samples should be placed in a 60C bath (Note 1). After 60C equilibrium is reached in about 30 min, the samples should be transferred to a dry-ice and acetone slurry ($<-$60C) and held for not less than 15 min (Note 2). The samples should then be put in a 10C constant temperature bath ($\pm$0.02C). After approximately 1 hour each sample should be removed, wiped dry, and placed in the 10C temperature-controlled NMR probe. The sample weight should be dialed into the integrator and the mv/g signal determined. After approximately 1 hour in a 20C bath, the NMR signal should be determined as before but with the NMR probe controlled at 20C. The same sequence should be repeated for temperatures of 30, 40, 50, and 60C (Note 3). The percentage of solids is calculated for each sample and temperature by using Equation 2.

Note 1. An experienced analyst can read the NMR signals at six different temperatures on 40 samples in an eight-hour day. On the previous day approximately four hours are required for weighing and tempering the samples, and an additional four hours are needed for calculating and recording the data. Also, an additional weighing should be made of one of the samples. This is used for "tuning" the NMR and is handled throughout the procedure like the samples. Tuning is absolutely necessary at each new temperature to insure optimum precision. This sample is used first in the NMR probe at each new temperature, and the NMR is then tuned to maximum meter deflection.

Once the NMR signal is reasonably well-centered, repeated sweeps are made with additional adjustments to tuning so that the over-modulated deflection point of the NMR signal is centered at the 5.0 mv recorder scale. Then, with additional sweeps, incremental adjustments of the centering potentiometer are made so the reverse sweeps give read-out signals which agree within 0.03 mv. Once this centering adjustment is made, it is normally not necessary to readjust it during the day. Succeeding temperatures can be tuned in through centering of the over-modulation deflection point of the signal at 5.0 mv recorder scale.

Note 2. This stage provides a convenient stopping-point if a large number of samples are analyzed. They can be stored over-night or longer at a temperature $<$10C.

Note 3. To optimize precision it is necessary that the results for all temperatures be determined on the same day. Also, most samples will be liquid at 50C, and 60C measurements would not be required. The calculation may be referred to.

Results and Discussion

Seventeen samples with a wide range of solids content were analyzed in duplicate on three different days by two analysts. One of the analysts was inexperienced, having used this procedure and the NMR on only one previous occasion. The data were examined statistically, and the overall average standard deviation by both analysts was 1.0, giving a 95% confidence limit of $\pm$2.0% solids. There was no significant difference between the experienced and inexperienced operators in their over-all standard deviations. Likewise there were no significant differences either within or between analysts in the within-day (duplicate) results, the reproducibility between days, the reproducibility across samples and days, and the reproducibility between the different temperatures which were used. Since no significance could be attached to any of these interactions, it was concluded that the method and the NMR operational technique were satisfactory and could be successfully employed even by inexperienced operators.

Table I lists the NMR results and the SFI data for three samples of low, medium, and high solids content. Sample 1 is an hydrogenated soybean oil; Sample 2 is a blend of hydrogenated soybean and cottonseed oils; and Sample 3 is a blend of animal fat, hydrogenated vegetable oil, and emulsifiers. The results by the two different laboratories agree reasonably well despite differences in equipment capabilities and operation.

Our data support prior conclusions (6) that no over-all correlation for all samples and all temperatures can be made between the SFI results and the NMR results. The variation for some specific cases is so great that the values from one (SFI) cannot be estimated from the other (NMR) with acceptable accuracy. However a pattern is indicated between the SFI and NMR values, whereby for specific samples of the same kind and history it should be possible to develop an acceptable correlation for each and every temperature of interest.

The precision attained, SD = 1.0 by a single NMR measurement, is within acceptable limits and com-

TABLE I

Comparative NMR and SFI Values for Three Samples of Different Solids Content

	Percentage of Solids					
	NMR (Swift)			NMR (Pillsbury)		
Sample	1	2	3	1	2	3
10C	87.6	70.1	47.5	87.1	68.9	46.9
20C	66.5	40.7	28.5	62.8	37.6	24.0
30C	40.9	15.7	15.8	29.9	11.7	12.7
40C	10.3	0.8	6.1	7.5	2.2	5.3

SFI (Swift)			
Sample	1	2	3
10C	47.6	36.8	24.5
21.1C	32.4	21.0	16.9
26.7C	25.8	14.1	12.9
33.3C	13.2	3.2	12.3
37.8C	6.2	0.3	8.5

TABLE II

Variations in Sample Temperatures after Transfer from Controlled Baths to Ambient NMR Probe

Time in 40-ml probe (22.2C)	Temperature						
	10C	20C	30C	40C	50C	60C	70C
1 minute	13.2	20.5	28.0	35.1	41.2	50.0	55.6
2 minutes	14.8	20.8	26.8	32.5	36.8	43.6	47.5
3 minutes	15.4	20.9	26.4	31.2	34.7	40.4	43.6
4 minutes	15.9	21.0	26.0	30.4	33.4	38.4	40.8

parable with the precision reported by Pohle et al. (5), which was calculated from the average of two consecutive NMR measurements. The variation of results between laboratories is anticipated to be greater than that within laboratories, but if the conditions are properly standardized, this increase is expected to be slight.

The temperature of the sample must be controlled within the NMR at the time of measurement if accuracy and precision are to be optimal. With an NMR probe at room temperature, this effect becomes particularly important when the temperature of the sample differs appreciably from that of the probe, especially for those samples where the slope of the melting curve is large. Although the effect of temperature variation on the measured percentage of solids was not actually determined, the temperature changes which could occur in transfer between the control baths and spectrometer were measured by a thermistor placed one-sixteenth of an inch from the inner glass surface of the cell. These are recorded in Table II. The temperatures reflect the changes that are taking place in the sample, mainly at the walls of the sample tube. Such changes must be recognized and taken into account when the solid content of fats is determined.

The general principles of this method could be applied as a rapid quality-control procedure with about the same precision and accuracy. A sample should be weighed, and a constant temperature bath should be available for each temperature of measurement. After tempering, each weighing should be placed in one of the baths, and after one-half hour the NMR signals should then be read. This would require about 1½ to 2 hours to complete the analysis, at five temperatures, for a single sample.

ACKNOWLEDGMENTS

Samples, NMR data, and SFI analysis were supplied by W. D. Pohle of Swift and Company; advisory support by D. K. Cunningham, statistical evaluations by B. Drew, and assistance by Pillsbury Analytical Department.

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[Received May 15, 1967]