Study of the Polymorphism and the Crystallization Kinetics of Tripalmitin: A Microscopic Approach

M. Kellens[a,1], **W. Meeussen**[b] and **H. Reynaers**[a,*]
[a]Laboratory of Macromolecular Structural Chemistry, Catholic University of Leuven, B-3001 Leuven, Belgium and [b]n.v. Vandemoortele Coördination Center, R&D Department, Izegem, Belgium

The morphology and kinetics of crystallization of tripalmitin have been examined in detail by optical microscopy. The α-crystallization process is characterized by a fast heterogeneous nucleation and spherulitic growth, even at low undercooling, resulting in intense birefringence and smooth spherulitic entities. Four different β′-microstructures have been found—grainy, fibrous, feathery and lamellar. Around 47°C, a clear change from a grainy to a fibrous β′-microstructure is observed. This transition seems to take place without a drastic change in nucleation or in crystal growth. At 50°C, both nucleation and crystal growth exhibit a clear discontinuity, indicating interference from β-crystallization. Around 52°C, the β′-form changes again from a fibrous to a more feathery microstructure; the transition is accompanied by a distinct decrease in crystal growth rate. The lamellar β′-structure exhibits the highest stability and can be obtained only *via* an accelerated nucleation at low temperature, followed by further growth at elevated temperature near the melting temperature of the β′-form. Determination of the β-form on the basis of its microstructure is not always precise, because the microstructure strongly depends on whether the β-crystals are obtained from a transformation of α or β′, or whether β-crystallization occurs directly from the melt. Clear confirmation of the polymorphic nature of the solid state can be obtained from melting point determination.

KEY WORDS: Kinetics, microscopy, polymorphism, tripalmitin.

The crystallization of triglycerides, the major constituent of oils and fats, is a key step both in the manufacture of fat products and the fractionation of fats and oils (1). The crystallization behavior is complex because triglycerides are characterized by the existence of multiple crystalline states. Because of the commercial importance of edible fats, intensive studies have been carried out on the crystallization properties of triglycerides for more than a century (2,3).

Determination of the polymorphic identity of a solid state is not always easy. Differential thermal analysis, X-ray diffraction and infrared spectroscopy are most commonly used to characterize the morphological form. Although polarizing microscopy has already found wide appreciation in the food industry, the technique has gained little attention in polymorphism and crystallization studies of fats. In many applications of edible fats, however the morphology and number of glyceride crystals determine the suitability of the fat for a given purpose.

The present paper is part of a fundamental study on the polymorphism of monoacid triglycerides (4). In this work, the polymorphic behavior of the triglyceride tripalmitin is studied by the optical microscopic technique. The study focuses on the relation between the crystallization behavior of the different polymorphic forms and their morphologic appearances.

In previous papers, X-ray diffraction and thermal analysis studies have established the existence of three different basic polymorphic forms (α, β′ and β) for tripalmitin, with the characteristics of the β′- and β-form being strongly affected by the crystallization conditions (5,6). As for the β′-form, the existence of multiple subforms has been claimed (7,8). No definite proof, however, could be given to substantiate both submodifications. Early microscopic studies of the polymorphic forms of single-acid triglycerides have shown that the crystal forms exhibit a wide range of microscopic appearances (9,10). Quimby reported four basic textures for the three basic polymorphs, two being correlated with the β′-form (11). Recent studies with differential scanning calorimetry, X-ray diffraction and synchrotron radiation have led to a reassessment of these earlier reports (5,6). Most of these observations were found to be reproducible and can now be interpreted in terms of spherulite microstructure.

EXPERIMENTAL PROCEDURES

Tripalmitin (PPP) was purchased from Sigma Chemical Co. (St. Louis, MO). It had a purity of more than 99% and was used without any further purification. The optical microscope used was a Leitz Dialux 22EB equipped with a Wild Photomat MPS45 camera. The objective was a 4x (NA 0.12) or 10x (NA 0.25); the ocular was 12.5x for visual observation and 10x (TL 160 mm) for photography. Magnification was verified with a Reichert graduated plate (2 mm divided into 200 segments of 10 μm). The microscope had a fixed analyzer above a rotatable polarizer below the sample. Temperature control was achieved with a Mettler FP82 hot stage equipped with an FP80 controller. Sample thickness was not systematically monitored, but was estimated to be approximately 10 μm.

Identification of the crystal structures was done by studying the melting behavior immediately after crystallization by heating the samples at a rate of 5°C/min. The melting points were registered visually under the microscope, and were 44–46, 57–59 and 65–66°C for α, β′ and β, respectively.

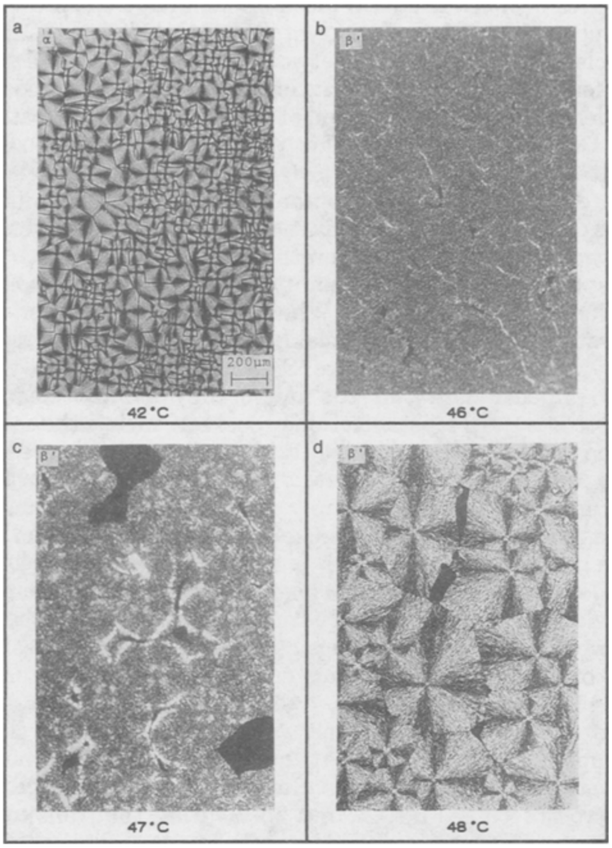
RESULTS AND DISCUSSION

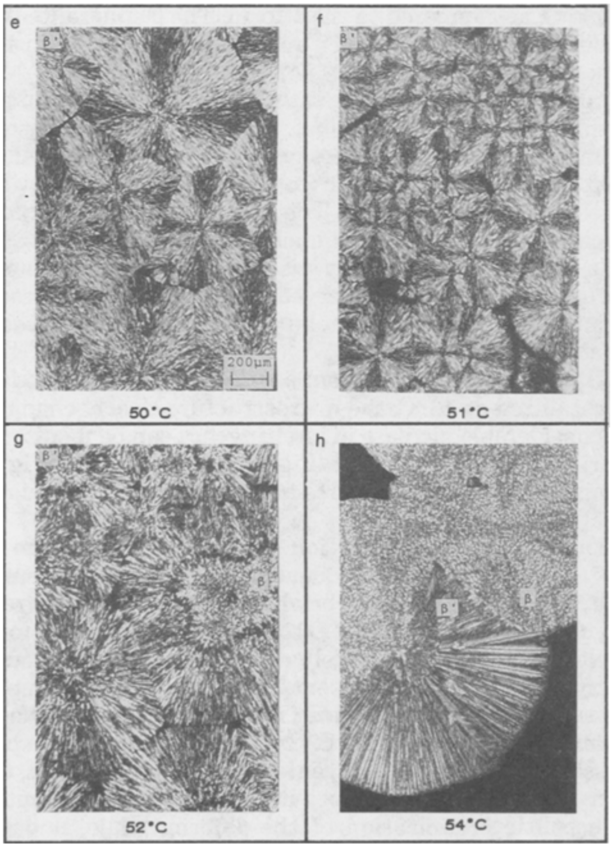
Morphology. Depending on the crystallization conditions, tripalmitin can crystallize into different polymorphic forms, each form being characterized not only by its typical structural and thermal properties but also by a morphological appearance. To find out how the morphology of the solid state is determined by the crystallization conditions, samples of tripalmitin were crystallized isothermally from the isotropic melt at temperatures between 42 and 58°C. Prior to the crystallization step, the samples were kept at 80°C for 3 min, then cooled at a rate of 5°C/min to 3°C above the crystallization temperature, and further to the desired temperature at a rate of 2°C/min to

---

[1] Present address: n.v. De Smet Engineering, Prins Boudewijnlaan 265, B-2650 Edegem, Belgium.

[*] To whom correspondence should be addressed at Laboratory of Macromolecular Structural Chemistry, Dept. of Chemistry, Catholic University of Leuven, Celestijnenlaan 200F, B-3001 Leuven, Belgium.





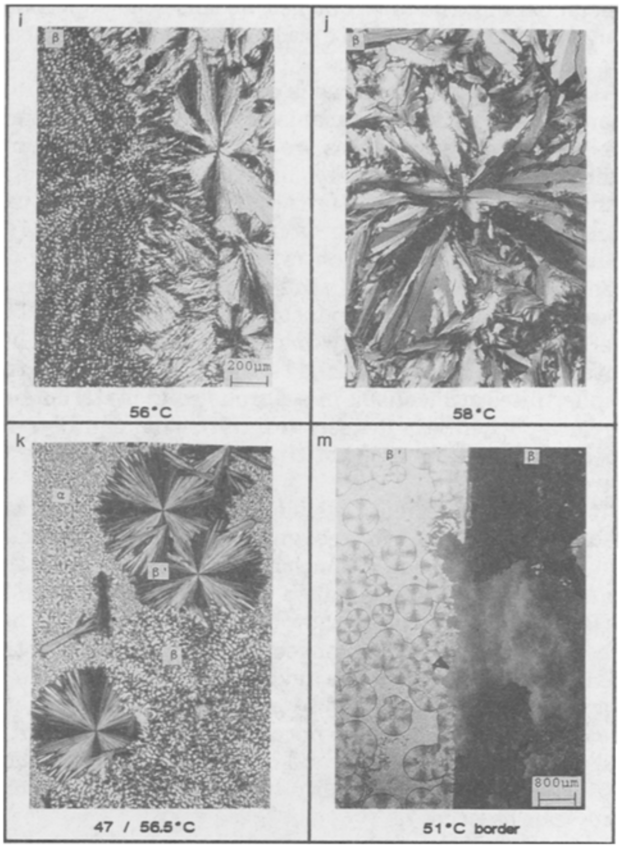


FIG. 1. Optical micrographs of the different polymorphic forms of tripalmitin after isothermal crystallization from the melt at 42, 46, 47, 48, 50, 51, 52, 54, 56 and 58°C (a–j) (magnification 80x; 1 cm = 400 μ). The β'-lamellar structure (k) was obtained by nucleation induction at 47°C for about 20 s followed by crystal growth at 56.5°C. After 30 min at 56.5°C, the sample was quickly cooled to 25°C in order to stop both the β'- and β-crystallization processes. Photo m was taken at the edge of the cover-glass during the isothermal crystallization of tripalmitin at 51°C (magnification 20x; 1 cm = 1,600 μm).

avoid temperature undershoot. Optical micrographs of the different crystal structures were taken at the crystallization temperature and are represented in Figure 1.

At 42°C, tripalmitin is crystallized quickly into the α-form. The α-crystallization process is determined by a fast heterogeneous nucleation. The α-form is characterized by a bright spherulitic pattern (Fig. 1a). The spherulites fit tightly together, which results in sharp and straight edges. The spherulite size is strongly dependent on the nucleation density and can vary considerably from one place to another in the sample.

At 46°C, a dense packing of β'-spherulites is observed. The anisotropy of the spherulites is much less than observed for the α-form, and they barely stand out against the background (Fig. 1b). The contours of the spherulites can be observed only vaguely. Photography of fast-growing spherulites under these circumstances is hampered by the requirement of long exposure times. When crystallization is completed, the spherulites are fused into a featureless mass of tiny, irregular grains with random polarization. This lack of preferred orientation within the spherulite can explain the low contrast, because the superposition of grains with opposite orientation results in a near-zero path length difference.

At 47°C, the same material is produced, but growth rate is lower. Also, an increase in anisotropy (double refraction) can be seen; the spherulites are contrasted more sharply against the background (Fig. 1c). At 48°C, a spectacular change in spherulite structure occurs. The spherulites can be observed clearly and show a fibrous structure (Fig. 1d). The fibers are severely contorted but clearly run outward radially. At 50°C, a similar β'-structure is found. However, obtaining a sharp focus is difficult (Fig. 1e). The fibers seem to be densely packed, but the spherulite centers are less well organized then at 48°C. At 51°C, the β'-spherulites still consist of a fibrous solid material, but they are packed less densely (Fig. 1f). This can be seen especially at the borders of the spherulites (absence of sharp edges).

At 52°C, two different crystallization processes are manifested—a β-polymorph, recognizable as nonspherulitic clumps of bright small grains, and β'-spherulites (Fig. 1g). Nucleation is more frequent in the β-polymorph than in the β'-form. The β-clumps show a fast increase in size, not by regular growth but by convection of fluid, carrying the small grains away from the nucleation site. This mode of growth, however, is stopped by an overgrowth of β'-fibers. The sample then reverts to normal spherulitic β'-growth. These β'-fibers are thicker and less densely packed than at 51°C, and an open, feathery, branched structure sometimes can be seen.

At 54°C, a simultaneous production of β- and β'-crystals still occurs. The β-fibers are now of a featherlike nature and are even less densely packed (Fig. 1h). The grainy β-form now grows as fast as the open-feather β'-spherulite that harbors it, and the β-grains can sometimes break out in convective flows. The β-form can show different degrees of organization inside the β'-spherulites.

At 56°C, β'-nucleation is impractically slow, and β-crystallization has now become dominant. The β-form can exhibit different microstructures. When originated *via* β'-induction, it is present as small but intensely refracting grainy pellets (Fig. 1i, left part). The initial β'-spherulitic pattern can still be observed weakly. In other parts of the sample, β-nucleation appears directly in the isotropic melt without being induced by a β'-nucleation, and it is followed by a clear spherulitic growth (right part of Fig. 1i). At 58°C, the melt directly crystallizes to β, but at an extremely slow rate, resulting in large, leaflike lamellar structures (Fig. 1j).

β'-Crystallization at high temperatures (above 54°C) can be favored by keeping a molten sample first at a low temperature for a short period (10 s) to accelerate nucleation, and then allowing further crystal growth at an elevated temperature. Nucleation at 47°C for 10 s, followed by growth at 56.5°C, gives rise to large lamellae, appearing isolated or arranged in a spherulitic pattern (Fig. 1k). The lamellar β'-form is characterized by high stability. Even at such a high crystallization temperature, recrystallization to β was found to take place very slowly; after 2 h at 56°C, β'-lamellae could still be observed in the sample.

In a previous paper, the crystallization behavior of tripalmitin was studied with differential scanning calorimetry (DSC) under analogous conditions (5). Some similarities between the DSC measurements and the present results can be found—α-crystallization below 44°C, β'-nucleation and growth between 44 and 50°C and interference from β-crystallization above 50°C. A striking difference is the proliferation of the β-form once nucleated in DSC; in microscopy, this proliferation seems to be halted by overgrowth of a β'-layer. Between the glass plates, the β'-crystallization process is found to be much less disturbed by β-crystallization than at the edges of the cover-glass. Even at temperatures up to 56°C, growth of β'-spherulites could be monitored thoroughly between cover- and carrier-glass. When uncovered, however, β-crystals were developed quickly above 50°C, resulting in a typical bloomed structure (Fig. 1m). Consequently, the two-dimensionality of the microscopy sample clearly obstructs the β-crystallization process. If such is the case, then the apparent instability of the β'-form (that prevents production of large β'-crystals in the bulk) is not an inherent β'-property, but simply a consequence of the fast, nonspherulitic, three-dimensional growth mechanism of the competing β-form.

Dependent on the sample history, the β-form can exhibit a wide variety of microstructures (Fig. 2). To study the morphologic changes during the transition of α to β, a sample of tripalmitin, after being crystallized into the α-form at 42°C, was heated from 42–60°C at different rates (2 and 20°C/min, respectively), and then kept at this temperature for 15 min. At low heating rate (2°C/min), crystallization to β occurs almost without a change in microstructure (Figs. 2a and 2b). During the transition to β, small cracks and voids appear in the crystal phase; this is a consequence of the higher density of the β-crystal packing as compared to the α-form. The β-spherulites exhibit a somewhat rougher surface as compared to the smooth α-crystals.

Upon heating the α-form at a high rate (20°C/min), the α-spherulitic structure is lost. Due to the fast increase in temperature, the α-melt does not immediately recrystallize to β, but becomes isotropic (complete loss of intensity between crossed polarizers). The β-recrystallization process is characterized by a high nucleation density and occurs in a random way; a highly disordered and grainy microstructure can now be observed (Fig. 2c). The voids result from a contraction of the solid phase during the recrystallization to β.

Upon crystallizing β from a grainy β'-form, previously crystallized at 46°C, the microstructure clearly changes. A considerable increase in birefringence can be monitored during the β'-β-transition (Figs. 2d and 2e). The bright, grainy β-form already starts to appear during melting of the β'-material.

During the crystallization process of the β'-form at 49°C, small β-crystals frequently appear in the centers of the β'-spherulites (Fig. 2f, bright spots). When slowly raising the temperature to 60°C (2°C/min), the β'-crystal form melts but is not immediately recrystallized to β, although β-crystals are already present; the β-recrystallization process occurs at a much slower rate as compared to the β'-form crystallized at 46°C. Two β-recrystallization processes are evident: At the centers of the β'-spherulites, the β-crystals slowly develop with retention of the initial spherulitic organization of the β'-form, while at other places, where the β'-crystal structure is completely molten,

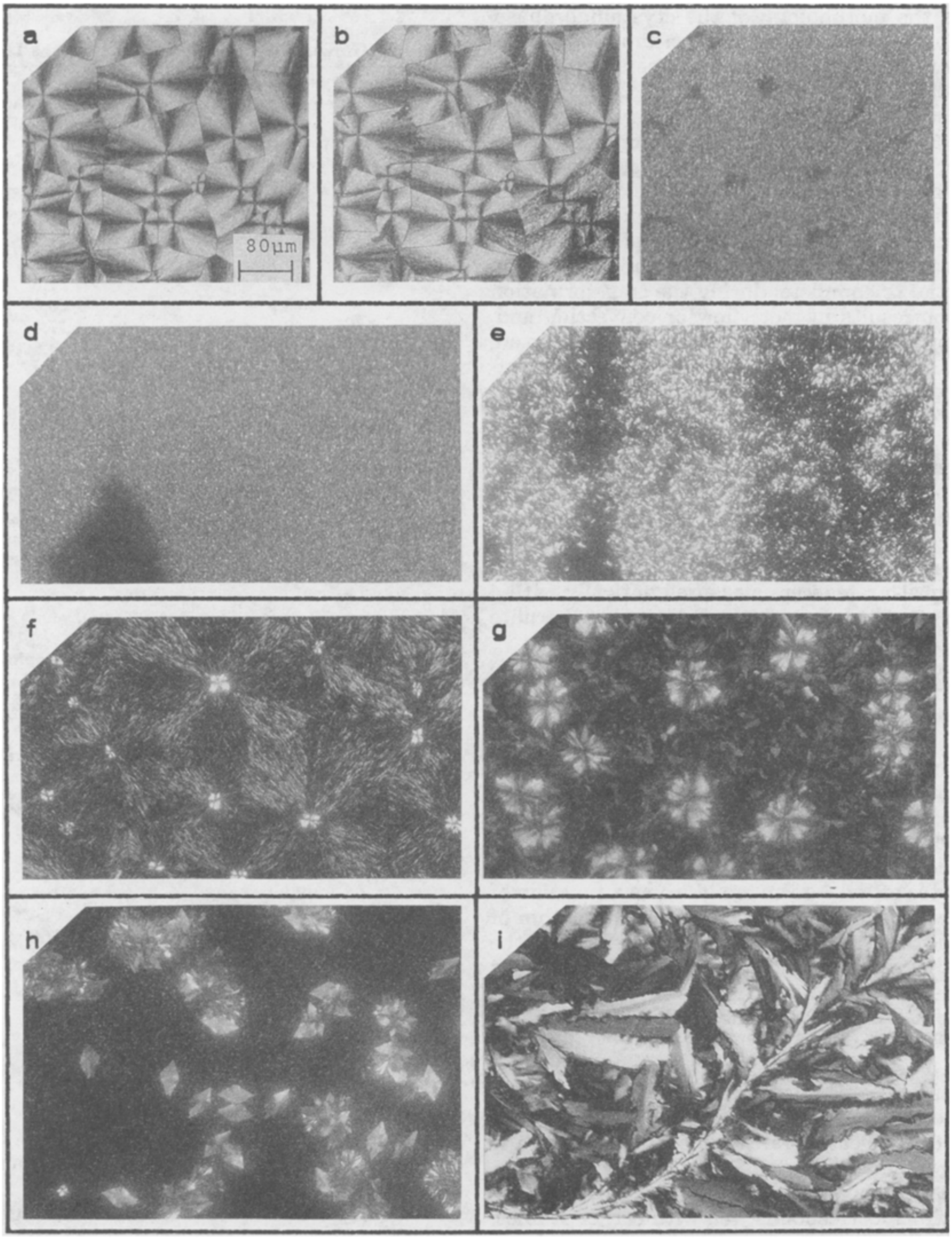


FIG. 2. Optical micrographs of different β-microstructures (magnification 133x, 1 cm = 106 µm). a. α-Form, obtained upon cooling an isotropic melt from 90–40°C at a rate of 5°C/min. b. β-Form, after slowly heating an α-form from 40–60°C at 2°C/min and keeping at 60°C for 15 min. c. β-Form, after quickly heating an α-form from 40–60°C (>20°C/min) and keeping at 60°C for 15 min. d. β′-Form, crystallized from the melt at 46°C for 10 min. e. β-Form, obtained after slowly heating the β′-form from 46–60°C at 2°C/min and keeping at 60°C for 15 min. f. β′-Form, crystallized from the melt at 49°C for 20 min. g. β-Form, obtained after slowly heating the β′-form from 49–60°C at 2°C/min and keeping at 60°C for 15 min. h. β-Form after crystallization from the isotropic melt at 58°C, with a nucleation induction (20–30 s) at 46°C. i. β-Form, directly crystallized from the isotropic melt at 58°C (magnification 53x; 1 cm = 266 µm).

a less intensely birefringent and more disordered β-form appears (Fig. 2g).

When the isotropic melt is left for 20–30 s at 46°C, followed by a further crystallization at 58°C, diamond-shaped β-crystals can be monitored which, upon prolonged stay at 58°C, result in spherulitic structures built up of lamellar entities (Fig. 2h). Nucleation induction at 46° clearly accelerates the β-crystallization process which, in turn, affects the morphology of the crystalline phase. Crystallization of β from the isotropic melt at 58°C, without nucleation induction at a lower temperature, results in large, leaflike lamellar structures (Fig. 2i).

The microstructure of the β-form strongly depends on whether the β-crystals are obtained *via* recrystallization from the α- or β'-form, or whether the β-crystallization occurs directly from the isotropic melt. The heating rate during the transition process, as well as the nucleation density, strongly determines the morphology of the β-crystal form; a higher heating rate causes a more pronounced melting (partial or complete) during the transformation process, which results in more flow or convection and, hence, more pronounced destruction of the microstructure of the initial phase. Clear confirmation of the polymorphic identity can be achieved by melting point determination.

Nucleation and growth rates. During the isothermal crystallization processes of tripalmitin at different crystallization temperatures, nucleation and spherulite growth rates also have been monitored. The nucleation rate was obtained by counting the number of nuclei appearing per unit of time in an observation field of 700 by 1000 μm. Spherulite growth rates were measured optically with a graduated occular by following the increase in spherulite radius as a function of time.

The crystal growth rate of the α-form is difficult to measure due to the high crystallization rates. The α-form is characterized by an extremely fast heterogeneous nucleation, even at low undercooling. At 43.5°C, the crystallization rate was sufficiently slow to allow photographic measurement of the α-spherulitic growth rate, about 140 μm/min. The crystallization kinetics of the β'-form are strongly dependent on the temperature and, hence, on the degree of undercooling. In Tables 1 and 2, the growth and nucleation rates of the β'-form are given as a function of the crystallization temperature. When the logarithm of the linear growth rate is plotted against the inverse of the undercooling, three linear segments can be observed which intersect around 50 and 52°C (Fig. 3b) (12). The undercooling is defined as the difference between the highest β'-melting temperature (58.5°C) and the crystallization temperature. For the nucleation rate (as established by counts of nuclei) a similar picture, with two linear segments intersecting at 50°C, is obtained by plotting the logarithm of the nucleation rate against the inverse of the undercooling squared (Fig. 3a) (12).

TABLE 1

Spherulite Growth Rate of Tripalmitin from the Isotropic Melt

Crystallization temperature (°C)	Growth rate (μm/min)
45.0	30.9
46.3	25.8
47.0	27.8
47.2	21.8
47.6	24.2
47.8	22.8
48.3	21.6
48.5	20.0
49.1	17.4
50.0	14.6
50.5	14.4
51.0	14.4
51.5	12.8
52.0	11.7
53.0	10.2
54.1	8.6
55.0	7.5
56.5	4.0

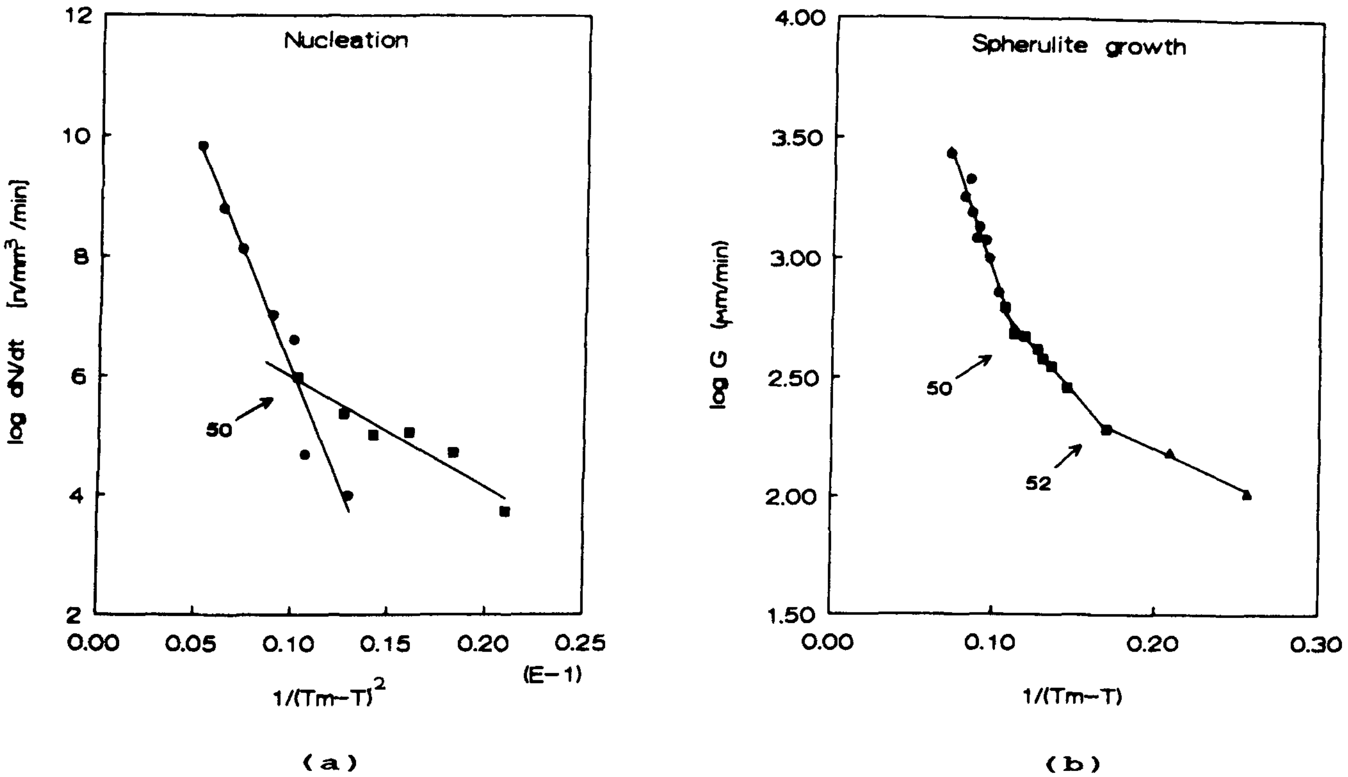
TABLE 2

Nucleation Rate of Tripalmitin from the Isotropic Melt (number of nuclei counted per min in an area of 700 × 1000 μm and a sample thickness of 10 μ)

Crystallization temperature (°C)	Nucleation rate (nuclei/mm^3/min)
45.0	18710
46.3	6640
47.2	3410
48.3	1130
49.2	108
50.1	54
48.9	750
49.0	400
50.0	214
50.5	150
51.0	157
51.5	111
52.0	41

Both nucleation and crystal growth show a discontinuity at 50°C, which corresponds well with earlier studies, where β-crystallization was found to become dominant above 50°C (5,13). The crystallization kinetics, as reflected by DSC, however, point to an almost exclusive β-crystallization above 50°C, with β'-crystallization being almost completely suppressed (5). In microscopy, on the other hand, the fast nonspherulitic growth of the β-form is restricted by an overgrowth of β'-spherulites. It seems that this containment allows thin samples to crystallize in the β'-polymorph at higher temperatures than is possible with bulk samples. Another factor favoring β'-crystallization in thin samples is the increased β'-nucleation rate, due to the larger surface-to-volume ratio. The intersection at 52°C, observed in the spherulite growth rate, corresponds to the transition of a fibrous to a feathery β'-form. Consequently, the radial growth rate seems to be negatively affected by a growth in the tangential direction.

Microscopic observation of spherulite nucleation and growth as function of temperature has confirmed the interpretation of earlier DSC experiments (5). The change from β'- to β-crystallization at 50°C is confirmed, and is shown to be caused by a nonspherulitic growth mechanism for the β-form at this temperature. This fast growth of the β-form, characterized by convective liquid flow, has also been observed in microscopy; in the latter case, however, it is restricted by an overgrowth of β'. It seems that this containment allows thin samples to crystallize in the β'-polymorph at higher temperatures than is possible with bulk samples. Another factor favoring β'-crystallization in thin samples is the increased β'-nucleation rate, due to the larger surface-to-volume ratio. The α-crystallization process is characterized by a fast heterogeneous nucleation and spherulitic growth rate, even at low under-



(a) (b)

FIG. 3. Nucleation and crystal growth rates of tripalmitin. a. Logarithm of the nucleation rate of tripalmitin, measured during isothermal crystallization at different temperatures between 45 and 55°C *vs.* the inverse squared undercooling (reference melting temperature of the β′-form, 58.5°C). b. Logarithm of the linear growth rate of the β′-form of tripalmitin, measured during isothermal crystallization at different temperatures between 45 and 55°C *vs.* the inverse undercooling.

cooling, resulting in intense birefringence and smooth spherulitic entities.

Four different β′-microstructures have been observed—grainy, fibrous, feathery and lamellar. Around 47°C, a drastic change from a grainy to a fibrous β′-microstructure has been detected. The transition seems to take place without drastic change in nucleation or crystal growth. Around 52°C, the β′-form changes again, from a fibrous to a more feathery microstructure. The fibrous-feathery transition is accompanied by a distinct decrease in crystal growth. Consequently, the radial development of the β′-spherulites is negatively effected by a growth in the tangential direction.

The lamellar β′-structure exhibits the highest stability and can only be obtained by an accelerated nucleation at low temperature, followed by further growth at elevated temperature near the melting temperature of the β′-form. Determination of the β-form on the basis of its microstructure is not always precise, because the microstructure of the β-form strongly depends on whether the β-crystals are obtained from a transformation process of α or β′, or whether β-crystallization occurs directly from the isotropic melt. The mobility of the molecules during the transition processes, as well as the nucleation density, strongly determines the morphology of the β-crystal form; the more pronounced the melting process (partial or complete) during the transformation process, the more flow or convection occurs, and the more the structural pattern of the initial crystal phase is wiped out.

The wide range of morphologies makes it difficult sometimes to identify polymorphism on the basis of microscopic appearance. Clear confirmation of the polymorphic identity of the solid state can be achieved by melting point determination.

REFERENCES

1. Timms, R.E., *Prog. Lipid Res. 23*:1 (1984).
2. Small, D.M., *The Physical Chemistry of Lipids: From Alkanes to Phospholipids*, Plenum Press, New York, 1986.
3. Garti, N., and K. Sato, *Crystallization and Polymorphism of Fats and Fatty Acids*, Marcel Dekker, Inc., New York, 1988.
4. Kellens, M., Ph.D. Thesis, University of Leuven, Belgium (1991).
5. Kellens, M., W. Meeussen and H. Reynaers, *Chem. Phys. Lipids 55*:163 (1990).
6. Kellens, M., W. Meeussen, C. Riekel and H. Reynaers, *Ibid. 52*:79 (1990).
7. Simpson, T.D., and J.W. Hagemann, *J. Am. Oil Chem. Soc. 59*:169 (1982).
8. Hernqvist, L., and K. Larsson, *Fette Seifen Anstrichm. 84*:349 (1982).
9. Weygand, C., and W. Grüntzig, *Anorg. Allgem. Chem. 206*:304 (1932).
10. Grüntzig, W., *Ibid. 240*:313 (1939).
11. Quimby, O.T., *J. Am. Chem. Soc. 72*:5064 (1950).
12. Wunderlich, B., in *Macromolecular Physics. Vol 2: Crystal Nucleation, Growth, Annealing*, Academic Press, New York, 1976.
13. Sato, K., and T. Kuroda, *J. Am. Oil Chem. Soc. 64*:124 (1987).

[Received February 18, 1991; accepted March 10, 1992]