

# Fat Content and Liquid-to-Solid Ratio of Chocolate by Wide Line Nuclear Magnetic Resonance

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## Abstract

Wide line nuclear magnetic resonance (NMR) was used for fast and accurate determination of the fat content and liquid-to-solid ratio of chocolate. The transition range of solid to liquid was found to be extremely narrow. The temp effect on the NMR signal is discussed and correction methods are suggested to that effect. A method was developed for the separate determination of butter fat and cocoa fat in addition to the total fat content determination in milk chocolate.

## Introduction

THIS PAPER INTENDS to demonstrate that the method of wide line NMR can be used as a fast and accurate method for the analysis of fats and oils in chocolate.

Shaw, Conway and others (1,2) have shown the applicability of a wide line NMR method for the determination of moisture in various solids. Conway, Ferren, Chapman and others (3-6) have obtained the same degree of success in the determination of the oil content of various substances.

The success of this method lies in the fact that an NMR instrument can differentiate between mobile hydrogens as in liquids like water or oil and bonded hydrogens, e.g., hydrogens which are part of a crystalline substance like solid sugar, cellulose or solid fat (7). The ratio of the two signals obtained from the NMR instrument then can give the solid-to-liquid ratio of a given system like margarine or chocolate (5,6).

In this work a method was developed to determine the total fat content and the solid-to-liquid ratio of chocolate at any temp. The actual analysis time is between 0.5 and 2 min and the results are obtained directly in percentage fat or liquid when the liquid-to-solid ratio study is done.

## Theory

NMR is a form of spectroscopy, but unlike other forms like IR, visible and UV, the energy levels are not built into the molecules or atoms but are created by an applied magnetic field. These are sometimes called magnetic Zeeman levels. The separation between these levels and consequently the resonance frequency for transitions between them are a function of the applied magnetic field. The energy levels created by applied magnetic field are formed at the nuclear level rather than at the atomic or molecular level as in UV or IR.

The resonance frequency lies in the megacycle range, and can be calculated if the applied magnetic field,  $H_0$ , is known by using the following formula,

$$\omega = \gamma H_0 \quad [1]$$

where  $\gamma$  is the magnetogyric ratio of the nucleus. Each magnetic nucleus has a unique value for  $\gamma$  and these have been tabulated (8).

In the present experiments, the proton resonance of the oils was observed and the proton  $\gamma$  value is 4.258 Mc/Kilogauss.

In a basic NMR spectrometer the desired frequency

is supplied by a radio frequency oscillator to a small coil which is wound around a cylindrical tube. At resonance, the inserted sample absorbs energy which is subsequently dissipated to the surroundings as thermal energy. The absorption of energy is detected, amplified and recorded on a graphic recorder. The amt of energy absorbed is proportional to the number of nuclei and so by integrating the absorption curve, quantitative information can be obtained on the number of nuclei in a given system. In our case the number of hydrogens served as a quantitative measure of the number of molecules of fat, given, of course, that the number of hydrogens/molecule of cocoa fat is constant which is the case to a very good approximation.

## Experimental

The instrument used in this experiment is the Varian PA-7 wide line NMR with V-4221 integrator and V-4220 variable temp accessory with temp range -175-250C. The proton signal was observed at a frequency of 7.313 mc using a field strength of 1717 Gauss.

Fourteen samples were chosen to give a wide range of different kinds of chocolates; eight were dark chocolate and six were milk chocolate. The samples were divided into two groups; in one group  $CCl_4$  was used as solvent while in the other group the samples were run without solvent at 24 and 80C. The details of the experimental results are tabulated in Table I. Solid samples were prepared as cylinders made by using a cork borer in the solid chocolate chunks. The piece so taken was put inside the sample tube.

The signal obtained by the PA-7 is the first derivative of the absorption curve. For quantitative analysis where the V-4221 integrator is used, the first derivative signal has to be overmodulated. In this case each part of the first derivative curve resembles the original absorption curve and the integrated signal is proportional to the number of protons present (see Fig. 1).

The accuracy of the PA-7 is  $\pm 1\%$  full scale. In the present experiment this amounts to 0.15-0.30% fat. This accuracy is far better than that of the average chemical methods. The same sample run by two

TABLE I  
Percentage Fat and NMR Signal of Various Chocolates<sup>a</sup>

Sample	% Fat	NMR signal <sup>b</sup>		Description
		24C	80C	
DC1.....	32.2	51.0	41.0	Dark chocolate
DC2.....	33.1	52.5	42.0	Dark chocolate
DC3.....	36.9	59.0	46.0	Dark chocolate
DC4.....	41.6	70.0	53.0	Dark chocolate
MC1.....	31.2	94.0	40.0	Milk chocolate
MC2.....	32.8	116.0	44.0	Milk chocolate
MC3.....	31.7	132.0	44.0	Milk chocolate
DC1C.....	32.6	50.5		Dark chocolate in $CCl_4$
DC2C.....	33.3	51.0		Dark chocolate in $CCl_4$
DC3C.....	37.1	59.0		Dark chocolate in $CCl_4$
DC4C.....	41.9	66.0		Dark chocolate in $CCl_4$
MC1C.....	31.1	50.0		Milk chocolate in $CCl_4$
MC2C.....	32.9	52.0		Milk chocolate in $CCl_4$
MC3C.....	31.9	51.0		Milk chocolate in $CCl_4$

<sup>a</sup> The chemical analysis of the samples performed by Curtis & Tompkins, Ltd., San Francisco, Calif.

<sup>b</sup> Instrument Parameters:

RF attenuation	28 db	Time constant	2 sec
Modulation amplitude	0.5 gauss	Readout multiplier	10
Sensitivity	200	Signal multiplier	5
Sweep amplitude	1 gauss	Threshold	0.1 mv
Sweep time	2 min	Weight setting	As required

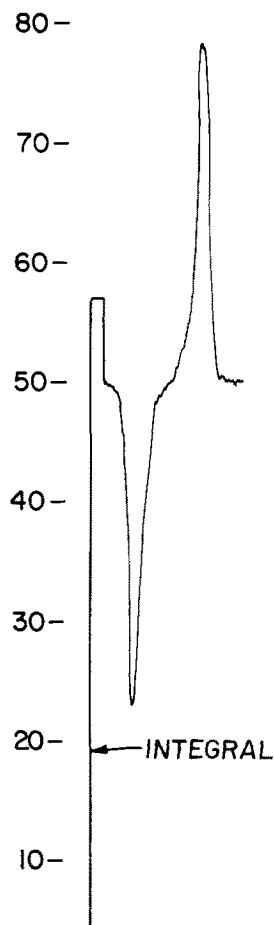


Fig. 1. An overmodulated first derivative spectrum of the PA-7. Each part of the first derivative signal is proportional to the absorption curve. The vertical line at the end of the run represents the integral or the area under the two curves.

different laboratories gave results that differed sometimes by more than 1.0%.

### Discussion

*Percentage Liquid in Chocolate.* For experimental and convenience reasons, it is easier to determine the percentage liquid in a sample than to determine the percentage solids or liquid-to-solid ratio. However, by basic arithmetical manipulations, the above two parameters can be obtained.

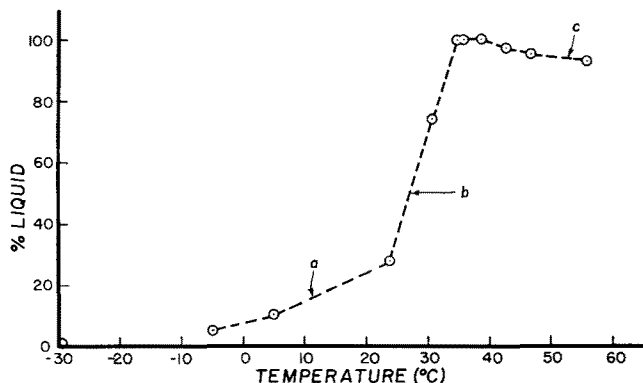


Fig. 2. Percentage liquid in dark chocolate vs. temp. Note the small slope of part "a" and the sudden increase in liquid-to-solid ratio represented by part "b." The exponential line shape, part "c" is due to the temp effects on the NMR signal.

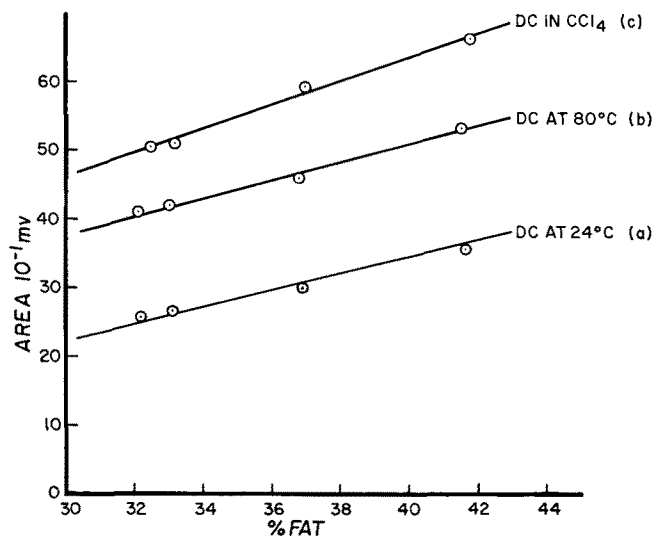


Fig. 3. A plot of the area under the curve vs. the percentage cocoa fat. The upper curve is of dark chocolate dissolved in  $\text{CCl}_4$  at 24°C; the middle curve is of whole dark chocolate at 80°C; and the lower curve is of whole dark chocolate at 24°C.

The percentage solid in the total samples is simply

$$S = 100 - L \quad [2]$$

where S is the percentage solids and L is the percentage liquid.

The liquid-to-solid ratio is then

$$\frac{L}{S} = \frac{L}{100 - L} \quad [3]$$

The percentage liquid of the fat present in a given sample can be obtained from the following formula:

$$\text{Percentage liquid of fat in sample} = \frac{L \times F}{100} \quad [4]$$

where L is as defined, and F is the percentage fat in the sample, obtained as described in the second part of the discussion.

It should be stressed, however, that in actual practice, if liquid-to-solid ratio is desired, for example, a calibration curve can be made as to read the above factor directly, even though the instrument signal is a function of the liquid phase. This is because of the simple relationships between the percentage liquid and the liquid-to-solid ratio and the percentage solids as described by formulas 2, 3 and 4 above.

In this experiment the liquid content of a sample of dark chocolate was determined in the range -30-

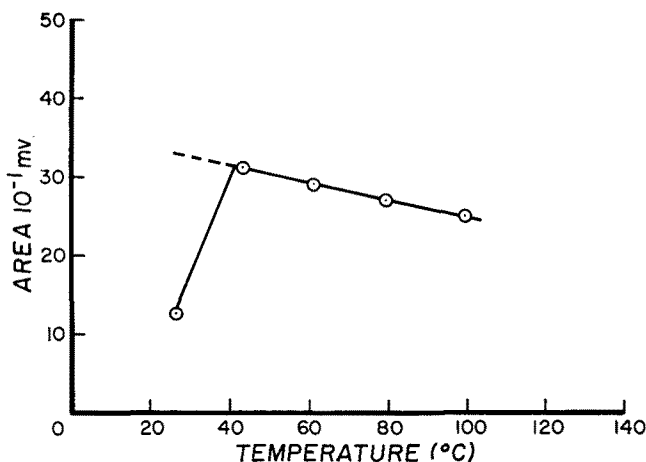


Fig. 4. The NMR signal of dark chocolate (sample 3X) vs. temp.

TABLE II  
NMR Signal Corrected for Temperature Effects

Sample	NMR signal		NMR signal corrected to maximum signal
	35C	80C	
1X.....		43.0	48.0
2X.....		46.0	51.0
3X.....		50.0	55.0
1Xc.....	47.5		
2Xc.....	50.0		
3Xc.....	55.5		

56C and the results are presented in Figure 2. In part "a" of the curve (range -30-23C) a very small change of the liquid content with change in temp can be seen. In part "b" (23-35C) a striking change can be seen indicating a sharp melting range so typical for cocoa fat. The exponential decrease in part "c" (over 35C) is due to the fact that the NMR signal is inversely proportional to the absolute temp; which means that a small correction factor should be applied if samples are run at various temp. This point was demonstrated experimentally in the second part of the experiment where the actual fat content of chocolate samples was determined. The correction factor can be determined easily by running a sample of oil dissolved in CCl<sub>4</sub> at various temp and plotting the results.

It was found that the percentage liquid of milk chocolate at room temp is much higher than that of dark chocolate. This was mainly attributed to the presence of butter fat in the chocolate, a fact which tends to increase the liquid fraction of the sample and accounts for the high NMR signal obtained at room temp for milk chocolate. This point is discussed in detail later on.

*Fat Content of Chocolate.* As we said in the introduction, an obvious application of wide line NMR is to the quantitative determination of fats in chocolate. In the present experiment the fat content of various chocolates was determined. Figure 3 exhibits extreme linearity which is an indication of the accuracy of the method. The lines for pure chocolate at 80C and chocolate in CCl<sub>4</sub> show the same linearity and slope. The line would have overlapped if the two sets of experiments had been run at the same temp. This fact was illustrated by an experiment, summarized in Table II, where six samples were analyzed. Three of the samples were pure chocolate samples and the other

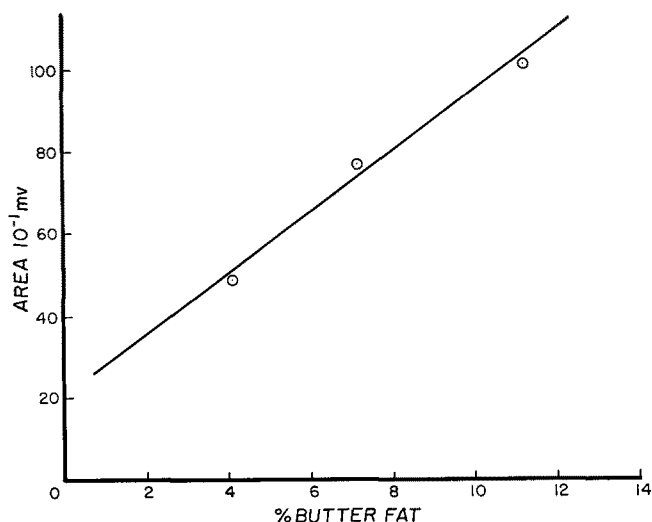


FIG. 5. NMR area after correction for cocoa fat content vs. percentage butter fat.

TABLE III  
Butter and Cocoa Fat Content of Milk Chocolate

Sample	% Cocoa butter	% Butter fat	NMR signal 24C	NMR signal* cocoa fat 24C	NMR butter fat
MC1.....	29.2	4.1	94	45	49
MC2.....	26.1	7.2	116	39	77
MC3.....	22.1	11.2	132	31	101

\* The values given in this column were obtained by multiplying the values derived from the calibration curve at 24C by a normalizing factor of 2.

three were the same samples dissolved in CCl<sub>4</sub>. Samples 1X, 2X and 3X were run at 80C, and samples 1Xc, 2Xc and 3Xc were run at 35C. Sample 3X served as the control sample for the temp study plotted in Figure 4.

It can be seen that if the values obtained at 80C are corrected to 35C, the corrected values are equal, within the experimental error, to the values obtained for the same samples in CCl<sub>4</sub> at 35C. The dotted line in Figure 4 shows the behavior of a sample in a case where there is only a temp effect and no change in solid-to-liquid ratio.

In Table I, values are also reported of the NMR signal of various chocolates at room temp (24C). It can be noted that the percentage liquid of the milk chocolate is higher at that temp. This is due, as mentioned before to the butter fat present in the milk chocolate. Actually, the butter fat content can be determined directly by the NMR method using the calibration curve of dark chocolate. The results of such a study are tabulated in Table III. In the case at hand the percentage fat was known before so the NMR signal due to the cocoa fat was found from the extrapolated graph 3(a). This value in turn was subtracted from the total signal at 24C and the resulting signal was plotted to give Figure 5—the percentage butter fat in the milk chocolate. In practice the percentage fat is the unknown. In that case the percentage butter fat can be found if the following formula is used in conjunction with the calibration curve in Figure 5.

$$B = (\Delta_{24} - 0.24A_{35})/0.76 \quad [5]$$

Formula 5 was obtained from the solution of two simultaneous equations where B is the corrected NMR signal reading for percentage butter fat,  $\Delta_{24}$  the NMR signal at 24C,  $A_{35}$  the NMR signal at 35C. The value B then is read on the calibration curve to give the fat content in percentage.

With careful handling of the experimental technique the total fat can be found also from Figure 3(a) provided one assumes that the liquid-to-solid ratio is always constant for a given temp (24% according to Figure 2).

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