Fully Automated Determination of Solid Fat Content by Pulsed NMR[1]

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ABSTRACT

Pulsed NMR has been developed into a quick, accurate fully automated method for the determination of the solid fat content in partially crystallized fats. Accepting a standard deviation of 0.3% solids, the percentage of solids is displayed on a digital voltmeter or printed out 6 sec after placing the sample into the sample holder. To allow for the dead time of the receiver, a correction factor has been introduced giving rise to only small errors (<1%) in the solid fat content. Due to the short measuring time, no temperature control of the sample holder is needed between 10-45 C. Pulsed NMR values can be converted into dilatations and vice versa.

INTRODUCTION

The determination of the solid fat content plays an important role in the fat industry. The solid fat content is used for process control, e.g. hydrogenation, interesterification, and blending. Important properties of margarines and shortenings also require close control of the solid fat content. Therefore, a quick routine method which is easy to handle is of great importance for the fat industry.

In the past, several methods, such as differential thermal analysis, differential scanning calorimetry, and dilatometry, were used for the determination of the solid fat content in fats. Out of these, dilatometry is the most generally accepted method. However, dilatometry is a laborious, time consuming method. Many workers have, therefore, studied the possibilities of replacing dilatometry (1-5). During the last 5 years, the possibilities of wide-line NMR have been investigated in detail. Wide-line NMR offers some advantages over dilatometry regarding speed and ease of operation. Nevertheless, important disadvantages remain. (A) Only the narrow peak arising from the liquid can be used for the determination of the solid fat content; and, therefore, the solid/liquid ratio must be determined indirectly, usually from the liquid signal at the measuring temperature and the liquid signal after melting the sample. (B) As the spectrometer is mostly used under so-called saturation conditions to obtain a sufficiently strong signal to noise ratio, the liquid signal is dependent upon the relaxation times and consequently on viscosity and temperature. Even if a well chosen reference sample, such as olive oil, is used, these systematic errors are only partly eliminated. (C) Since the sample must be melted, the possibilities for automation are rather poor.

Although pulsed NMR should give an answer to these problems, no attempt had been made to use pulsed NMR for the solid fat determination until a few years ago because of the high cost of pulse apparatus. Recently, relatively cheap instruments have been developed and now a highly automated instrument is commercially available. This article describes the development of pulsed NMR into a quick, accurate method for the determination of the percentage of solids in a partly crystallized fat.

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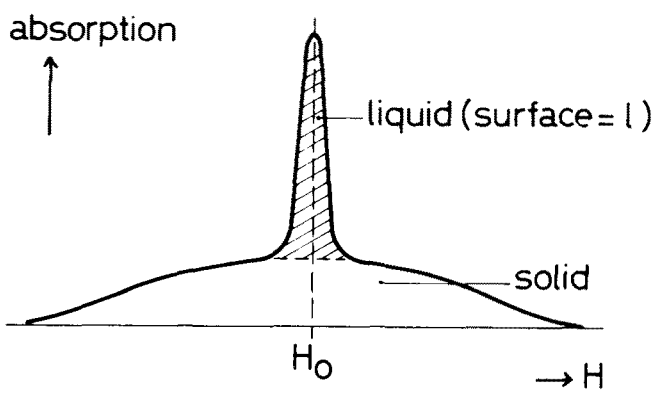

FIG. 1. Wide-line NMR spectrum of a partly crystallized fat.

EXPERIMENTAL PROCEDURES

We modified a version of the minispec, i.e. process analyzer type p 20 ex Bruker Physik AG which operates at 20 MHz. The 90° pulse is ca. 3 μs, and the dead time of the receiver is ca. 7 μs. To avoid the use of hydrogen containing material, the sample coil was made of copper wire without insulation, fixed by means of Teflon tape to a glass tube (inner diameter 10.5 mm). From considerations of long term stability, the diode detection is linearized by adding a direct current of 0.4 v to the signal. The temperature of the magnet is stabilized at 33 C. The sample quantity is ca. 1 g. Only for the indirect method (see below) did we use a sample height smaller than the receiver coil to prevent the fat from coming out of the measuring coil due to expansion.

The measuring procedure is automated fully and is started as soon as a sample is put in the sample holder. The signal from the minispec is processed electronically, as described elsewhere (6). After 6 s, the percentage of solids is displayed directly on a digital voltmeter or printed out.

Using an air stream through the sample holder the temperature of the sample can be kept constant to within 0.1 C at fixed levels (0, 5, 10, 15 C etc.) between 0-60 C. The temperature of the sample holder without temperature control is ca. 33 C due to thermal contact with the temperature stabilized magnet room.

All samples were stabilized and tempered in the same way. The melted samples (60 C) were kept in melting ice for 90 min and subsequently kept for 30 min at each measuring temperature in closely fitting holes of a metal block thermostated in a waterbath, starting at 10 C in steps of 5 C upward.

PULSED NMR

Hydrogen nuclei have a magnetic moment and can be considered as small magnets (7,8). These elementary magnets can be polarized by means of a large static magnetic field H_0. At thermal equilibrium, most of the elementary magnets will be parallel to H_0 and give rise to a resultant magnetization M_0. M_0 is proportional to the total number of hydrogen nuclei. This equilibrium can be disturbed by a radio frequency (rf) field H_1 (ω) perpendicular to H_0 if the resonance condition $\omega = \gamma H_{tot}$, where γ is the gyromagnetic ratio and H_{tot} the total magnetic field experienced by the nuclei, is fulfilled. The total magnetic field is the sum of the external magnetic field H_0 and the local field from the

surrounding nuclei. This internal local field depends upon the distance between the elementary magnets and their positions and will, therefore, differ for different nuclei. This causes the resonance frequency to differ for different nuclei.

In wide-line NMR we apply a continuous relatively small rf field (H_1) and sweep H_0 to measure the absorption of all nuclei (7,8). We then obtain a spectrum with one or more absorption peaks (Fig. 1). In pulsed NMR, on the other hand, a very short and strong rf pulse, covering the whole range of the resonance frequencies of the nuclei, is applied to the sample. The advantage of pulsed over wide-line NMR is that all nuclei are excited within a relatively short time interval (pulse width). The irradiation by means of pulses is a considerably more efficient mode of excitation than continuous irradiation, which results in a better sensitivity.

The effect of H_1 pulses can be described in the so-called rotating rrame (7-9). In this frame, M_0 is forced to rotate around the H_1 direction. We use only the 90° pulse which rotates M_0 90° around H_1 (Fig. 2). The signal induced in the receiver coil, with its axis perpendicular to H_0, is proportional to M_0. Neglecting the inhomogeneity of H_0, the decay rate after the 90° pulse is determined by the spin-spin relaxation time T_2. T_2 depends upon the distances between the nuclei and their positions and in particular upon the mobility of the nuclei. T_2 increases with mobility. T_2 of solids is ca. 10 μs and T_2 of liquids is larger by a factor of 10^4. It is this difference in T_2 which offers us the opportunity to measure the solid fat content in partially crystallized fats. Figure 3 shows the magnetization decay of a partially crystallized fat after a 90° pulse. The tail of the magnetization decay of the liquid fat protons is determined by the inhomogeneity of the magnet, rather than by spin-spin relaxation.

The solid fat content now can be determined with an indirect and direct method. The indirect method is used as a reference method for the quick direct method.

Indirect Pulse Method

The indirect method is analogous to the wide-line method. Only the signal of the liquid fat is taken into account. The magnetization of the solid fat protons decays very fast and is far less than 0.1% of the initial value at 70 μs after the 90° pulse, whereas the decrease of the magnetization of the liquid fat protons, neglecting the influence of H_0 inhomogeneity is negligible at 70 μs. The signal height at 70 μs after the 90° pulse is, therefore, proportional to the number of protons in the liquid (1 in Fig. 3). A measure of the number of solid fat protons can be obtained by melting the sample. The signal height then will be proportional to the total number of protons. If we correct this signal for the temperature dependence of both M_0 and the Q factor of the receiver coil (by means of the signals of a reference oil at both temperatures) and neglect the difference in proton content of liquid and solid fat, the solid fat percentage N^P_{ind} at temperature t will be:

$$N^P_{ind} = \left\{ 1 - \ell(t)/c\,\ell(t_m) \right\} 100\%, \qquad [1]$$

where $\ell(t_m)$ is the signal height of the melted sample at temperature t_m and $c = \ell_{ref}(t)/\ell_{ref}(t_m)$. The index reference indicates a reference sample of oil being liquid at temperatures t_m and t.

Direct Pulse Method

The signal $c\,\ell(t_m)$ also can be determined directly from the magnetization decay of the solid fat protons and is equal to the signal immediately after the 90° pulse ($s + \ell$ in Fig. 3). However, the decay time of the solid fat protons is so short that it is impossible to measure the initial magnetization ($s + \ell$) but only the signal height ($s' + \ell$) a

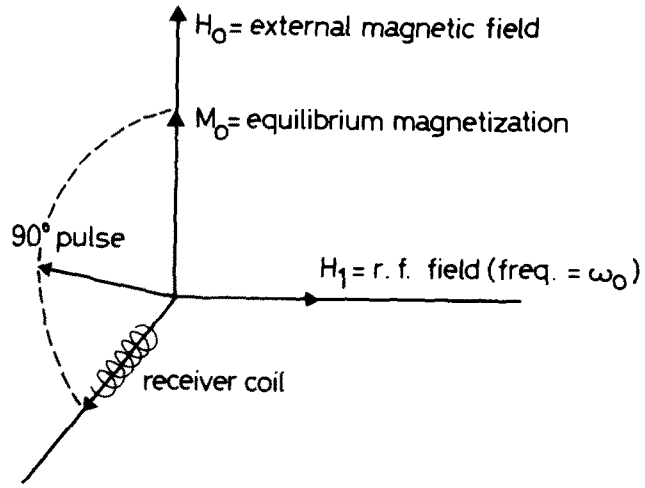

FIG. 2. Motion of magnetization caused by a 90° rf pulse.

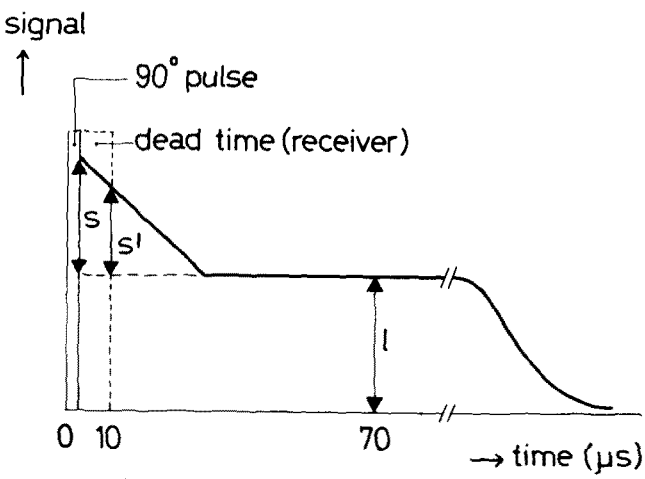

FIG. 3. Magnetization decay of a partly crystallized fat.

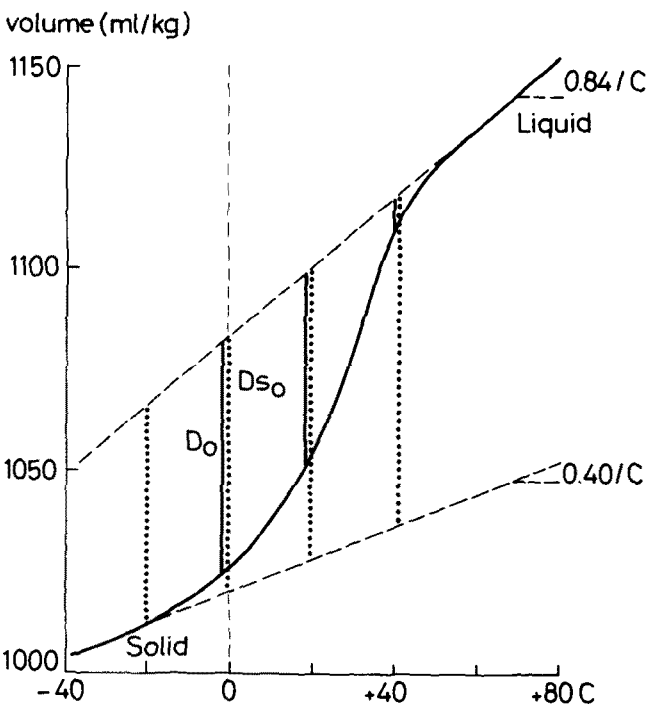

FIG. 4. Dilatometric curve showing change in volume with change in temperature.

TABLE I

Influence of Trigger-Time upon Solid Fat Content (% Solids) Measured by Direct Pulse Method

Sample[a]	10 C			20 C			30 C			40 C		
	1 s	2 s	4 s	1 s	2 s	4 s	1 s	2 s	4 s	1 s	2 s	4 s
Edible tallow	32.6	32.1	32.3	28.8	29.0	28.4	15.8	16.8	16.5	4.2	5.6	5.0
Palm oil	47.9	47.9	48.0	25.7	27.0	26.3	8.1	9.4	9.2	0.7	0.7	0.3
Coconut oil	77.4	78.1	78.1	---	34.6	35.3	0.5	0.3	0.9	0.1	0.9	0.1
Hydrogenated coconut oil, 31 C	84.6	85.3	84.9	53.6	55.4	55.4	6.6	8.3	7.1	1.8	2.0	1.2
Hydrogenated fish oil, 37 C	67.3	67.4	67.2	53.4	55.0	54.7	24.4	27.0	27.4	1.1	0.1	0.5
Hydrogenated whale oil, 37 C	80.0	80.3	80.2	63.8	64.4	64.5	27.4	30.2	30.3	0.2	0.1	0.5
Hydrogenated rapeseed oil, 45 C	88.1	88.2	87.7	86.8	87.1	86.6	67.9	69.3	69.6	22.4	25.6	26.6

[a]The samples indicated in Tables I, II, and III have been taken from different charges.

certain time after the 90° pulse due to the dead time of the receiver. We have to know signal s which can be obtained by multiplying s′ by a correction factor of f for the dead time which is dependent upon T_2 of the solid fat protons. The solid fat content can be expressed in the signals s′ and ℓ according to Figure 3:

$$N^P_{dir} = \left\{ f\, s'/(\ell + fs') \right\} 100\%. \qquad [2]$$

The correction factor f (s/s′) can be determined with the indirect method as a reference method. Before the direct measurement, we have to adjust an arbitrary value for f (f_{adj}) for which we often use f_{adj} = 1.37. From the difference of the solid fat contents measured with the direct and the indirect method, we calculated the f value (f_{calc}) which we would have to adjust to measure the same percentage of solids with the direct and indirect pulse method:

$$f_{calc} = f_{adj}\,(100/N^P_{dir} - 1)/(100/N^P_{ind} - 1). \qquad [3]$$

DILATOMETRY

The solid fat determination by dilatometry is based upon the difference in density between solid and liquid fat (4). Figure 4 shows the expansion curve of a sample of fat. The first part of the dilatation curve represents the expansion of the solids; the last part, the expansion of the liquid. The drawn vertical lines are called the dilatation (D) and the dotted lines, the total melting dilatation (D_s). This leads for the solid fat content to the relation:

$$\%\ \text{solids} = 100\ D_t/(D_{so} + \alpha\, t) = c_t\, D_t, \qquad [4]$$

D_t, dilatation at t C; D_{so}, melting dilatation at 0 C (ml/kg); α, difference in slopes of the solid and liquid line. For D_{so} and α of margarine blends and shortenings the values of 65 and 0.44, respectively, normally are used. However, the true melting dilatation may vary between 50-90 depending upon the nature of the triglycerides. Moreover, the melting point of the remaining solids will increase during melting, and, hence, D will increase more rapidly than indicated by the factor 0.44. So important systematic errors can influence the results if the above mentioned values 65 and 0.44 are used.

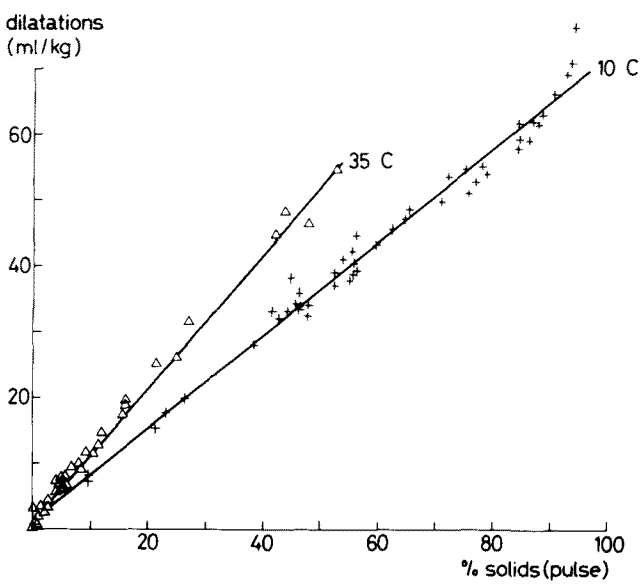

FIG. 5. Relation between dilatations and pulse values.

RESULTS AND DISCUSSION

Measuring Time and Saturation

The measuring time depends upon the standard deviation required. The standard deviation of a single dilation determination is 1-2% solids; however, a standard deviation of 0.6% solids or less, which can be achieved by a determination in duplicate or triplicate is often aimed at. The standard deviation using the pulse method is ca. 0.5% if only one 90° pulse is applied. We decided to improve the accuracy to 0.3% solids by applying three 90° pulses.

The measuring time will be dependent upon the time interval between the pulses (trigger-time) which we chose to be as small as possible. This time interval is limited by saturation. Before applying the next pulse, the whole nuclear spin system has to reach equilibrium again. The speed of return to this thermal equilibrium depends upon the spin-lattice relaxation time T_1. If the time interval between two pulses is smaller than ca. 5 T_1, the signal after the last pulse will decrease.

Using the indirect pulse method, we have only to deal with T_1 of the liquid fat which is of the order of 100-200 ms. To avoid saturation, the time interval between the 90° pulses must be at least 0.5 s. Using the direct pulse method, we have to deal with both T_1 of the liquid and solid fat. T_1 of solid fat is often considerably longer than T_1 of liquid fat and varies from ca. 0.4 s for crystals with poor ordering to ca. 8 s for crystals with better ordering. In general, T_1 of α crystals will be shorter than that of β crystals. Under crystallization conditions, as normally used for the determination of the solid fat content, the crystals will mostly be rather imperfect and correspond with T_1 values lower than 1 s. A time interval of a few seconds between the 90° pulses is expected to be sufficient to avoid saturation.

TABLE II

Effect of Temperature Control of Sample Holder on Solid Fat Content (% Solids)
(A with control; B without control) Measured with Direct Pulse Method

Sample[a]	10 C		20 C		30 C		40 C	
	A	B	A	B	A	B	A	B
Edible tallow	32.1	31.8	29.0	28.8	16.8	16.2	5.6	6.2
Palm oil	47.9	48.2	27.0	25.8	9.4	8.9	0.7	1.3
Coconut oil	78.1	77.6	34.6	34.6	0.3	0.9	0.9	1.1
Hydrogenated coconut oil, 31 C	85.3	86.1	55.4	51.5	8.3	6.5	2.0	2.0
Hydrogenated fish oil, 37 C	67.4	67.6	55.0	53.4	27.0	27.1	0.1	0.2
Hydrogenated whale oil, 37 C	80.3	80.4	64.4	64.3	30.2	29.3	0.1	0.1
Hydrogenated rapeseed oil, 45 C	88.2	87.7	87.1	86.7	69.3	69.2	25.6	25.8
Average difference[b]	-0.14		-0.48		-0.30		+0.10	

[a]The samples indicated in Tables I, II, and III have been taken from different charges.
[b]These values have been calculated from the solid fat content of 32 fat samples.

Under saturation conditions the solid fat nuclei will be more saturated than the liquid fat nuclei due to the longer T_1 of the solid fat nuclei. The decrease of the solid fat signal will, therefore, be larger than the decrease of the liquid fat signal, and the measured solid fat content will be too small (equation [2]).

To investigate the influence of saturation as a function of the trigger-time, we measured the solid fat contents of 32 fats between 10-40 C at trigger-times of 1, 2, and 4 s (Table I). All measurements at a trigger-time of 2 s are within the measuring accuracy equal to those at a trigger-time of 4 s, indicating that no saturation occurs at a trigger-time of 2 s. However, the solid fat contents measured at a trigger-time of 1 s are, in most cases, somewhat smaller due to saturation. The influence of saturation often increases with increasing temperature, as can be explained from the temperature dependence of T_1 of the solid fat. The measuring time corresponding with a standard deviation of 0.3% then will be 6 s.

Temperature Control of the Sample Holder

For long measuring times (wide-line NMR), temperature control of the sample holder is needed. To measure samples tempered at different temperatures, the temperature control has to be switches over to other temperatures, thus losing time, e.g. 5 min/temperature.

As the measuring time with the direct pulse method is short, so that probably no temperature control of the sample holder is needed, we measured the solid fat contents of 32 fats between 10-45 C with and without temperature control (Table II). We would expect that the effect of measuring without temperature controller increases with increasing difference in temperature between sample and sample holder. However, Table II shows the reverse below 30 C. At 30 C, the difference in temperature between sample and sample holder is almost 0, so that we did not expect a difference in solid fat contents measured with and without temperature controller. However, the solid fat contents measured with temperature controller proved to be 0.3% larger. Although the same stabilizing procedure was applied in both cases, small differences in the adjustments of the thermostats apparently can give differences in solid fat content of this magnitude. Therefore, we conclude that the effect of measuring without temperature controller is smaller than the average differences in Table II and, therefore, less than ca. 0.2% solids, which is within the measuring accuracy.

Solid Fat Factor f

The solid fat factor f makes allowance for the decrease

TABLE III

Percentages of Solid Fat Determined with Direct (f = 1.37) and Indirect Pulsed NMR Method and Calculated f-Factors

Sample[a]	10 C			20 C			30 C			40 C		
	$N^P_{Indirect}$	N^P_{Direct}	f	$N^P_{Indirect}$	N^P_{Direct}	f	$N^P_{Indirect}$	N^P_{Direct}	f	$N^P_{Indirect}$	N^P_{Direct}	f
Margarine a	54.0	55.2	1.29	19.5	20.2	1.29	4.1	4.6	1.20	0.5	0.1	6.78
Margarine b	9.3	9.5	1.32	5.7	6.4	1.19	2.5	2.9	1.16	0.3	0.4	1.01
Palm oil	52.0	53.4	1.28	25.5	26.5	1.28	8.1	8.1	1.35	0.4	0.7	0.77
Coconut oil	75.5	75.4	1.36	29.5	28.3	1.43	0.7	0.4	2.37	0.0	0.0	---
Hydrogenated coconut oil, 31 C	90.8	90.5	1.40	55.2	54.6	1.38	5.4	5.1	1.43	0.5	0.4	1.69
Hydrogenated fish oil, 37 C	77.3	77.8	1.31	55.8	56.0	1.34	24.5	24.1	1.38	0.3	0.4	1.01
Hydrogenated rapeseed oil, 45 C	85.4	86.8	1.20	86.4	86.9	1.29	66.4	66.7	1.33	24.1	22.8	1.45
Hydrogenated olive oil, 28 C	63.0	64.2	1.28	29.1	29.7	1.31	4.7	4.9	1.29	0.8	0.2	5.43
Beef tallow	43.6	45.7	1.24	44.2	44.6	1.33	24.7	24.2	1.39	10.9	9.6	1.56
Palm kernel oil	70.7	70.7	1.35	41.7	40.0	1.45	0.6	0.1	8.14	0.2	0.1	2.70
Hydrogenated safflower oil, 31 C	85.9	86.2	1.32	58.9	58.6	1.37	9.4	8.3	1.55	0.4	0.5	1.08
Hydrogenated sunflower seed oil, 34 C	87.5	87.8	1.31	63.5	63.6	1.34	17.2	15.8	1.49	0.0	0.0	---
Hydrogenated peanut oil, 31 C	46.0	47.4	1.28	16.5	16.8	1.32	1.7	2.1	1.09	0.2	0.2	1.37
Mean f[b]			1.31			1.34			1.42			1.61

[a]The samples indicated in Tables I, II, and III have been taken from different charges.
[b]The results of 48 fat samples have been included in the mean f values.

in magnetization due to the dead time of the receiver. This decrease in magnetization depends upon the characteristic decay time and the spin-spin relaxation time T_2 (7-10). Lattice defects often give rise to a high degree of mobility of the molecules which results in an increase of T_2. Different fat compositions will crystallize in a different way and, therefore, correspond with different T_2s. As the mobility of the molecules and the distribution of the fat components in the liquid changes with the temperature, we expect T_2 to be temperature dependent.

We investigated the dependence of f upon temperature and fat composition by measuring f for 48 different fat compositions between 10-45 C (Table III). The f values have been calculated by means of equation [3]. Since the absolute measuring accuracy of the solid fat content is almost independent of the solid fat content, the measuring inaccuracy of f increases with decreasing solid fat content (see equation [3]). In particular, the f values, calculated from percentages smaller than 5%, are rather inaccurate. Since f is always larger than or equal to 1.00, the calculated f values smaller than 1.00 have no physical significance, except that the measuring inaccuracy was high in these cases.

For two reasons the mean f value increases with the temperature. (A) After rapid cooling at 0 C, α crystals corresponding with small f values, due to the high degree of mobility, may be formed which gradually melt or transform into β' (or β) crystals at higher temperatures. β' and β crystals correspond with better ordering and less mobility, and the f value of β' and β crystals will, therefore, be somewhat larger. (B) Components melting at higher temperature (the saturated ones) often give rise to fewer imperfections than components melting in the low temperature region (the unsaturated ones). The contribution of the saturated components, corresponding with rather large f values, increases relatively at increasing temperature.

We could take into account this temperature dependence by readjusting f for each temperature, which, however, is time consuming and easily leads to mistakes. The use of one mean f for all fats and temperatures would be the ideal situation. The increase of f from 10 to 30 C is only ca. 0.1. If we use a mean f value of 1.37 in this temperature range, systematic errors, due to differences in f values, are ca. 1% solids at most for fat contents of 50% and become 0 for fat contents approaching 0 and 100%. Above 30 C, the systematic error is hardly ever greater than 1%, as even appreciable deviations of f in this temperature range cause relatively small systematic errors. In general, these small systematic errors do not cause serious problems.

Comparison of Pulsed NMR and Dilatometry

The dilatation D (4) is related to the percentage of solids according to equation [4]. If we assume D_{so} and α to be independent of the fat composition, we expect a linear relationship between D and the percentage of soilds at a certain temperature. The relationship between the dilatations of 48 different fats and the percentages of solids measured with the direct pulse method, using f = 1.37 at 10 and 35 C proves, apart from the scattering, to be linear (Fig. 5). The slope of the line is strongly temperature dependent and increases with the temperature. This follows from equation [4], in which c_t is temperature dependent.

The relation between both methods, the temperature dependence included, can be represented by:

$$N^{P}_{dir} = 100\ D/(60 + 1.2\ t) + 0.7\%. \qquad [5]$$

The standard deviation around the regression line is 1.6 dilatation points ($\approx$ 1.7% solids). The fan of lines represented by equation [5] does not pass through the origin, contrary to what would be expected from equation [4]. This is due to the fact that, in particular, in the range of large amounts of solids, the increase in dilatation at increasing solid fat content is somewhat smaller than the increase in percentage of solids as measured by the pulse method. Comparing equation [5] with equation [4], the melting dilatation D_{so} proves to be 60 which agrees with the value of 65 normally used for margarine blends and shortenings. The temperature factor α, however, proves to be almost a factor of three larger than we would expect. As mentioned before, the melting point of the remaining solids will increase during melting so much that the temperature dependence of D increases by almost a factor of 3.

The standard deviation (σ) around the regression line ($\sigma \approx$ 1.7% solids) is considerably larger than would be expected from the standard deviations in the dilatations ($\sigma \approx$ 0.6% solids, 2 measurements) and in the pulse values ($\sigma \approx$ 0.3% solids). To investigate to what extent systematic errors as a consequence of different f values for different fat compositions contribute to the standard deviation around the regression line, we compared the indirect pulse values, being independent of f, with the dilatation. However, the remaining standard deviation was still ca. 1.5% solids.

Differences in fat composition give rise to differences in D_{so} and α values. The rather large standard deviation must, therefore, be ascribed to the dependence of D_{so} and α on fat composition and temperature and not to the inaccuracy in the NMR measurements. If either pulsed NMR or dilatometry is used for the solid fat determination these systematic errors do not present serious problems. Difficulties may arise if pulsed NMR values have to be converted into dilatations and vice versa.

To improve the conversion accuracy it will be necessary to split up the different fats into groups with similar fat composition. Some preliminary experiments showed that an improvement can, indeed, be obtained.

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