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POLYMORPHISM OF TRIGLYCERIDES
A CRYSTALLOGRAPHIC REVIEW

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Abstract

In order to understand the role of fat in food systems detailed knowledge about the arrangement of triglyceride molecules is useful. The triglyceride molecules in a fat can be packed in alternative ways, each crystal form having different melting points. This phenomenon is called polymorphism.

Based mainly on X-ray studies (single-crystal, powder diffraction, scattering) and Raman spectroscopy the structure of the different polymorphic forms, α, β', β', and β, are explained. The emphasis is on work done in the author's laboratory.

There is a close relationship between the polymorphic forms which is persistent also in the liquid state - the molecules are arranged two and two in a bilayer. The differences between the polymorphic forms are due to: (i) the hydrocarbon chain packing, (ii) the tilt of the chains versus the methyl end group plane, and (iii) differences in the methyl end group region.

From a technical point of view the polymorphic transitions of fats is of the highest importance. This paper describes a mechanism behind the β' → β transition - the chain mobility in the methyl end group region causes this transition.

Introduction

The functional properties of fats are very important in many food systems. The type of dispersion, the solid-liquid ratio and the occurring crystal form often have a strong influence on the behaviour of the entire system. The triglyceride molecules in a fat can be packed in the solid state in alternative ways, each crystal form having different melting points. This phenomenon is called polymorphism.

The evolution of the present knowledge of the polymorphism in triglycerides can be studied in detail in various articles [2-4,6,9,15-19,21,22,24,25,28]. Up to this date no other triglyceride crystal structure than the β-form is completely determined [15,19].

Since all the different polymorphic forms of fats occur and influence the physical properties of various food systems, knowledge about the structure of triglycerides in the different crystal forms is important.

The present review deals with the polymorphism of triglycerides - the structure of the different forms and the transitions between the forms.

Materials and Methods

The triglyceride samples used in the different studies were either purchased or prepared by standard methods [cf. 9]. Crystals for single-crystal work were grown from acetone at 20°C. A Weissenberg camera was used for the X-ray single crystal work, a diffraction-pattern versus temperature (DPT) camera [9,27] was used to study the polymorphic transitions. The DPT camera was also used for the powder-diffraction and the scattering studies. The X-ray films were examined with a photodensitometer. Raman spectra were recorded with two different systems: A Cary 82 spectrophotometer and a Dilor RT10 Laser Raman with triple monochromator, both systems using Argon laser for excitation.

Polymorphic transitions of triglycerides

Triglycerides are known to crystallize in three typical polymorphic forms namely α, β' and β. Fig. 1 shows a somewhat more complex picture of the polymorphic transitions. The two β'-forms are quite similar which will be discussed below. The sub-α-form is normally not obtained, since very low temperatures are needed. As indicated in the figure
all polymorphic forms can be obtained directly from the liquid state except the sub-α-form. So far only the β\textsubscript{1}- and β-forms have been obtained from solvents. The transition liquid → α → β\textsubscript{2} → β\textsubscript{1} → β is the complete way for triglycerides to find the optimum packing of the molecules. In a fat both chain length and degree of unsaturation can vary a lot but the same polymorphic forms are obtained. The main difference between different fats or between different pure simple or complex triglycerides is how fast the transition goes. As indicated in Fig. 1 the transitions need not to follow the complete way.

**The chain packing of hydrocarbons**

Regardless which kind of functional group that is present, all hydrocarbon chains in long chain compounds are packed in one of a few possible ways. The best method to describe the chain packing is to use the subcell corresponding to the smallest repetition unit within the chain layer. All subcells have been discussed thoroughly in a review by Abrahamsson and coworkers [1]. In simple triglycerides only three different subcells occur: the hexagonal (H) subcell (α); the orthorhombic (O.L) subcell (β\textsubscript{2}, β\textsubscript{1} and sub-α) and the triclinic (T\textsubscript{1/2}) subcell (β).

**The chain orientation**

Based on X-ray powder diffraction Clarkson and Malkin [5] proposed that two acyl chains in a triglyceride molecule are arranged adjacent and the third one points in the opposite direction. This principal structure of triglycerides is illustrated in Fig. 2a. The hydrocarbon chain can then be close packed according to the different subcells mentioned above but still be orientated according to the principal packing in Fig. 2a. The molecules are packed in a way that two "chair like" molecules build up a dimeric unit. The bilayer structure illustrated in Fig. 2a consists of four triglyceride molecules or two dimers.

![Figure 1](image1.png)

*Figure 1.* Complete picture of the polymorphic transitions of triglycerides. All transitions are irreversible except the α → sub-α transition. The dotted lines indicate the possibility of a direct crystallization in other forms than the α-form. The possibility of growing crystals from solvents is also indicated [9].

![Figure 2](image2.png)

*Figure 2.* Principal structure of triglycerides, double chain layer (a) and triple chain layer (b).

![Figure 3](image3.png)

*Figure 3.* Schematic orientation of the proposed triglyceride dimers of the α-, β\textsubscript{1}- and β-forms in the ca-projection shown together with respective chain packing subcell and the cb-projection where the chain directions are indicated.

In 1948 Lutton [23] proposed an additional structure - the triple chain layer which is seen in Fig. 2b. This structure can occur when one specific chain in a triglyceride differs in length by four or more carbons. The triple chain layer also occur if one specific chain is unsaturated, i.e., 1,3 di-stearoyl-2-oleoyl-glycerol (common in cocoa butter).

In Fig. 3 a schematic comparison between α, β\textsubscript{1} and β is illustrated. The structures are in three planes showing both the close relation of dimeric units building up bilayers and the difference in hydrocarbon close packing. The dimeric units are in fact more or less consistent even in the liquid state.
Liquid state

Based on X-ray technique and Raman spectroscopy [10,16] a proposed structure of triglycerides in the liquid state has been developed. In the liquid state the triglycerides are arranged in the chain-shaped conformation (see Fig. 4) and most often the dimeric unit is present. The model is dynamic, i.e., both size and orientation of the lamellar units vary with diffusion rates of the molecules. When the temperature is increased the size of the lamellar units decreases. The degree of order within the lamellar units, however, is proposed to be almost constant. When the temperature of a melt is decreased the lamellar units are increased in size until crystallization finally takes place. Formation of a crystal nucleus can be regarded as the limiting point when formation of adjacent all-trans chains within the lamellar unit dominates over gauche conformation.

The α-form

Fig. 5 shows the α-form [12] of triglycerides. The proposed structure is based on evidence from X-ray diffraction and Raman spectroscopy studies. The chain axes are indicated as lines, since the main part of the hydrocarbon chains is oscillating. The molecules are arranged perpendicular to the methyl end group plane and the hydrocarbon chains are hexagonally (H) close packed.

From a molecular packing point of view the α-form is unsatisfactory because of the irregular methyl end group region. Due to this irregularity the hydrocarbon would be expected to possess a high degree of mobility. This mobility, together with the hydrocarbon chain oscillation, normally induces a rapid transformation from the α-form to one with better chain packing.

The β'-forms

Fig. 6 shows the main features of the proposed β'-form of the triglyceride triundecanoin. The structure has been derived from a single crystal study [12] which has been recently further extended [8]. Measurements of the (ab) lattices in the β'-1- and β'-2-forms indicate that the glycerol group region in the β'-1-form can have the same structure as in the β-form. If the α-form is compared with the β'-1-form it can be seen that the methyl groups are better packed in the β'-1-form. This is due to a tilt of the hydrocarbon chains in relation to the methyl end group plane. The hydrocarbon chains are arranged according to the orthorhombic (O.L) subcell.

The a-c projection of the β'-1-form is closely related to the a-c projection of the β-form [cf. Fig. 7]. The main difference between the β'-1 and β-forms is seen in the two other projections (see Fig. 3).

The β'-1-form shows two directions of chain tilt in the c-b projection. This change in tilt is assumed to take place at the methyl end group plane. A second β'-form, β'-2, of the triglyceride triundecanoin has been observed [12]. It differs from the β'-1-form in its alignment in the different planes. The β'-2-form is probably tilted only in the c-a plane and vertical in the c-b plane.

The β-form

Fig. 7 shows the crystal structure of trilaurin [19]. The β-form is in fact the only polymorphic form so far to have its structure completely determined.
The chains are tilted in relation to the methyl end group planes in the c-a projection as well as in the c-b projection (see Fig. 3). In the β-form the hydrocarbon chains are arranged according to the triclinic T121 subcell.

**The β' → β transition**

From a technical point of view the β' → β transition is most important. When producing margarine it is important that the fat crystals are stable in the β-form. This is, however, not always the case. If, e.g., hydrogenated rapeseed oil with low content of erucic acid (LOBRA) is used the β-form is developed very rapidly [7]. As a consequence the texture of the margarine becomes unacceptable due to the presence of large crystals [29]. This rapid β' → β transition can be hindered by using additives like diglycerides [11, 14] or sorbitan tristearate [20], plant breeding [13, 26] or just by blending the hydrogenated LOBRA oil with other oils.

When the β' → β transition occurs, the methyl end group planes of two adjacent bilayers need to slide in a way that all the bilayers are tilted in the same direction. This transition is strongly dependent upon the structure of the methyl end group plane. Consequently some triglyceride molecules give a stable β'1-form while others are rapidly transformed to the β-form.

![Diagram](image)

**Figure 6. Proposed structure of the ca-projection of the β'1-form of triundecanoin [8]. The glycerol part is proposed to be arranged in the same way as in the β-form of trilaurin [cf. 12].**

![Diagram](image)

**Figure 7. Molecular arrangement in the β-form of trilaurin [19].**

![Diagram](image)

**Figure 8. Comparison between the proposed structures of the β'1-form of triundecanoin (a) and tridecanoin (b). Shaded areas indicate a higher degree of hydrocarbon chain mobility.**

Fig. 8 shows the proposed structure of the β'1-form of triundecanoin and tridecanoin. The β'1-form of tridecanoin is derived from the β'1-form of triundecanoin by "cutting" one carbon from every acyl chain [8]. Furthermore the bilayers are adjusted in order to get a similar distance in the methyl end
group region as in the case of triundecanoin. The difference between the two triglycerides what concerns the methyl end group region is rather obvious. Tridecanoin shows "empty areas" - shaded in Fig. 8. This indicates a higher mobility in the case of tridecanoin. Since the $\beta' \rightarrow \beta$ transition involves sliding as mentioned above, tridecanoin consequently should undergo the $\beta' \rightarrow \beta$ transition faster compared with triundecanoin. This is also the case. In fact all simple triglycerides with odd acyl chains are stable in the $\beta'$-form and all even acid simple triglycerides are stable in the $\beta$-form.

The structures and polymorphic behaviour discussed above are based on investigations of simple saturated triglycerides. X-ray diffraction data, however, indicate that these structures are valid also for most complex triglycerides as well as mixtures of triglycerides. In a natural fat both the chains length and the degree of unsaturation can vary, but nevertheless the same polymorphic forms are obtained.

**References**

12. Hernqvist L, Larsson K. (1982). On the crystal structure of the $\beta$'-form of triglycerides and structural changes at the phase transitions liq. $\rightarrow \alpha \rightarrow \beta' \rightarrow \beta$. Fette-Seifen-Anstrichmittel 84, 349-354.

**Discussion with Reviewers**

P. Birker: The author proposes that triglyceride melts contain liquid crystalline regions, and that the size of these regions varies with temperature. This model is based on his own work published in reference 10, i.e., the observation of a very broad reflection in the diffraction pattern of e.g., liquid trimeristin at about 22 °A; I accept that this reflection can indicate liquid crystallinity but there is no proof that the size of liquid crystalline regions change with temperature. The observed difference (ref. 10) in half peak value of this reflection of 25 °A at 50°C and 26 °A at 60°C are not significant. What is the proof or experimental support for the proposed variation of size and orientation of ordered regions with diffusion rates?
I agree that the difference in half peak values, 25 Å at 50° and 26 Å at 80°C is small but nevertheless it has been observed. I suggest further investigations. If this difference is considered to be existing it must be due to a reduction of the lamellar units. The possibility of using line broadening to estimate the size of the domains in a L2 phase in a liquid crystalline phase was used 1983 (Fontell K, Hernqvist L, Larsson K, Sjöblom J (1983) On structural relations between lipid mesophases and isotropic reversed micellar (L2) solutions. J. Coll. Interface Sci. 93 453-460).

The alternation of chain direction occurs in the c-b projection which is given in Fig. 3. In this figure the alternation is proposed to occur at the methyl end plane. The question as to whether the chain tilt alternates in the glycerol group region or at the methyl end plane cannot be unambiguously determined. In an earlier discussion of the β'-form (ref. 16) the space groups was proposed to be P212121. The new single-crystal data (ref. 12), however, show that the earlier observed apparent orthorhombic symmetry was due to twinning, the correct space group is P21/c.

Concerning the bend in the chain tilt, see answer given above to P. Birker. The alternation of chain tilt only exists in the c-b projection, not in the c-a projection. The presence of voids in the c-b projection of the β'-form of tridecanoin (Fig. 8b) indicates that tridecanoin (an even triglyceride) easily can be transformed to the β-form. When the β' → β transition occurs, the methyl end-group planes of two adjacent bilayers need to slide in order to get the same tilt in all bilayers, both in the c-a and the c-b projections. The importance of the methyl end-group plane has been pointed out earlier (Larsson K (1966) Alternation of melting points in homologous series of long-chain compounds. J. Am. Oil Chem. Soc. 43 559-562) and discussed in the case of β-forms (6).