Dynamic Polymorphic Phase Transitions in a Model Binary Triglyceride System Measured by Position-Sensitive X-ray Diffraction Methods

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X-ray diffraction measurements have been made on a binary mixture of pure tripalmitin and tristearin which undergoes particular polymorphic phase transitions on heating from the solid. The results were obtained using a position-sensitive detector which can record the whole diffraction pattern in time periods less than a minute. This allows many patterns to be recorded during the transformations where heating rates can be up to several °C/min. As such the transitory existence of the intermediate β′ phase has been unambiguously identified. The results serve to exhibit the usefulness of the X-ray apparatus and indicate its potential for future studies in dynamic crystallization and melting.

KEY WORDS: Fats, melting kinetics, phase transitions, polymorphism, position-sensitive detector, triglycerides, X-ray diffraction.

The polymorphic behavior of fats has been known for a long time and good reviews are given by Timms (1) and by Garti and Sato (2). Initially observations of the phenomenon were made on samples which underwent multiple melting during the course of heating from the original solid. Later on, distinct crystal phases were identified using X-ray powder techniques, and the energies of the various transitions could be detected using calorimetric methods such as Differential Scanning Calorimetry (DSC). Another technique useful in supplying supporting evidence of polymorphic phase has been that of Raman spectroscopy. These techniques are described by Chapman (3). More recently ^{13}C-Nuclear Magnetic Resonance (NMR) has been used in this laboratory employing the technique of cross polarization/magic angle spinning to facilitate the study of solid-state samples. These experiments resolved the main α and β forms, and were also able to distinguish the chain orientations in two pure triglycerides (4,5).

Polymorphism is an important phenomenon and aspect of fats. This is because the stability of individual phases has serious influence on the processing (exemplified by solidification) of fats in food materials, the final presentation of the food (hardness, for example, and texture) and indeed the longer term storage (continued crystallization and resulting changes in microstructure such as bloom in chocolate and sandiness in margarine).

Assignment of the individual polymorphic phases of fats is described fully elsewhere (1,2). Classification is based largely on the X-ray diffraction patterns which are characteristic for each phase. Generally accepted for the α structure is a single very broad diffraction line at a d-spacing of about 0.415nm. The β' phase shows two lines usually at about d-spacings of approximately 0.38nm and 0.42nm, and so on for the other phases. In general the angular region for defining the polymorphic phase of triglycerides is between about 15° and 30° in 2θ. This is based on the use of the conventional X-ray wavelength of 0.154nm.

The measurement of melting point to determine phase is a poor technique. Only very pure materials exhibit melting and phase change sufficiently sharply to give accurate and meaningful results. When using DSC, the presence of any phase can only be assumed after it has been transformed to another more stable phase or melted completely. No phases can be identified after crystallization without subsequent destruction by melting. Spectroscopic techniques, such as infrared and Raman, measure the vibrations of molecule groups, knowledge of the polymorphic state is required *a priori*. NMR experimentation, as it stands, requires fairly long experimental times to accumulate good data. Indeed the most powerful method of phase identification is by a diffraction technique. This is because it is the only probe which is susceptible to the relative spatial arrangement of the atoms rather than the motion of atoms or the energies liberated on changing the structure.

Up until recently the collection of the scattered intensities in X-ray diffraction has been achieved by exposing films or by stepping a single electronic counter round the sample and stopping for measured periods at each angular setting. Both methods have drawbacks, and often the problem is the length of time required to perform the measurement of the complete angular region of interests. In the case of the film, the time is dictated by adequate exposure to give sufficiently good resolution. So the technique is not so good for elucidating structures in which very subtle changes in diffraction occur. In the case of the single counter sufficient intensity which gives reasonably good statistical accuracy of the data can only be obtained after counting for a min or so per degree in 2θ. With a scan from 15° to 30°, collection of data takes at least 15 min. This is fine for static samples which undergo no crystallographic modifications in that time.

To circumvent the need to scan through the diffraction angles sequentially with a single detector, apparatus is now available which can record the whole diffraction pattern covering all angles at once. These are the so-called multi-detectors, comprised of large banks of single counters, or the less brutal and highly sophisticated Position Sensitive Detector (PSD). Both types of counting systems produce large amounts of data recorded in digital form rather than analogue (as with the film). This makes it highly amenable to manipulation and analysis. Instead of collecting data point by point over the 15° in 2θ taking a total of 15 min with these devices we can now record fifteen separate patterns for about 1 min each. This allows "snap shots" of the crystal structure during a change in that structure.

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Of long-standing interest has not only been the identification of individual polymorphic phases but also the transitions between them. In addition the kinetics of formation have attracted much study but never before has the rate of collection of data by any technique been sufficiently fast to identify transitory phases during crystallization or during melting. This problem has recently been restated in the work of Garti *et al.* (6) who have been looking at the phase changes in mixtures of pure triglycerides in the presence of emulsifiers.

The objective of the study reported here was to identify the presence of the transitory intermediate β' phase in a solidified mixture of pure triglycerides as it was heated to liquid using the dynamic X-ray technique.

EXPERIMENTAL

Materials. From the work of Garti *et al.* (6), a single sample was chosen on which to make the dynamic X-ray measurements. Pure tripalmitin and pure tristearin were mixed in proportion of seven parts to three. The pure triglycerides were purchased from Sigma Chemicals (Poole, England) (at better than 99% purity) and used without further purification or chemical analysis. Mixing was effected by thoroughly stirring about 10 g of the finely ground powders together. This was heated to about 90°C and then left to stand cooling slowly and forming the solid.

X-ray diffraction apparatus. The X-ray apparatus used comprised a standard Philips generator PW1120 producing Cu K_α radiation with a wavelength 0.154nm. Collimation of the X-ray beam was achieved by using a two-slit system producing a beam of maximum area about 1×10 mm^2. Flat samples (about 20mm diameter and 0.5mm thick) were mounted on an aluminum plate in the horizontal plane. The counter was of the PSD type (the CPS 120 manufactured by INEL, Buc, France) and was curved with a radius of about 250mm and spanned 120° of arc. The workings of the detector are fully described elsewhere (7). It was supported such that the sample was situated at the center of curvature. The plane in which the counter curved is vertical, and this defined the scattering plane.

The counter's construction is based on a rigid wire supported in a chamber through which gas can flow. The wire itself is held at high electrical potential. As X-ray photons pass into the chamber, after being scattered by the sample, ionization of the gas occurs which discharges on the wire. The location of the discharge (and hence the exact angular point on the detector where the X-ray photon arrived) can be accurately determined by measuring the fluctuations in voltage which subsequently occur at the ends of the wire. In electronic terms, the wire can be separated into about 4k detection channels thereby achieving an angular resolution of about 0.03° in 2θ. The speed of detection is very high, and so count rates can be tolerated of up to about 5k events per sec for an individual peak or 100k per sec over the whole detector. Calibration of the angular settings is achieved by using a standard sample of known crystallographic d-spacings, such as sodium chloride, which gives strong diffraction in the region of interest.

The experiment is controlled by a dedicated computer which starts and stops data acquisition, dumps data sets to disk and analyzes, manipulates and displays results. Design and construction of the particular diffractometer was carried out by SPECTROLAB, Newbury, England, from whom similar installations can be obtained commercially.

Experimental procedure. Samples of the triglyceride mixture of about 250mg in weight were placed on the aluminum plate. They were heated to liquid on a hot plate and then cooled rapidly while being held above a dry ice and ethanol mixture. The sample on the plate was transferred to the diffractometer where it was located on a copper block. Temperature control of the sample was then effected by circulating water through the block. Heat sink cream (high thermal conductivity zinc oxide-filled silicone compound) was smeared between the plate and the block to effect as good a thermal contact as possible. Heating of sample was achieved by circulating hot water through the block, the temperature of which was limited by a programmable controlling device in the water bath. The temperature of the sample was measured by a thermocouple situated in the aluminum plate. Since the mass of the metal block and plate far exceeded that of the triglyceride sample, thermal equilibration was assumed.

Experiments were performed by raising the sample's temperature in a linear fashion with time. Simultaneously with the heating process, X-ray diffraction was recorded, and new patterns were taken at one minute intervals for a duration of just under one minute. Heating rates of 1 and 5°C/min were used. A second type of experiment was performed in which a quenched sample was heated to a temperature in the region of 55°C and held. Then at this elevated temperature development of diffraction was monitored again at one minute time intervals.

RESULTS

Results of the diffraction experiments using the heating rate of 1°C/min are shown in Figure 1 plotting X-ray intensity as a function of diffraction angle. The data are displayed sequentially in time behind one another in isometric projection viewed from two different positions. This gives the appearance of a surface. The time axis is equivalent to temperature as patterns were recorded each minute during which the temperature was raised by 1°C. So, for example, the first pattern is shown after one minute and the temperature at the start of data collection was 25°C and 26°C at termination.

At low temperatures the diffraction pattern shows only one strong line at 2θ of about 21° (equivalent to a d-spacing of about 0.42nm). Then after about 30 min, when the temperature had risen to about 55°C, the pattern disappears abruptly and is replaced by weaker diffraction consisting of two lines. These are found at 2θ positions of about 20.8° (d = 0.43 nm) and 23.0° (d = 0.39nm). After 40 min or so the pattern changes again when the temperature is about 65°C. The new diffraction pattern is altogether sharper and now has one very strong line at an angular position of 19.1° and two others at 23.0° and 23.8° (d = 0.46, 0.39 and 0.37nm). Weaker features are also seen elsewhere. After about 50 min when the temperature is about 75°C, the sample melts and the diffraction pattern remaining is one characteristic of a liquid having only a broad feature of low intensity between about $10° < 2\theta < 30°$. In Figure 2 are shown

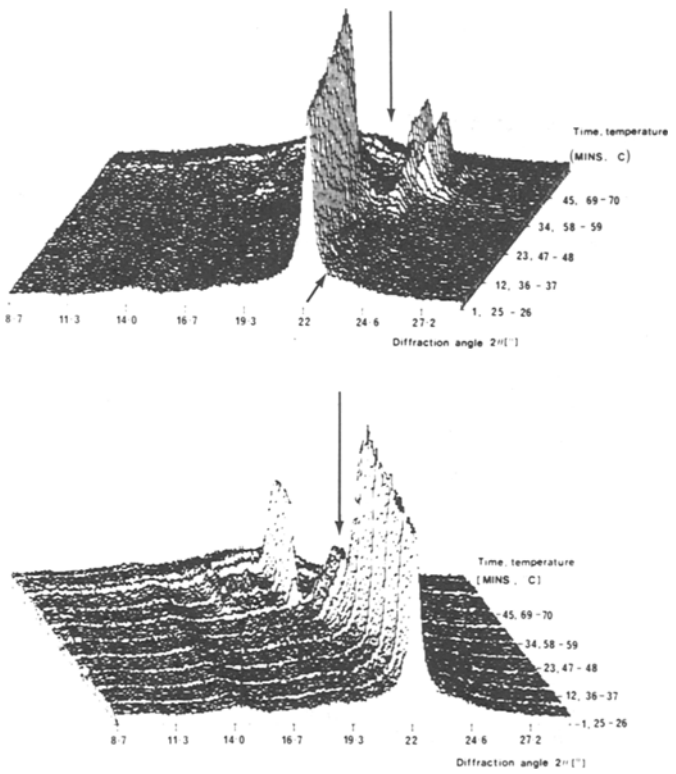

FIG. 1. Progress of the diffraction pattern of the mixture of tripalmitin/tristearin 70/30 as the sample is heated at 1°C/min. Two isometric views are shown as certain features are hidden behind the dominant α peak. On the upper figure the two arrows indicate the position of the 0.38nm diffraction line characteristic of the β′ structure. In the lower part of Figure 1 the single arrow indicates the position of the other characteristic β′ diffraction line corresponding to about 0.42 nm.

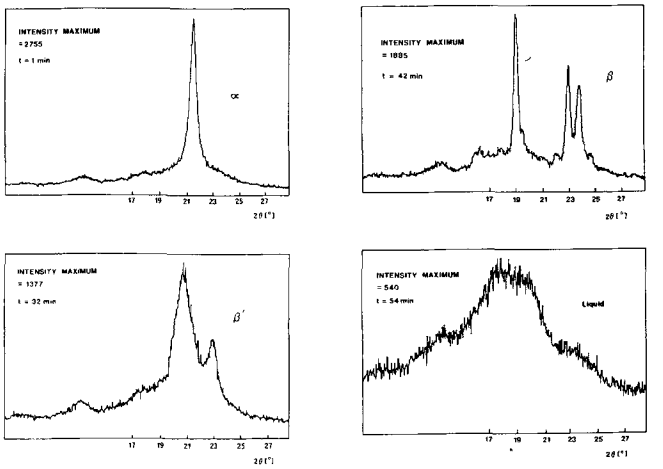

FIG. 2. Individual diffraction patterns are shown for easy comparison at the times of 1, 32, 42 and 54 min during the heating process and these correspond to temperatures of 26, 57, 67 and 81°C, respectively. The diffracted intensities are plotted as a function of diffraction angle, and it should be noted that ordinate scales are different for each pattern. The peak positions for the three solid phase index very well with the generally recognized classification of the α, β′ and β polymorphic forms.

individual diffraction patterns of the four separate phases at times of 1, 32, 42 and 54 min corresponding to temperatures 26, 57, 67 and 81°C.

Very similar data were produced for the heating rate of 5°C/min. However diffraction lines were broader than for the slower heating rate. This reflects the fact that during the faster heating rate the sample experiences a wider temperature range during the min exposure time to X-rays. Nevertheless four distinct patterns were recorded.

Heating rates of faster than 5°C/min were not attempted. Firstly, this was because even heating of the sample could not be ensured. Secondly, at such high heating rates the diffraction patterns collected in one min could contain information by more than one phase. Collection of data for periods of less than one min does not allow sufficient numbers of counting events to occur. This then begins to deteriorate the statistical quality of the data.

In the second experiment attempts were made to stabilize a sample at elevated temperature by melting the first phase. The sample was heated with great care, and its temperature not raised above 55°C. The diffraction patterns showed in every case that transformation to the β phase could not be stopped and was complete in less than 20 min. Therefore stabilization of the β′ phase could not be achieved.

DISCUSSION

Using the conventional classification for the polymorphic phases in fats, the first three patterns correspond reasonably well to diffraction from a sample showing the α, β′ and β phases, respectively. These data thus confirm the presence of the intermediate β′ phase during heating of the α form to the β form. This, according to Garti *et al.* (6), has been hitherto shown by "only a few weak indications."

Other observations of β′ phases in triglycerides have of course been made. In pure tristearin, for example, two separate β′ phases have been identified (8). What is more, both these phases were isolated and held stable at room temperature. Such results were not evident in this work nor, indeed, were the effects searched for. This is not necessarily in conflict with the claims of Simpson and Hagemann (8). However, it is most likely that the quality of the X-ray data collected by the apparatus used here can not tolerate further analysis than already attempted here. Recourse to very bright X-ray sources, such as a synchrotron, coupled with a PSD system will be necessary for resolution of these structures during dynamic melting. With such an apparatus of high photon flux, the time resolution could be further improved or the quality of the patterns ameliorated.

Other attempts to measure the β′ to β transition have also been made with X-rays (9). There a single counter X-ray diffractometer was used with the counter positioned stationary at one angle, 2θ, of about 19.1°. In this region there is no diffraction signal from the β′ phase, and growth of the β form was deduced by the growing intensity observed as a function of time. In the isothermal experiments reported (9) on pure triglyceride mixtures, transition times ranged up to at most 2 hr and were usually much shorter. Whereas the triglyceride systems were different, similar transition times were observed in our limited number of experiments. Using only a single counter allows the danger of not knowing what is

happening elsewhere in the diffraction arc, and this problem is eliminated with the PSD.

Two major conclusions can be drawn from the experiments described here.

Existence of intermediate phase. In the mixture studied here and by other workers, the previously **assumed** existence of the β' phase is now **confirmed**. This has implication for the understanding of the polymorphism of fats in general, and gives more confidence in explaining the complex behavior of this class of materials.

Potential of position-sensitive X-ray diffraction. Diffraction techniques are the only methods available for comprehensively and unambiguously assigning polymorphic phases in fats. This laboratory scale PSD X-ray diffraction machine performs that task more rapidly and comprehensively than has been done before. It greatly extends our ability to measure the dynamics of crystallization and melting. The capability of the equipment permits a whole range of studies in dynamics previously not tenable. This is true for studies, as here, on pure triglycerides and their mixtures which are vital in contributing to our understanding of more practically applied systems of fats. Use could be made to provide data for the α to β' transitions described by Hagemann and Rothfus (10). The technique can be equally well used to characterize the behavior of practical fat blends under conditions close to those encountered in conventional processing. In addition it is now feasible to relate DSC crystallization and melting behavior to polymorphism as measured by this new technique. Until now unambiguous interpretation of DSC data has not been possible.

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REFERENCES

1. Timms, R.E., *Prog. Lipid Res. 23*:1-38 (1984).
2. Garti, N., and K. Sato (eds.), *Crystallization and Polymorphism of Fats and Fatty Acids*, Surfactant Science Series *31* (1988), Marcel Dekker, New York.
3. Chapman, D., *The Structure of Lipids*, Methuen and Co. Ltd., London, 1965.
4. Norton, I.T., C.D. Lee-Tufnell, S. Ablett, and S.M. Bociek, *J. Am. Oil Chem. Soc. 62*:1237 (1985).
5. Bociek, S.M., S. Ablett and I.T. Norton, *Ibid. 62*:1261 (1985).
6. Garti, N., J.S. Aronhime and S. Sarig, *Ibid. 66*:1085 (1989).
7. Ballon, J., V. Comparat and J. Pouxe, *Nuclear Instruments & Methods 217*:213-216 (1983).
8. Simpson, T.D., and J.W. Hagemann, *J. Am. Oil Chem. Soc. 59*:169 (1982).
9. Gibon, V., F. Durant and Cl. Deroanne, *Ibid. 63*:1047 (1986).
10. Hagemann, J.W., and J.A. Rothfus, *Ibid. 65*:1493 (1988).

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