NMR Relaxation Studies to Explore the Role of Emulsifier in Tristearin Polymorphic Transformation

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Proton magnetic resonance measurements were performed on α and β polymorphs of tristearin crystals. T_1 relaxation time was measured with the Bruker PC Minispec at 20 MHz and 37° on the pure triglyceride crystals and therefore on tristearin crystallized in the presence of different solid surfactants. A significant negative correlation for T_1 values and different compositions of α and β polymorphs in a mechanical mixture was found. The shortening of T_1 relaxation time of the β form was found to be different for the various types of the added surfactants while they have no effect on the relaxation time of the α form. A model was proposed according to which the presence of additives, such as surfactants, generates vacancies within the crystal lattice of triglyceride and enhances the freedom of rotation of the fat molecule about the terminal carbon-carbon bond.

Nuclear magnetic resonance (NMR) relaxation time measurements constitute a very sensitive probe for the study of the molecular environment. The pulse techniques usually are used to obtain information from spin-lattice relaxation time (T_1) and spin-spin relaxation time (T_2). The comparison of relaxation time values for various phases of a certain chemical compound leads to conclusions concerning the mobility of the molecule in the respective phases. The T_2 measurements have been used as a superior method in the determination of liquid/solid fractions of fats (1-4), mainly in commercial products.

The differences in mobility in polymorphic forms of fats are slighter than those between the liquid and solid fractions. Nevertheless, the NMR pulse technique, especially the T_1 relaxation times, can be applied to distinguish between various polymorphs of fats. To the best of our knowledge Norton et al. (5) were the first to report T_1 values for α and β crystal forms of tristearin and tripalmitin.

The β-form is more densely packed than the α polymorph. The T_1 for the β-form of tristearin was reported to be larger than that for the α-form by a factor of about 7(5), confirming that the molecules in the α-form have a higher freedom of mobility than in the β-form.

The high sensitivity of the NMR spin-lattice relaxation times to slight differences in the environment of fat molecules has been used in the present work to study the plausibility of defects generation in the fat crystals by compatible surfactants. The crystallization processes of fats are known to be modified when surfactants are used as additives (6-9). The surfactants, although not detectable by X-ray diffraction of the fat crystal, strongly affect the kinetics of polymorphic transformations. Recently, a study of heat capacity of tristearin which was crystallized in the presence of solid surfactants led to the deduction that the additive may generate defects in the tristearin crystals (10). The mobility of the hydrocarbon chains was most probably enhanced by the defects. The present study, applying the spin relaxation time (T_1) to tristearin crystallized with various surfactants, was undertaken in order to confirm the generation of defects by the surfactants. This may lead to suggestions concerning the way in which emulsifiers are blended into fats, on a molecular level, thus providing a basis for better understanding of their functions in polymorphic transformations.

MATERIALS AND METHODS

Tristearin 99% pure was purchased from Sigma Chemical Co., St. Louis, Missouri, and the emulsifiers from Grindsted Products, Denmark.

The following emulsifiers were tested: sorbitan monostearate (SMS), sorbitan tristearate (STS), sorbitan monolaurate (SML), monoglyceride of stearic acid (GMS), citric acid ester of monoglycerides (CGMS) and triglycerol-1-stearate (3GlS). The emulsifiers were added at percentages ranging between 1 and 15 wt%; the mixtures were blended in the molten state and quenched in order to obtain the α-form. Subsequently they were aged at 45 C for a few days to obtain the stable β-form.

NMR measurements. The measurements were performed on 50 mg crystalline tristearin samples with a Bruker Mini-Spec model PC-20 NMR spectrometer using the standard inversion recovery pulse sequence. The composite proton signal was observed at 20 MHz, at 37±1 C. The acquisition conditions were: T_1 relaxation times were determined by the 180°-τ-90° method and a 3-parameter fit. These parameters were T_1, pulse width and saturation magnetization value. τ Was varied in geometric progression in eight steps. The first τ value was from 0.012 sec to 1.526 sec. The last value was from 0.064 sec to 8.19 sec. The recycle times were at least five times the T_1 value. Every point was scanned nine times to increase the signal-to-noise ratio.

X-ray. The X-ray powder diffraction measurements were performed using a Philips diffractometer with monochromatized CuK radiation. The samples were scanned in the 2θ range of 18-25°.

RESULTS AND DISCUSSION

The T_1 relaxation times of α and β forms of pure tristearin were measured. In Table 1 the values obtained in the present study are compared to those of Norton (5). The values reported by Norton are higher, but with the same trend. The differences between the results probably are due to the dependence of T_1 on the strengths of the magnetic fields used in the two studies: Norton used a field of 60 MHz, whereas in the present study a 20 MHz field was used. However, the ratios of the T_1's of the two forms in both studies are very close (Table 1), accentuating the clear distinction between the α and β forms.

The X-ray diffraction pattern of the hexagonal α-form shows a single reflection within the 2θ range of

TABLE 1

Spin-Lattice (T_1) Relaxation Times Recorded for Tristearin in the α and β Crystal Form

Authors	α form (sec)	β form (sec)	$T_1(\beta)/T_1(\alpha)$
Norton et al.	0.38	2.8	7.36
Present study	0.25	1.7	6.8

$18<2\theta<25$ with a d spacing of 0.314 nm (11). In this form the molecular configuration of a tristearin molecule is symmetrical with respect to the hydrocarbon chain axis. The mobility freedom of the hydrocarbon chain is relatively high, as some rotation around its own axis is allowed due to the low density of their packing. In the β-form, obtained after aging of α, the symmetry of the molecules is lower, conforming with the denser packing. Consequently, the mobility of the hydrocarbon chains, which was shown to be related mainly to terminal methyl groups in the α-form (12), must be restrained in the β-form. It is known that the T_1 relaxation time varies in different substances because of differences in their lattices (13). In solids the spin-lattice relaxation time is long due to poor energy exchange between the processing protons and the lattice. Therefore, it can be inferred that denser lattices have higher T_1 values than lower density lattices. Hence, the difference between T_1 values of α and β forms seems to be caused by their different mobilities in the hydrocarbon chain regions.

In order to evaluate T_1 relaxation time for a random composition of α and β forms, a series of mechanical mixtures at various ratios was prepared and analyzed by the Mini-Spec. A linear correlation ($r = -0.991$) was found between the T_1 values and the composition of α and β mixtures (Fig. 1a). The mechanical mixtures were tested by powder X-ray diffraction (Fig. 1b) to confirm their composition. It can be seen that the T_1 analysis enables the detection of the presence of two polymorphs in a mixture of pure fat.

Figure 2 summarizes the T_1 values of α and β tristearin crystals formed in the presence of several solid surfactants. The lower curve shows that the surfactant presence does not affect the relaxation time of the α form.

In the β-form, with any emulsifier added the T_1 reaches values shorter by factors of 1.8–3.2 than those of pure triglyceride in the same polymorphic modification. This reduction seems to be significant because both measurements were carried out under identical conditions. The reduction in T_1 is most pronounced in the presence of 3GlS and the least in the presence of SMS. A regression analysis of T_1 vs the percentage of the additives did not fit a linear relationship. In all cases the changes in T_1 values follow a visible hyperbolic pattern. This indicates that the effect at a low level of admixture is relatively strong, reaching saturation at higher percentages of surfactants.

In order to ascertain that the T_1 times obtained with the addition of the surfactant are not the result of a weighted average obtained from the two materials,

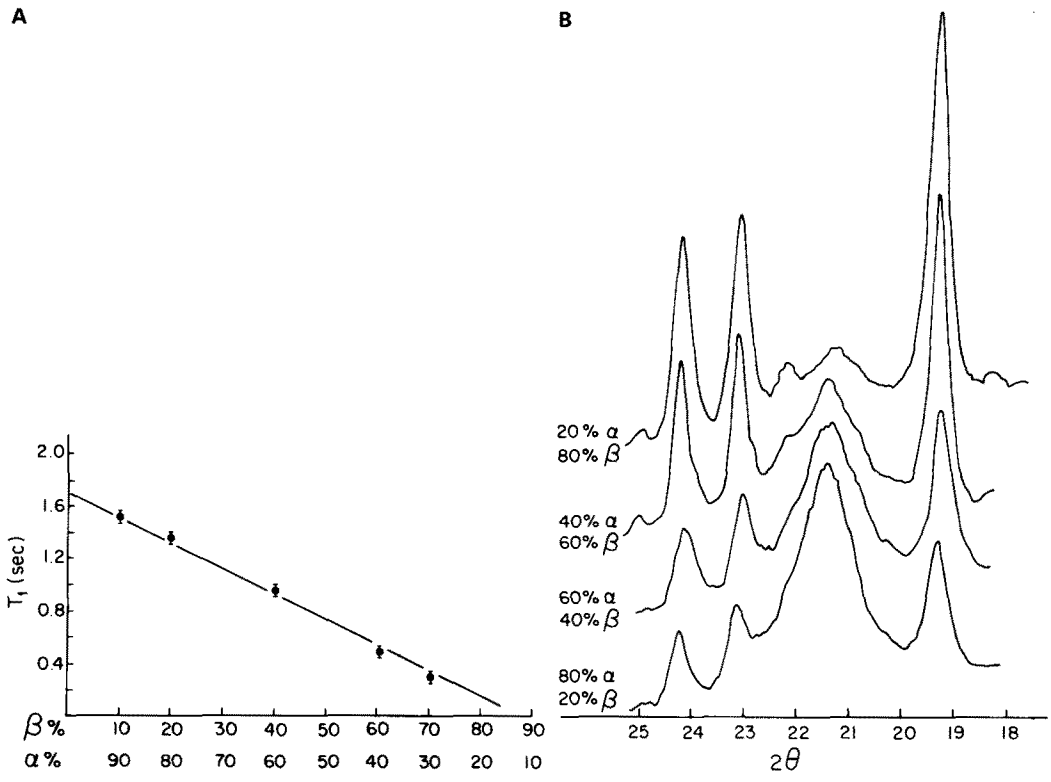

FIG. 1. A. T_1 relaxation time vs different ratios of α and β forms of tristearin crystals. B. Diffractograms of different ratios of α and β forms of tristearin crystals.

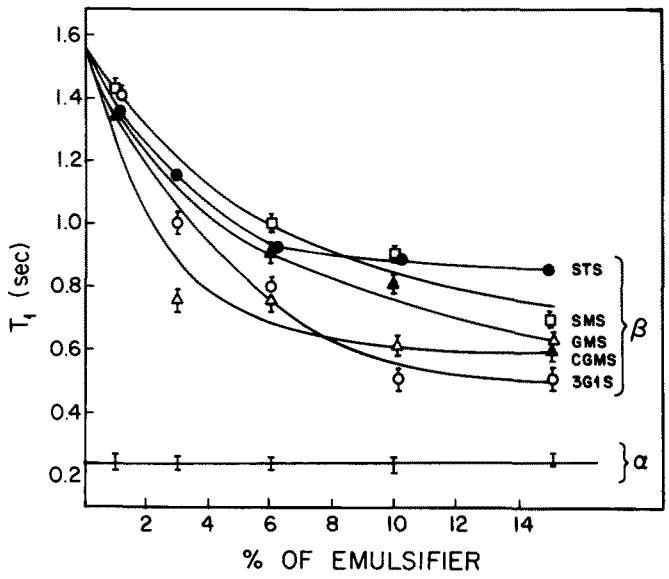

FIG. 2. T_1 relaxation time of tristearin crystals vs different amounts (% w/w) of added surfactants.

mechanical mixtures of tristearin with GMS and SMS were prepared independently and analyzed by the Mini-Spec. The values obtained (Table 2) confirm that the results presented in Figure 2 are not weighted averages of the two materials. It follows that those were obtained from a homogeneous mixture of fat and emulsifier.

The reduced values of T_1 with additives might have been ascribed to an incomplete aging from α to β during the sample preparation (see Materials and Methods). T_1 values of 0.5–0.8 sec correspond to the composition of about 50% α and 50% β (Fig. 1). However, the X-ray data of these samples showed that the material was pure β without any detectable trace of α.

Because the experimental evidence indicated that the reduction in T_1 yielded by tristearin with emulsifier is not due to an incomplete aging of the fat, the pronounced decrease must have another cause. A more plausible explanation for the decrease of T_1 can be suggested considering the extent of rotation about the terminal carbon-carbon bond of the tristearin molecule. The incorporation of the emulsifier may generate vacancies between adjoining hydrocarbon chains. This seems to occur because the surfactant molecule is supposed to be posed in a place normally occupied by a triglyceride molecule but, being smaller, it leaves an unfilled space. These vacancies enhance the mobility of the neighboring long tristearin molecules, thus reducing the relaxation times. A schematic illustration of the incorporation of the emulsifier within the fat is shown in Figure 3. These suggested structural effects would not cause any significant changes in the X-ray diffraction pattern as confirmed in Figure 4 because the defects are randomly spaced in the fat. On the other hand, heat capacity (Cp) measurements of tristearin crystallized with the addition of emulsifiers led to the conclusion that in their presence the freedom of mobility of the fatty hydrocarbon chains increased (10). The good agreement between the results of Cp and spin-lattice measurements supports the hypothesis of vacancies generation as a result of emulsifier incorporation in fat.

TABLE 2

Spin-Lattice Relaxation Times (T_1) Recorded for Mechanical Mixtures of β Tristearin Crystals and Solid Surfactants

Sample	T_1 (sec)
GMS 100%	1.37 ± 0058
GMS/Tristearin 10/90	1.40 ± 0.15
GMS/Tristearin 90/10	1.55 ± 0.13
SMS 100%	0.090 ± 0.004
SMS/Tristearin 10/90	1.46 ± 0.11
SMS/Tristearin 90/10	0.103 ± 0.016

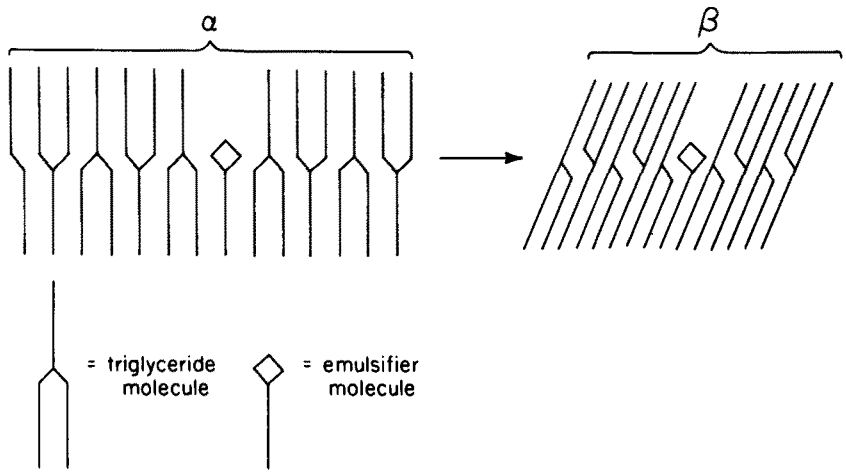

FIG. 3. Schematic illustration of the incorporation of surfactant within the fat crystals.

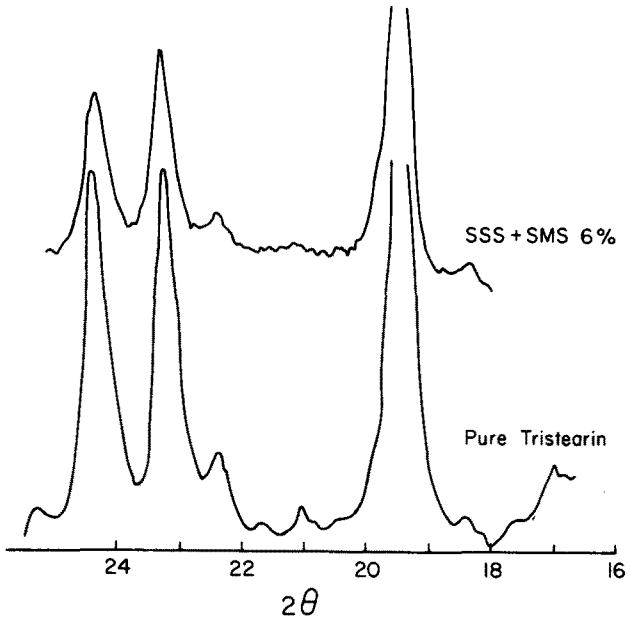

FIG. 4. Comparison between diffractograms of pure tristearin crystals and tristearin crystallized in the presence of 6% (w/w) SMS.

In contrast with the recognizable effect of surfactants on the β-form, their presence does not affect the T_1 values of the α-form (Fig. 2). It can be inferred that the lack of change is due to the similarity between the spacings of the fat and the surfactant when both are independently quenched from the melt. In the polymorphic structure the presence of the additive does not increase the rotational freedom of the terminal carbon groups beyond their inherent mobility in the nondensely packed crystal.

The variation of the curves in Figure 2 can be ascribed to the different structures of the additives. Cp measurements (10) and phase diagrams (14) implied that SMS has a better structural compatibility with the triglyceride than the other emulsifiers used. On the other hand, 3GlS, which has a bulky hydrophilic head, probably will generate larger vacancies than SMS, showing more pronounced shortening of T_1. Thus it appears that the shortening of T_1 depends upon the hydrophilic moiety of the additive and on the dimensional fit with the fat molecules.

The present results indicate that with relatively low concentration of additive the reduction in T_1 is more accentuated than in the higher range. This may indicate that the increase in mobility is not confined to the nearest neighbors but is shared by more distant molecules, i.e., that a relatively low number of vacancies will provide an effect of saturation.

Though the measurements of spin-lattice relaxation time constitute a very sensitive technique for comparing the relative freedom of mobility in the terminal parts of a fat molecule, it cannot be used for the recognition of polymorphs unless the purity of the sample is known.

From the present study, and on the basis of previous information (10-14), the following model for the incorporation of an emulsifier in fat may be suggested. On entering into the crystal structure, the surfactant molecule aligns itself along the longer fat molecule with its polar functional group in the close vicinity of the fat glycerol moiety causing the formation of a gap between two adjacent triglyceride molecules (Fig. 3). The formation of vacancies accounts for the reduction of spin-lattice relaxation times reported in this study.

The conclusions presented are in good agreement with those drawn from the thermal study (10). They are also consistent with other results which relate to the effect of surfactants as dynamic controllers of polymorphic transformations (14). A better understanding of the mode of the additive incorporation will improve our comprehension of the mechanism of its action as controller of polymorphic transformations.

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