Characterization of a Semisolid State in Milk Fat Through T_2* Resolved T_1 Distributions by Time Domain Nuclear Magnetic Resonance

D. Le Botlan*, L. Ouguerram, L. Smart, and L. Pugh

Laboratoire d'Analyse Isotopique et Electrochimique de Métabolismes,
CNRS UPRES A Q6006, Faculté des Sciences, F-44322 Nantes Cedex 03, France

ABSTRACT: The solid-to-liquid ratio is an important parameter in the study of fats. Many methods can be used: dilatometry, differential scanning calorimetry (DSC), and nuclear magnetic resonance (NMR). However, below approximately 20°C, NMR gives much lower solid-to-liquid values than DSC. This difference can be attributed to the presence of a semisolid state, whose T_2 value would be of the order of 50–200 μs, and which should give an NMR signal of 14 to 88.5% of the total signal of this phase at a time when the signal of the liquid phase is measured. Thus, such a state is seen partially as a liquid by NMR. In a previous study using time domain NMR, we have shown that in milk fat samples an intermediate component state clearly exists between the solid and liquid phases, constituting only about 6% of an aged milk fat. The T_2* distribution of these components in this intermediate state shows two peaks at about 60 and 170 μs. We have shown from the T_2* resolved T_1 distribution of the peak, corresponding to a T_2* of approximately 60 μs, that there is in the continuity in the crystalline phase. This first intermediate component state does not exist in pure triglyceride or in cocoa butter, and is scarcely present in a tristearin crystal/soy oil suspension. We have attributed this first intermediate component to fatty acid residue extremities that protrude from the crystalline phase and/or to chain ends at the edges of holes created by short chains.

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KEY WORDS: semisolid phase, T_1 and T_2* relaxation times, time domain NMR.

The solid-to-liquid ratio in a fat is one of the most important physical quality control measurements currently used in the fat industry. The properties of a food product can be affected by fat crystallization and, as far as the consumer is concerned, taste, texture, and shelf life are very important (1). If the milk fat, for instance, is in a liquid state then the taste will be buttery; when a large quantity of crystalline fat melts in the mouth it leaves a cool impression. However, when there are large fat crystals the product feels gritty or sandy (2). Many smaller crystals remaining unmelted in the mouth will produce a sticky taste (3). The consistency of a fat is a function of both its composition and thermal history (2,4). Food fats are principally mixtures of a great number of triglycerides. As physical systems, they are liquid crystals; they exhibit polymorphism and form solid solutions of mixed crystals (2,3).

The fat industry has a great need to monitor its raw materials as well as its finished products, and several methods have been developed over the years. The two most practical techniques are differential scanning calorimetry (DSC) (5–7) and low-resolution nuclear magnetic resonance (LR-NMR) (8–10), which is considered the best method (3). The principal advantage of LR-NMR is that it is a nondestructive observation technique which can be carried out by non-skilled staff. Measurements made do not disturb the sample, so it preserves its thermal history, and the quantity of material analyzed (1–2 g) is more representative of the sample than measurements made by DSC.

However, NMR and DSC methods give different values when the solid fat index (SFI) is examined (11–13); NMR values are much lower than those given by DSC below 20°C. For example, at 5°C, with milk fat, DSC gives 78.1% as solid and NMR 43.7% (14). A part of this difference is due to the fact that DSC measurements generally take the same average melting enthalpy into account for each entity; using a linear variation of melting enthalpies improves results (15).

Furthermore, because these methods probe different physical features of the material, the correlation between the NMR and the DSC results is only satisfactory in certain solid-to-liquid ratio ranges, i.e., between 0 and 20% and between 25 and 90% for "raw" milk fat (16). The parameters obtained vary according to the sample type (high or low melting point fractions). On the other hand, the determination of the absolute crystallinity by X-rays necessitates painstaking measurements and the "crystallinity index" which is often used is, at best, only proportional to the degree of crystallinity (17).

The observed difference could be explained by the presence of an amorphous phase which, due to its melting enthalpy, is seen as a solid by the DSC method. If its T_2 relaxation time is in the 50–200 μs range (16), this amorphous phase will be seen partly as a liquid by NMR; with a T_2 value of the order of 100 μs, for example, at time t = 70 μs (measurement time of the liquid phase in "direct" and "indirect"

*To whom correspondence should be addressed at Laboratoire d'Analyse Isotopique et Electrochimique de Métabolismes, CNRS UPRES A Q6006, BP 92208, Faculté des Sciences, F-44322 Nantes Cedex 03, France.
E-mail: denis.lebotlan@chimbio.univ-nantes.fr

NMR method) 61% of its total signal is measured, which means that 61% of this amorphous phase is regarded as a liquid phase and 39% as a solid phase by NMR.

Previously, by analyzing the free induction decay (FID) NMR signal obtained with a low resolution NMR spectrometer, two intermediate components between the solid and liquid phases ($T_2^* \approx 60$ μs and $T_2^* \approx 170$ μs) were observed.

The aim of this work is to characterize this first intermediate component using T_1 measurements resolved from the T_2^* values. This intermediate component could be: (i) an actual amorphous phase, (ii) the extremities of chains having a greater mobility than those inside crystals (flaws inside and/or on the periphery of crystals); or (iii) a liquid phase on the surface of the crystals, between the needles of the fractal structure of crystals (18,19) or in aggregates, which have reduced mobility, and liquid crystals.

EXPERIMENTAL PROCEDURES

Samples. The samples used were supplied by ELVIR (Condé Sur Vire, France). We concentrated essentially on anhydrous milk fat and on fractions with 10 and 41°C dropping point temperatures. Each sample was prepared in the following way: melted (10 min at 70°C), cooled to room temperature for 1 h, and then stored at 0°C (ice-water mixture) or 20°C (± 0.5) for 24 h. Tristearin was Sigma (St. Louis, MO) 99% commercial grade. The SFI for the 41 and the 10°C fractions were, respectively, 78.6 (0°C), 63.4% (20°C), and 18.0 (0°C) and 100% (20°C); standard deviation was approximately 0.5%.

NMR. All measurements were made using a Bruker (Karlsruhe, Germany) PC 120 LR-NMR spectrometer (operating frequency 20 MHz) using a 10 mm probe (10 VTS). Measurements were made at 0 (±0.5), 7, and 20°C. The sampling of FID (90° pulse width ≈ 1.1 μs) was made using the TEAM 490 program and BE490 acquisition card (12 bit) at 1 MHz (Bakker Electronics, Dongen, The Netherlands). The acquisition parameters were: relaxation delay, 3 s for milk fat samples; attenuation, 35 db; mode detection, diode for FID signal acquisition; filter, 100 KHz. The inversion–recovery method was used for the T_1 measurements: phase sensitive detection mode (PSD); 25 data points; 16 acquisitions. The SFI was determined by the "indirect NMR method," which consists of measurements at a given temperature and also at 70°C, 70 μs after the 90° pulse on the samples, plus a reference oil (20); sample height was 20.0 ± 0.5 mm.

T_1 measurements. The distribution of the relaxation time T_1 was determined for the milk fat at 7°C, for the "10°C and 41°C" fractions of the milk fat samples at 0°C, and for the fraction 41°C at 20°C as well. In order to separate the T_1 distributions that corresponded to particular phases (solid, semisolid, and liquid) according to their T_2^* relaxation time, measurements were made at different times τ_j (11, 40, 50, 70, 80, and 150 μs) after the 90° observation pulse.

The inversion–recovery sequence was as follows:

$$180°x - \tau_i - 90°x - \tau_1 - S_{i1} - \tau_2 - S_{i2} - \tau_3 - S_{i3} \cdots \qquad [1]$$

with S_{ij} = measurements corresponding to the τ_i delay and to the τ_j measurement times. Given that the FID NMR signal is Gaussian in shape in an inhomogeneous magnetic field, the signal of a component is practically zero at a time equal to 2.5 times its T_2^* $\{\exp[-(2.5)^2] = 1.9\ 10^{-3}\}$. Table 1 shows the percentages of two signals of $T_2^* = 60$ and 170 μs measured at different times; at time 40 μs the signal of a solid phase ($T_2 \approx 18$ μs) is nil. We can see that if the intensity of the two components of $T_2^* = 60$ and 170 μs are of the same order of size, the difference in signal $S_{40–70}$ and $S_{50–80}$ corresponds principally to the short component ($T_2^* = 60$ μs).

To take into account the 180° pulse imperfection, the following relation was used :

$$I_i = I_0\left[1 - (1 - \cos\theta)\ \exp\left(\frac{-\tau_i}{T_1}\right)\right] \qquad [2]$$

where I_i, signal corresponding to the interpulse τ_i, I_0 total signal corresponding to τ_i greater than $7 \cdot T_1$, and θ is the real angle of the 180° pulse; the obtained value was about $1-\cos\theta = 1.75$.

RESULTS AND DISCUSSION

A Carr-Purcell-Meiboom-Gill (CPMG) sequence cannot be employed to show the existence of an intermediate phase, whose T_2^* is in the range of 50–600 μs, since the Bruker Minispec has certain technical limitations. The emitter system cannot handle a pulse sequence with pulse spacing lower than 50 μs. Since measurements are taken at the top of the even echoes, in order to benefit from the imperfection correction of the 180° pulse, the first measurement is only possible at $4.\tau = 200$ μs. Few data points are then available to determine such relaxation times. However, we have shown the possibility of obtaining quantitative information about intermediate states from NMR FID signals (21).

The magnetic field used in low resolution NMR is very inhomogeneous. This inhomogeneity is often represented by a term called the "T_2 of inhomogeneity" (T_{2inh}), which is of the

TABLE 1
Percentage of Signal (Gaussian in shape) Measured at Times 40, 50, ..., and 150 μs (S_{40} ...) and Between 2 Times ($S_{40–150}$ ...), According to 2 T_2^* Relaxation Times

T_2^* (μs)	S_{40}	S_{50}	S_{70}	S_{80}	S_{150}	$S_{40–150}$	$S_{40–70}$	$S_{50–80}$
60	64	50	26	17	0	64	38	33
190	96	93	87	84	54	42	9	9

order of 1–3 ms for low-resolution NMR spectrometers. The apparent relaxation time T_2^* is linked to T_2 and T_{2inh} by the relation (22):

$$\frac{1}{T_2^*} = \frac{1}{T_2} + \frac{1}{T_{2inh}} \quad [3]$$

For the liquid part of a milk fat sample, the hydrogen atoms' relaxation can be represented by two or three average T_2 values of the order of 30–110 ms at 5°C (23). The term $1/T_2$ is therefore negligible in comparison to the $1/T_{2inh}$ term. Thus, whatever the chemical composition may be, the liquid phase will give the same FID signal, except if the dissymmetry of the sample modifies the local magnetic field B_o homogeneity. Due to this B_0 inhomogeneity, the liquid part of the FID signal is Gaussian in shape,

$$I = \sum_i I_i \cdot \exp[-(t/T_{2i}^*)^2] \quad [4]$$

instead of exponential.

On the other side of the mobility range, a crystalline phase has a very short T_2 relaxation time of about 18 μs and its signal becomes close to zero after 40 μs. The parameters T_2 and population, determined beyond 40 μs, which differ from those obtained with liquid oil, are therefore attributable to semisolid phases.

T_1 relaxation time of crystalline phases. Contrary to the spin–spin relaxation times, T_2, the evolution of the T_1 parameter according to mobility is not monotone but has a U form. These two parameters represent the evolution of magnetic vectors in the *XY* plane (T_2) and along the *Z* axis (T_1). For a correlation time (τ_c) such that $(\tau_c * \omega_0)^2 >> 1$ (ω_0, angular frequency), the value of T_1 according to the equation

$$\frac{1}{T_1} = \frac{3}{5} \frac{\gamma^4 h^2}{r^6 \cdot \omega_0^2} \frac{1}{\tau_c} \quad [5]$$

γ, proton magnetogyric ratio
h, Planck's constant
r, internuclear distance

increases therefore with τ_c (24), with the lattice density of the solid phase; a liquid phase and a solid phase can have the same T_1 value. Thus for tristearin at 37°C and at 20 MHz, a T_1 of 250 ms was measured for the α-form (hexagonal) and of 1.7 s for the more compact β-form (triclinic) (25,26), and 380 ms (α) and 2.8 s (β) at 60 MHz and at room temperature (27). In our studies, we obtained a T_1 value of 5500 ms for commercial-grade crystalline tristearin and 148 ms for the same melted tristearin sample put into a water-ice mixture (Fig. 1, curve T); it is worth noting that the T_1 distribution of this sample had not changed after 1 wk at room temperature.

Magnetic vectors evolve according to the spin-lattice relaxation mechanism along the *Z* axis. Unfortunately, no measurement is possible along this axis. A 90° observation pulse is needed to move magnetic vectors in the *XY* plane, where, obviously, they proceed according to a spin-spin (T_2^*) relaxation mechanism. The determination of the T_1 parameter corresponding to a selected signal according to its T_2 is then possible. For the characterization of the crystalline phase in a heterogeneous medium, the choice of the measurement times is simple since the dead time of the receiver coil (10 μs) prevents any measurement before 11 μs. On the other hand, the FID signal of solid tristearin shows that the "solid" signal is nil at 40 μs. Therefore, the T_1 distribution of the solid phase was obtained by the treatment of the difference between the measurements taken 11 and 40 μs after the 90° observation pulse (S_{11-40}). The T_1 distribution of the solid phase of a 10° dropping point fraction of milk fat is presented in Figure 1 (curve M). This distribution corresponds to the most compact phase of the sample.

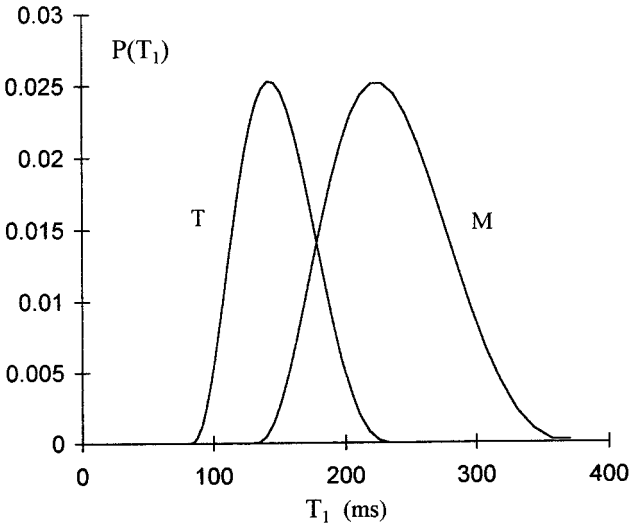

FIG. 1. T_1 distribution of a tristearin sample (T) and of the solid phase of a 10°C dropping point milk fat fraction (M) at 0°C (both samples cooled rapidly).

We can observe that the milk fat T_1 distribution, (S_{11-40}), is monodisperse and has, at a first approximation, a Gaussian form, like the distribution obtained for melted tristearin and prepared under the same conditions (Fig. 1). From the small difference between the T_1 values of the α-tristearin and the milk fat (148 ms and 220 ms), we can conclude that the average mobility of the solid phase of this milk fat sample is only slightly weaker than in α-tristearin. As it does not show a distribution with a short T_1 or a shoulder on the short T_1 side, it is therefore the T_1 distribution of the crystalline phase, without a significant amorphous phase. Such fractions with a low melting point are the most likely to show an amorphous phase, since they give the greatest difference between the DSC and NMR solid fat index (14). It is also worth noting that an amorphous phase can have a very short T_2. The NMR parameters of amorphous polyvinyl chloride, for instance, are, at 40°C, T_1 = 365 ms and T_2 = 19.6 μs. As an amorphous phase can contribute to the relaxation of a crystalline phase through a spin diffusion mechanism (28) if the two phases are coupled, we must say that there is no amorphous phase, with a short T_2, decoupled from the crystalline region.

Thus, we can conclude that the T_1 value can be a good parameter to describe the average mobility of a solid or a semi-

solid phase of a fat sample at heat balance, according to its thermal history or tempering.

Intermediate states. In order to obtain further confirmation that the distribution at about 60 μs corresponds to a true chemical entity and not to a mathematical entity, cocoa-butter was studied. The advantage of this product is that it has a simpler composition than milk fat. It is mostly constituted (about 72%) of saturated and unsaturated C_{18} fatty acids (29). The structure of cocoa-butter crystals must therefore be more regular than that of milk fat and it should not have so many flaws.

The FID signal analysis of aged and fresh melted cocoa-butter samples shows, in fact, the absence of a relaxation component at approximately 60 μs. With 11 samples prepared in three different ways, the shortest T_2 relaxation time of the nonsolid phase ($T_2 > 30$ μs) is in the range of 110 μs to 220 μs; six values are in the range of 130–160 μs.

Mixtures of oil and tripalmitin or tristearin crystals do not show any component in this range either.

This interpretation of the short T_2* component is also being confirmed by a study of native, partly hydrolyzed and litner (crystals) of different starches which show a similar T_2* component at 60–80 μs for the two first products only, in agreement with the model of fringed micelle (30) and of Blanshard (31).

The FID signals of milk fat samples (Fig. 2) were fitted to performing Gaussian functions using a visual semilogarithmic method. These relaxation parameters were then used as starting values in a global multi-Gaussian fitting by the NLREG program (32). The convergence of a multi-Gaussian fitting is impossible to achieve without good starting parameters. First a multi-Gaussian fitting was validated, as shown by the random scattering of residues (Fig. 3) obtained with a 41°C dropping point milk fat at 20°C with the following

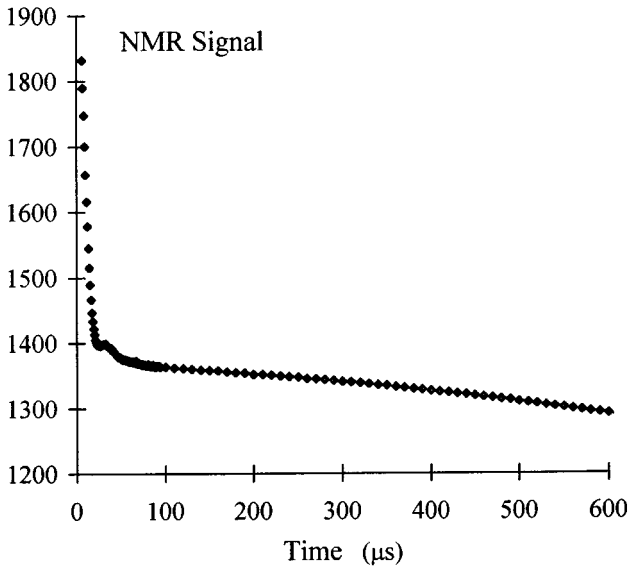

FIG. 2. Free induction decay (FID) nuclear magnetic resonance (NMR) signal of a 41°C dropping point milk fat fraction at 20°C (30.2% of solid phase).

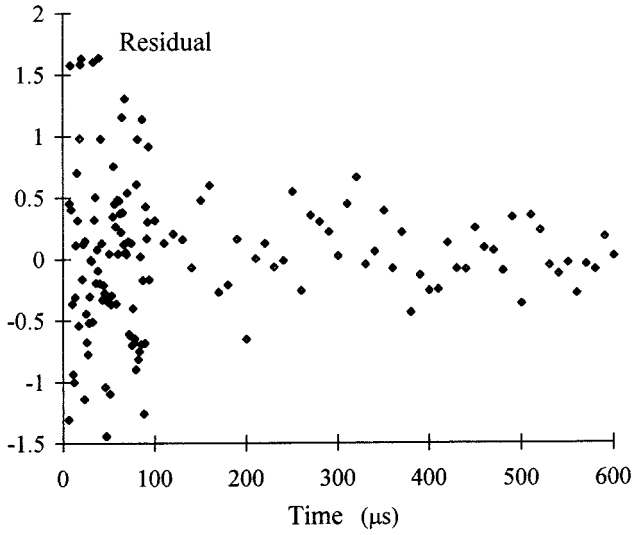

FIG. 3. Plotting of residuals obtained by a Gaussian fitting of the FID NMR signal shown in Figure 2, with the same arbitrary unit. For abbreviations see Figure 2.

NMR parameters: T_{2a} = 3620 μs, I_a = 58.0%, T_{2b} = 1321 μs, I_b = 10.3%, T_{2c} = 194 μs, I_c = 0.4%, T_{2d} = 55 μs, I_d = 1.1%, T_{2e} = 18.8 μs, I_e = 30.2% (variation coefficient 0.02 %). Then the CONTIN program (33) was used to determine the T_2* distributions of the intermediate and liquid entities. The T_2* distributions coming from the liquid phase of samples were determined by comparing the I_0 intensity of the CPMG relaxation curves to the intensity of different T_2* components. Generally, T_2* values greater than 600 μs, that is to say, T_2 greater than about 1 ms, correspond to a liquid phase. The fitting results are presented in Table 2. We note that the two long component T_2* values are of the order of those obtained for the reference oil. On the other side of the mobility range, the short T_2 distribution maxima correspond to the two T_2 values obtained by the discrete multi-Gaussian fitting, at about 60 and 170 μs for 1.5 to 7.3% of the total signal in milk fat samples or in fractions (Fig. 4) (Table 3); for four samples of the same milk fat with the same tempering, the variations in parameters were smaller (Table 4); the standard deviation is 0.45 point for the SFI result and 0.52 point for the total percentage of intermediate phase, PI.

The question is now whether the T_2* component at about 60 μs corresponds to a solid state or to a liquid one. As shown

TABLE 2
NMR Parameters of the Liquid Phase Obtained by Multi-Gaussian Fitting of FID NMR Signal for Different Samples (Sa), 10 and 41°C Dropping Point Milk Fat Fraction and Soy Oil (oil) at 0° and 20°C[a]

T (°C)	Sa	T^*_{2a} (μs)	I_a	T^*_{2b} (μs)	I_b
0	10°C	3030	1415	475	30
0	41°C	2490	488	483	45
0	oil	3105	1575	489	16
20	41°C	3080	1165	655	58
20	oil	2890	1790	449	14

[a]For abbreviations see Table 1.

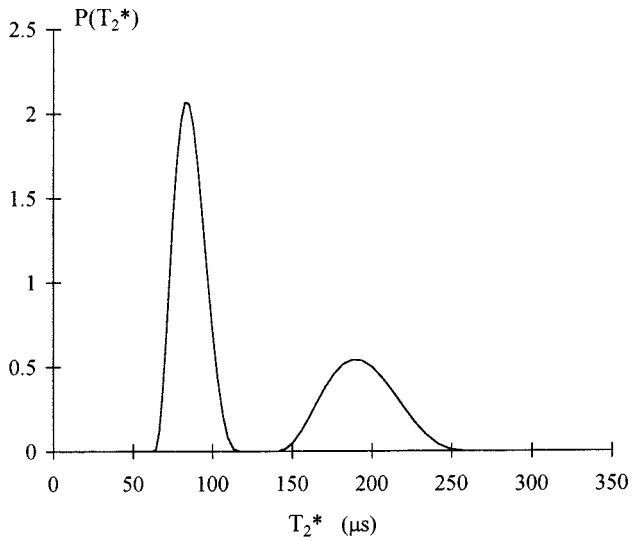

FIG. 4. T_2* distribution of the intermediate component in a milk fat sample at 7°C.

earlier, a T_1 study can provide valuable information. To determine the T_1 value of this phase, the differences between the measured signal intensities at 40 μs and 150 μs ($S_{40–150}$) were analyzed. Unfortunately, this partial signal ($S_{40–150}$) does not entirely correspond to the first intermediate component but takes into account about 64% of this one and about 49% of the second one.

To clarify the origin of this signal ($S_{40–150}$), we have also followed the evolution of the distribution of T_1 inside this range, from measurements taken at 40, 50, 70, and 80 μs (Fig. 5). The continuous decrease of the maxima 228, 150, and 100 μs in the increasing time range of measurements ($S_{11–40}$, $S_{40–70}$ and $S_{50–80}$) was typical of a continuous evolution of the mobility from the crystalline state. We have already seen that the T_1 values of a solid phase decrease when the mobility increases.

Such an evolution in mobility, from a quasi-crystalline state to a state resembling a liquid, is not dissimilar from the notion of "liquid-like-end-groups" presented by Hernqvist (18) and corresponding to the ends of long residues of fatty acids which protrude from the crystals and to chains at the edges of flaws (34). This structure is not surprising in milk fat, which consists of more than a hundred different fatty acid residues (2) and which can give rise to a mixed crystal formation (2).

To confirm this hypothesis, we considered that a single mixture of tristearin crystals and soy oil, not consisting of any solid phase at 0°C, should not present any significant quantity of "liquid-like-end-groups." Actually, measurements $S_{40–70}$ do not show a significant change in function of the pulse spacing τ_i; the treatment of these points by the CONTIN program did not converge due to a very poor signal-to-noise ratio.

The treatment of the signal $S_{150–400}$ gave a different distribution from the preceding one and also from that of the liquid phase, which was obtained conclusively by treating the measurements carried out at 1000 or 1500 μs. We are therefore faced with the possibility of a fourth state, different from the solid, semisolid, and liquid phases. The T_2* distribution can correspond to either an amorphous phase or liquid (i) overlapping with a star-like structure of the crystals (2,19), (ii) between radial needles (2,34), (iii) in aggregates, or liquid crystals. We are currently trying to solve this problem.

TABLE 3
Relaxation Times T_2* and Populations *P* of the Intermediate Components of Milk Fat (MF), 10°C (MF 10°), and 41°C (MF 41°) Dropping Point Fraction, According to Temperature and Tempering: Melted at 60°C Then (i) Cooled at 0°C in Water-Ice Mixture, (ii) Kept at Room Temperature for 24 h, (iii) Cooled Slowly (≈0.05°C/min) at Room Temperature, Then All Samples Were Kept at Measurement Temperature for 24 h

	T (°C)	T^*_{2I1} (μs)	P_1 (%)	T^*_{2I2} (μs)	P_2 (%)	SFI (%)
MF 41°a	0	52	4.0	167	3.3	64.4
MF 41°b	20	57	1.1	200	0.4	30.0
MF 10°a	0	61	2.3	227	1.1	19.4
MF c	7	45	3.0	151	1.3	45.0
MF b	7	60	2.4	149	1.1	44.2
MF a	7	55	3.6	141	1.4	51.9

TABLE 4
T_2 and Population of the Three Intermediate Components of Four Samples of Milk Fat; SFI, Solid Fat Index Determined from Populations of T_2* Relaxation Times (the T^*_{2I1} ≈ 55 μs Component has been Taken into Account in the Solid Phase and the Others in the Liquid Phase), Signal, Total Signal in Arbitrary Units per Gram of Fat; PI, Total Percentage of the Intermediate Phase

	SFI	T_{2I1}* (μs)	$P_{1\%}$	T_{2I2}* (μs)	$P_{2\%}$	T_{2I3}* (μs)	$P_{3\%}$	Signal	PI (%)
1	47.0	50	3.3	140	1.3	327	1.3	1505	5.9
2	46.0	50	3.5	150	1.4	372	1.3	1498	6.2
3	46.9	53	3.1	150	1.4	372	1.4	1499	5.9
4	46.6	64	3.1	194	1.2	327	0.7	1502	5.0

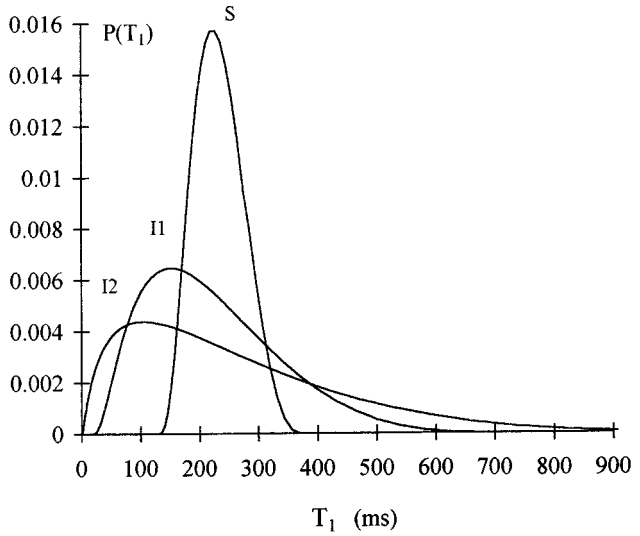

FIG. 5. T_1 distributions of the solid phase $S_{11–40}$ (S), and of the intermediate components $S_{40–70}$ (I_1) and $S_{50–80}$ (I_2) of a 10°C dropping point milk fat fraction at 0°C. The true intensity of these signals (distribution surface) are 445, 36.5, and 27.5 a.u. respectively; for the meaning of index 11–40, 40–70, and 50–80, see the text.

To conclude, in milk fat, the T_1 distribution corresponding to the first intermediate component whose T_2* is approximately 60 μs is in agreement with the "liquid-like-end-group" structure and/or with the occurrence of chain ends at the edges of holes created by shorter chains, which have a greater mobility than those inside crystals; if this T_2* component was a separate amorphous phase, it would have its own T_1 peak. Furthermore, these results show that semisolid states constitute less than 7% in milk fat.

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