The Effect of Lauric-Based Molecules on Trilaurin Crystallization

Paul R. Smith[a,*], **Deryck J. Cebula**[a] and **Malcolm J.W. Povey**[b]
[a]Unilever Research, Colworth Laboratory, Colworth House, Sharnbrook, Bedford MK44 1LQ, United Kingdom and [b]Procter Department of Food Science, The University of Leeds, Leeds LS2 9JT, United Kingdom

The crystal growth of trilaurin and the effect of partial laurates upon it have been investigated by using temperature gradient microscopy with differential scanning calorimetry and X-ray diffraction. The complex nature of trilaurin crystallization was demonstrated. It was found that the addition of monolaurin and lauric acid increased the crystal growth rate of trilaurin but decreased the facet and crystal size. Conversely, there was a significant reduction in growth rate upon the addition of dilaurin isomers. Crystal morphology was also altered, and the relative stability of the metastable phases was increased. The growth rate was lower for a sample containing 1-3 dilaurin than for one containing 1-2 dilaurin. It is postulated that the varying effects are caused by the varying sizes and shapes of the additive molecules.

KEY WORDS: Crystallization, habit, microscopy, modification, triglyceride, trilaurin.

The crystallization of triglycerides is a complex phenomenon that has interested researchers for many years. The process is complicated by the slow rate of crystal growth, caused by the polymorphic behavior of fats and the complex molecular shape. Despite this, the behavior is fairly well known, and a thorough review was provided by Garti and Sato in 1988 (1).

The crystallization of triglycerides is further complicated by effects of impurities, both glyceride and nonglyceride, upon the triglyceride. An early review showed that many kinds of emulsifiers tended to reduce the crystal growth rate of natural fat blends (2). Since then, some further work has been published on the effect of different emulsifiers on fat blends (3–6). However, there do not appear to be any published systematic works on model systems. Recently, Wähnelt *et al.* (7,8) have shown that the addition of diglycerides can retard the crystal growth of cocoa butter. Other researchers have investigated the effect of additives upon the polymorphic behavior of triglycerides (9,10). Aronhime *et al.* (11) have demonstrated that different additives can retard or enhance polymorphic transformations, depending upon their shape and how they fit into the triglyceride lattice. In particular, diglycerides have been found to retard transformations to β (11). In general, different additives have been shown to have different effects in different systems, and there has been little attempt at finding any underlying mechanisms.

Crystallization of fat blends is commercially important. In processes such as fractionation and products such as chocolate and margarine, control of crystal size and shape affect the product properties. Additives could have a role in controlling this, and, indeed, Hernqvist and Anjou (12) have demonstrated the use of diglycerides in stabilizing the β' form in high-oleic sunflower oil, making it suitable for use in margarine and fatty spreads by preventing the formation of the large and grainy β crystals.

Despite the effects that these materials can have on fat crystallization, there are no known rules for the effect of an individual impurity on a pure triglyceride, let alone complex mixtures of different additives on a fat blend or emulsion. Much current knowledge is empirical, and industrial application is often based more on art than on science.

The work presented here addresses this problem by studying a model system of a pure triglyceride (trilaurin) with known quantities of specific, chosen additives. In the first instance, various laurates were chosen as additives because they are molecules that are well characterized, with the same chainlength (C-12) as trilaurin. Therefore, we have an understanding of the sizes and shapes of these molecules, and can see how they interact with the trilaurin molecules.

EXPERIMENTAL PROCEDURES

Materials. Trilaurin, lauric acid, 1-monolaurin, racemic dilaurin, 1-2 dilaurin and 1-3 dilaurin were obtained from Sigma Chemical Co. (Poole, Dorset, United Kingdom) at better than 99% pure. The triglyceride was analyzed and found to be 99.9% pure with a trace of diglyceride present.

Methods. Mixtures of 1, 2 and 5% (by wt) of each additive in trilaurin were prepared. Samples containing 3 molar % of each additive were also made and a control sample of trilaurin was prepared. Samples were heated to approximately 80°C and complete dissolution of the additive was ensured.

Temperature gradient microscopy. A temperature gradient microscope stage has been designed and built after the manner of Hunt *et al.* (13) and Whittam and Rosano (14) (Fig. 1). It allows crystal growth to be monitored at any reasonable forced cooling rate. Cells are placed across the two plates and over the gap separating them. There is, therefore, a temperature gradient across the sample. The temperatures of the two plates can easily be controlled so that one is above the melting point of the sample, and the other below, creating a solid–liquid interface between the plates. The sample can then be moved at a controlled growth rate toward either of the plates, and crystallization or melting at fixed rates can be observed. In this case, the hot plate temperature was 70°C (±0.2°C) and the cold plate temperature was 10°C (±0.2°C). As the distance between the two plates is 6 mm, the temperature gradient is 10°C/mm.

Cells were made of two glass cover slips separated at the edges by a plastic spacer. Cell dimension was 22 mm × 22 mm. The sample thickness was always measured as 0.1–0.2 mm with a micrometer.

The sample was placed on the stage and allowed to equilibrate. It was then pulled through the temperature gradient at controlled forced growth rates of 1, 2, 5 and 10 mm/h (corresponding to cooling rates of 10, 20, 50 and 100°C/h). The microscope was a Leitz Ortholux II. During each run the observed position was only varied perpendicular to the growth direction (i.e., there was no movement of the field of vision parallel to the plates). Therefore, growth at one point on the interface was monitored, and the development of crystals at this point with time was seen. Photographs were taken at 5-min intervals for the first hour, then at 10-min intervals for the second hour and at 15-min intervals for the third hour for each run.

*To whom correspondence should be addressed.

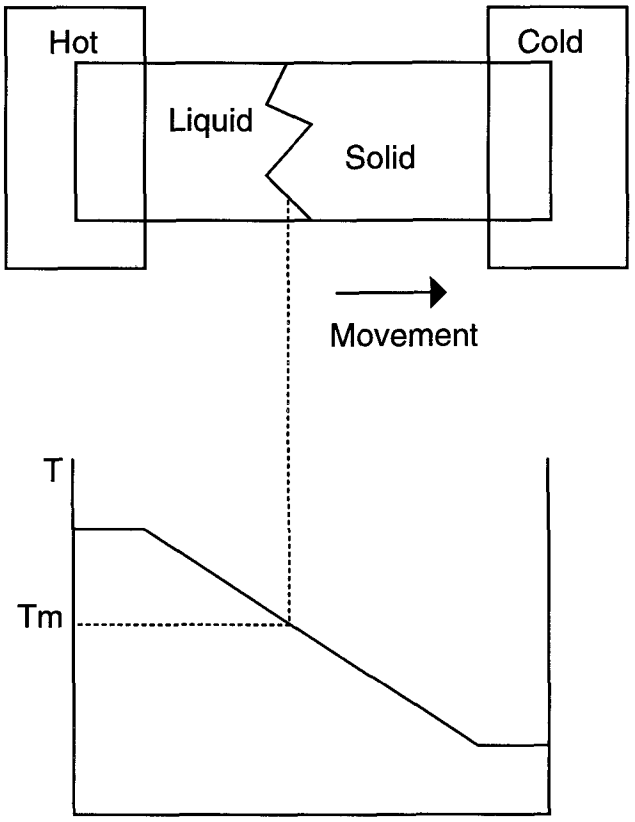

FIG. 1. The temperature gradient microscope stage.

Growth distance of the crystals was deduced from the position of the face at each time point. Undercooling at any particular time could also be calculated by knowledge of the growth distance, and of the distance moved by the initial interface away from the original position. Shapes and sizes of crystals were also noted from the photographs.

Calorimetry. Differential scanning calorimetry (DSC) was performed to determine transformation temperatures and polymorphic behavior with a Perkin-Elmer (Norwalk, CT) DSC 7 calorimeter. Samples were cooled from 60–20°C at 30°C per min, held for 5 min, and then heated to 60°C at 3°C per min.

X-ray diffraction. Dynamic X-ray diffraction measurements were performed with an Inel CPS-120 position-sensitive detector (Grenoble, France), according to the procedure described previously (15), to determine the polymorphic forms present during crystallization and transformations. Samples were cooled at 0.5°C per min from 60 to 0°C, and diffraction patterns were taken at 2-min intervals. X-ray diffraction was also performed with a Philips single counter detector (Eindhoven, The Netherlands) to allow accurate calculation of diffraction peak positions and lattice parameters.

RESULTS AND DISCUSSION

With the temperature gradient microscope stage, crystal growth was monitored for all four cooling regimes. The initial crystal growth rate was slower than the slide movement rate in all cases. Therefore, progressively increasing undercooling occurred, and crystal growth at a range of undercooling is illustrated.

For the pure trilaurin samples at slower slide-movement rates (1 and 2 mm per h), initial growth of long, thin, lath-like crystals occurred. These grew into the melt (Fig. 2). These crystals increased in size with time, and nearly perfect, large facets were seen. There was evidence of some twinning after two hours.

The addition of lauric acid caused a definite change to the morphology of the crystals, as can be seen from a comparison of Figures 2 and 3. There appears to be a broadening of the crystals, suggesting that the relative growth rates in the a and b directions are altered. A reduction in the facet size is also apparent, with the average size decreasing by a factor of at least two. Similar effects were caused by the addition of monolaurin. The twinning density was also increased in both cases, as evidenced by the increase in imperfection of the crystals.

Addition of any of the dilaurins causes the facet and crystal sizes to be reduced by a considerable amount (Fig. 4). After about 2 h they are so small that they cannot be observed. There is also a change in the crystal morphology

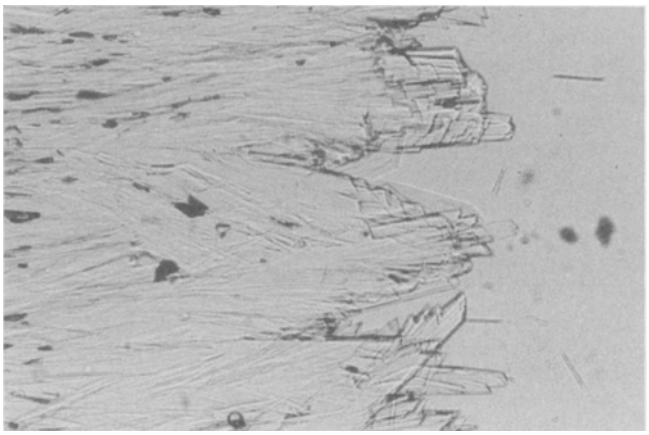

FIG. 2. Crystals of trilaurin, after two hours growth at a slide movement rate of 1 mm per hour.

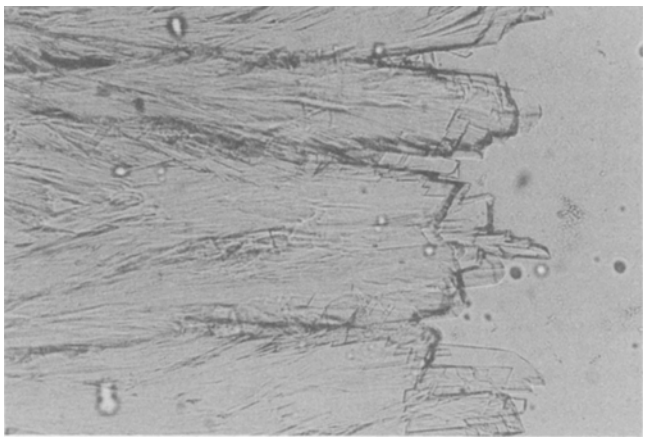

FIG. 3. Crystals of trilaurin plus 2% lauric acid, after two hours growth at a slide movement rate of 1 mm per hour.

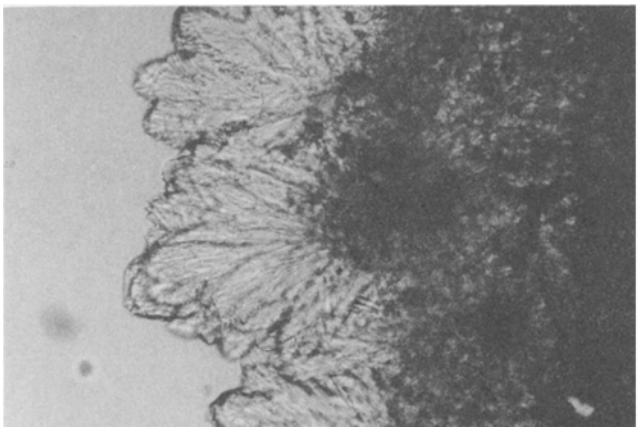

FIG. 4. Crystals of trilaurin plus 2% 1-3 dilaurin, after two hours growth at a slide movement rate of 1 mm per hour.

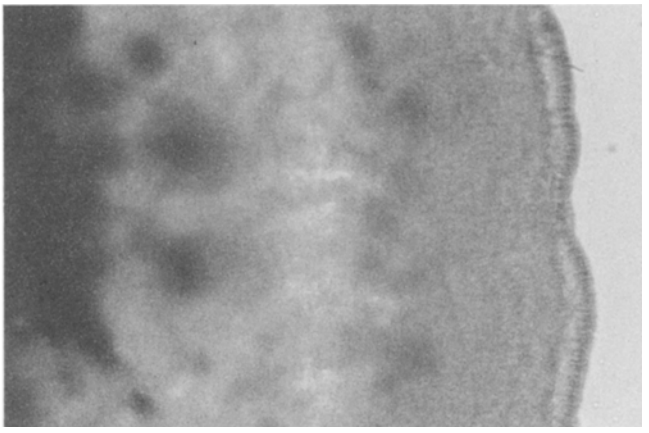

FIG. 5. Crystals of trilaurin, after 20 min growth at a slide movement rate of 10 mm per hour.

at that time, with much smaller crystals being produced. X-ray diffraction confirmed that these were still β crystals. The original, larger β crystals are also of a different morphology, being more pointed and showing a large change to the crystal habit. The twin density appeared to be increased as the crystals became darker.

Different effects were seen for the pure trilaurin sample at the faster movement speeds (5 and 10 mm per h) (Fig. 5), than at the slower movement speeds. Initially, the distinctive β plate-like crystals were seen. These had a higher twinning density and less perfect facets than the slower-grown crystals. This was followed by a change in the type of crystals seen, with much smaller, optically dense crystals being grown. Eventually, other crystals, less optically dense and more pointed, were seen. Finally, spherulites consisting of these crystals nucleated and grew in the liquid ahead of the main interface. This behavior was seen in all cases, although there was some variation in the times of the transformations. The major difference was that the dilaurates encouraged the formation of the two latter types of crystal (β and β') more quickly than in the other systems, with 1-2 dilaurin having the greatest effect.

Growth distance against time plots at a slide movement rate of 1 mm per h for all systems are shown in Figure 6. Both monolaurin and lauric acid increase the growth rate of trilaurin, whereas the dilaurates decrease it. 1-3 Dilaurin is particularly effective at retarding the growth

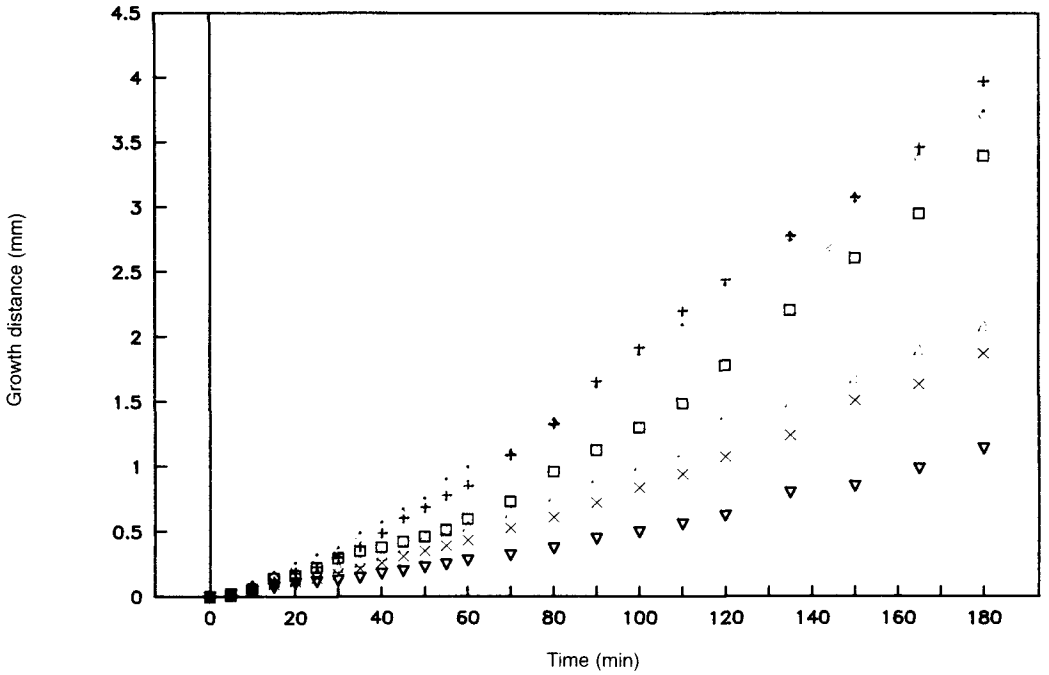

FIG. 6. Growth distance against time for all systems at a slide movement rate of 1 mm per hour (additive level 2% by wt). Trilaurin, □; trilaurin plus lauric acid, +; trilaurin plus monolaurin; ◇; trilaurin plus dilaurin, △; trilaurin plus 1-2 dilaurin, x; trilaurin plus 1-3 dilaurin, ▽.

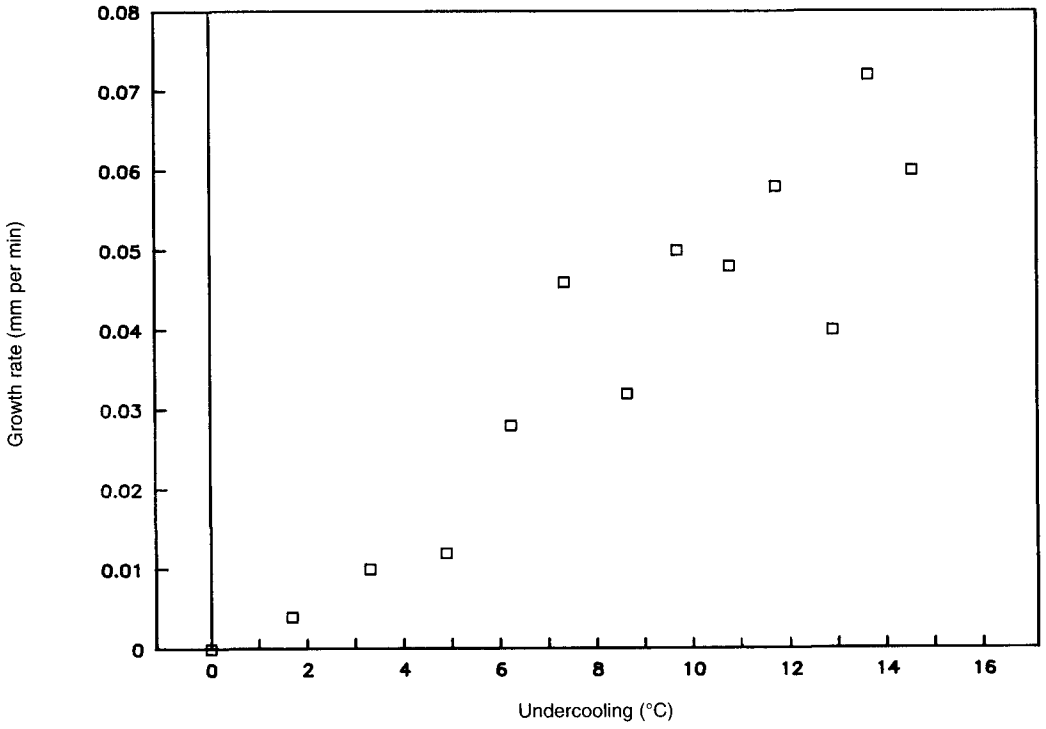

FIG. 7. Growth rate against undercooling for trilaurin at a slide movement rate of 5 mm per hour.

rate and reducing the total growth distance to about one-third of the initial amount. Increasing the concentration of the additive leads to greater retardation for the dilaurin-containing samples, with limited increases in growth rates for the monolaurin and lauric acid-containing samples. Consideration of results for similar molar concentrations of additive gives similar results.

From the shape of a plot of crystal growth rate against undercooling for a particular crystal face, it is possible to determine the crystal growth mechanism that is occurring. This can be done because the growth of the fastest growing face is always being measured for the large, lath-like crystals. Generally, there are four potential mechanisms. An overview is given in the next four paragraphs [for a more detailed explanation, the reader is referred to Boistelle (16)].

Continuous growth mechanism. An ideally rough interface with all sites being equal is assumed. The growth rate is proportional to the undercooling.

Island nucleation (birth and spread). For crystal growth to occur on a perfectly flat facet, it will be necessary for a new layer to nucleate on the facet, and then grow to cover the old surface. Nucleation of the new layer will be a rate-limiting step, and it can be shown that the growth rate is proportional to exp $\{-k/T\}$, where k is a constant. Therefore, growth is slow at low undercoolings.

Spiral growth mechanism. If a faceted crystal contains a screw dislocation, there will be a step in the facet. Molecules will add preferentially to this step because they will then be more strongly bound to the surface. The addition of molecules will cause the dislocation to rotate about its core and expand. In this case, the growth rate is proportional to the square of the undercooling.

Twin-plane mechanism (17). Crystal layers can be produced by nucleation at twin-plane reentrant corners. The reentrant corner lowers the energy of formation for a new plane. The growth rate is again proportional to the undercooling.

For pure trilaurin, with the cell moving at 2 mm per h, a graph of growth rate against undercooling is illustrated (Fig. 7). The rate of growth appears to be proportional to the undercooling. This suggests that growth is occurring either *via* the continuous growth mechanism or by the twin growth mechanism. In view of the fact that trilaurin is highly faceted and that triglycerides have high twinning densities, it is postulated that growth is occurring *via* the twin growth mechanism. Similar results were seen in all cases and show that this is the dominant mechanism in triglyceride growth. This confirms early work done by Albon *et al.* (18).

DSC showed that chilled liquid trilaurin solidified at 21°C, and the addition of 2% monolaurin leads to a slight increase in this temperature. The addition of 2% dilaurin decreases the solidification temperature, with the 1-3 isomer having a particularly large effect (Fig. 8A). On heating trilaurin, an endothermic trough is seen at 26°C (Fig. 8B). This corresponds to any α in the sample transforming to β' or β. Because of the low stability of the β', any α transforms quickly to β, and, therefore, a β melting peak is seen at 45°C. Monolaurin-containing samples are extremely similar. However the addition of dilaurin causes an upward temperature shift in the initial transformation, as well as a change in the nature of the transformation, especially with the 1-2 dilaurin. This suggests that, in this case, α is melting, before transforming to β. This result illustrates that the 1-2

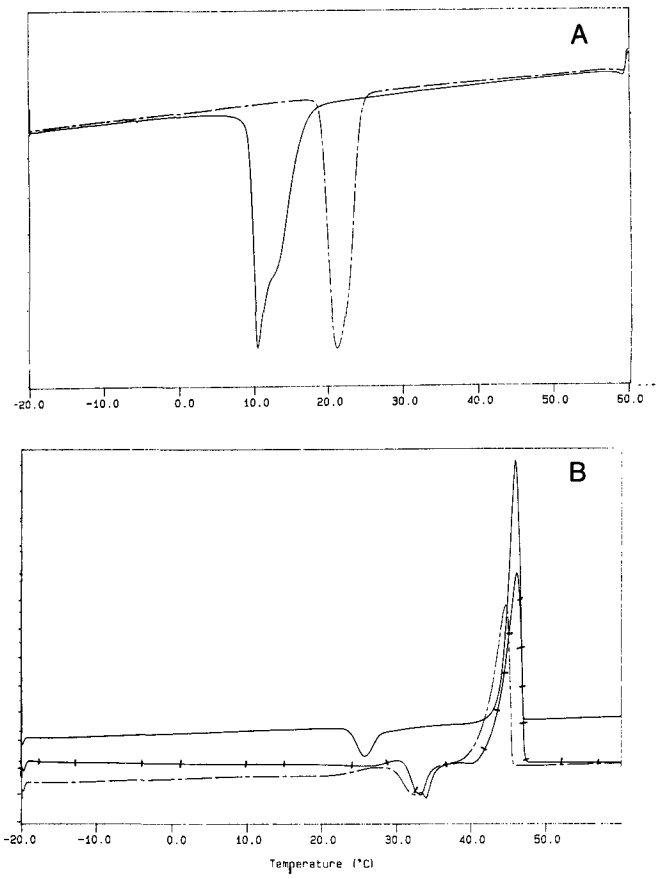

FIG. 8. Differential scanning calorimetry cooling curves at 3°C per min. A: i, Trilaurin, —·—·—·—; ii, trilaurin plus 2% 1-3 dilaurin, ——. B: i, Trilaurin, ———; ii, trilaurin plus 2% 1-2 dilaurin, —+—+—+—; iii, trilaurin plus 2% 1-3 dilaurin, —·—·—·—.

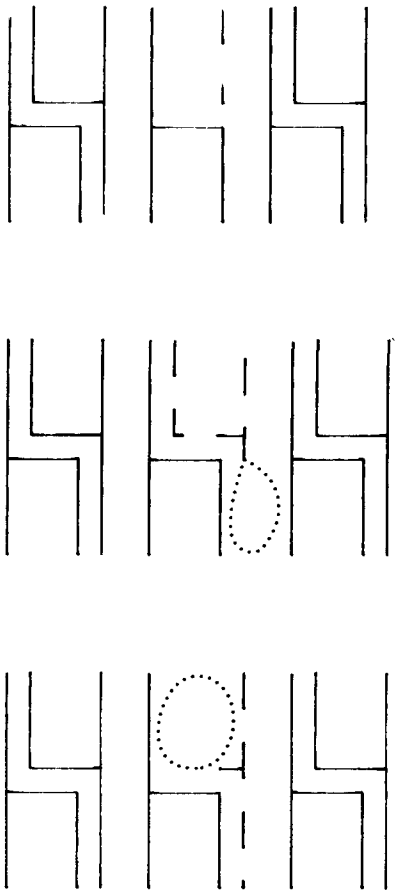

FIG. 9. Suggested incorporation mechanisms for the different additives. Top panel: lauric acid or monolaurin, gap filling. Middle panel: 1-2 dilaurin, gap generating. Bottom panel: 1-3 dilaurin, gap generating.

dilaurin is a stronger α and β' stabilizer than the 1-3 dilaurin.

Time sequence position-sensitive X-ray diffraction plots show that on slow cooling α and β' phases nucleate and grow. These always transform to β over several minutes. The additives do not have much effect, except for dilaurin, which causes crystallization to be shifted to lower temperatures.

These results illustrate the complex nature of triglyceride crystallization. It is affected by the additives in distinct ways. The crystallization rate is increased by the smaller monolaurin and lauric acid molecules. Facet and crystal size are also reduced by the smaller molecules. It is suggested that this is because they are both small molecules that can easily fit into the trilaurin lattice (as illustrated in Fig. 9). Therefore, they do not retard the growth rate and, in fact, may slightly increase it. The formation of the smaller, less perfect crystals is due to an increase in the defect density caused by the additives. Because of the larger size of the dilaurin molecules, incorporation into the lattice is more difficult. As illustrated in Figure 9, a blocking of growth sites can occur, which leads to the reduction in growth rate and, because of the different effects on different faces, to a change in habit. Because the diglyceride molecules have different shapes and fit into the lattice in different ways, the variation in effect between the two isomers can be explained.

The work shows the effect that particular partial glycerides can have on triglyceride crystallization. These materials are produced by the degradation of fats, and thus the importance of quality control of fats is illustrated.

ACKNOWLEDGMENT

The authors thank the management of Unilever Research for permission to publish this work.

REFERENCES

1. Garti, N., and K. Sato (eds.), *Crystallization and Polymorphism of Fats and Fatty Acids*, Surfactant Science Series 31, Marcel Dekker, New York, 1988.
2. van den Tempel, M., in *SCI Monograph 32*, SCI, London, 1968, p. 22.
3. Niiya, I., E. Moire, M. Imamura, M. Okada and T. Matsumoto, *Japan. J. Food Sci. Technol. 18*:583 (1969).
4. Niiya, I., T. Maruyana, M. Imamura, M. Okada and T. Matsumoto, *Ibid. 20*:182 (1971).
5. Sambuic, E., Z. Dirik and M. Naudet, *Rev. Fr. Corps Gras 28*:59 (1981).
6. Garti, N., E. Wellner and S. Sarig, *J. Am. Oil Chem. Soc. 59*:181 (1982).

7. Wähnelt, S., D. Teusel and M. Tülsner, *Fat Sci. Technol. 93*:117 (1991).
8. Wähnelt, S., D. Teusel and M. Tülsner, *Ibid. 93*:174 (1991).
9. Hernqvist, L., B. Herslof, K. Larsson and O. Podlaka, *J. Sci. Food Agric. 32*:1197 (1981).
10. Norton, I.T., C.D. Lee-Tuffnell, S. Ablett and S.M. Bociek, *J. Am. Oil Chem. Soc. 62*:1237 (1985).
11. Aronhime, J.S., S. Sarig and N. Garti, *Ibid. 65*:1144 (1988).
12. Hernqvist, L., and K. Anjou, *Fat Sci. Technol. 85*:64 (1983).
13. Hunt, J.D., K.A. Jackson and H. Brown, *Rev. Sci. Instrum. 37*:805 (1966).
14. Whittam, J.H., and H.L. Rosano, *J. Am. Oil Chem. Soc. 52*:128 (1975).
15. Cebula, D.J., and P.R. Smith, *Ibid. 67*:811 (1990).
16. Boistelle, R., in *Crystallization and Polymorphism of Fats and Fatty Acids*, edited by N. Garti, and K. Sato, Surfactant Science Series 31, Marcel Dekker, New York, 1988.
17. Tiller W.A., in *The Science of Crystallization: Microscopic Interfacial Phenomena*, Cambridge University Press, Cambridge, 1991, pp. 80–84.
18. Albon, N., D. Illingworth and R. Hull, *J. Cryst. Growth 2*:26 (1968).

[Received March 20, 1994; accepted August 30, 1994]