Polymorphism of Milk Fat Studied by Differential Scanning Calorimetry and Real-Time X-ray Powder Diffraction

E. ten Grotenhuis[a], G.A. van Aken[a,*], K.F. van Malssen[b], and H. Schenk[b]

[a]NIZO food research, Department of Technology, 6710 BA Ede, The Netherlands,
and [b]Institute for Molecular Chemistry (IMC), Laboratory of Crystallography,
Universiteit van Amsterdam, 1018 WV Amsterdam, The Netherlands

ABSTRACT: The crystallization behavior of milk fat was investigated by varying the cooling rate and by isothermal solidification at various temperatures while monitoring the formation of crystals by differential scanning calorimetry (DSC) and X-ray powder diffraction (XRD). Three different polymorphic crystal forms were observed in milk fat: γ, α, and β′. The β-form, occasionally observed in previous studies, was not found. The kind of polymorph formed during crystallization of milk fat from its melted state was dependent on the cooling rate and the final temperature. Moreover, transitions between the different polymorphic forms were shown to occur upon storing or heating the milk fat. The characteristic DSC heating curve of milk fat is interpreted on the basis of the XRD measurements, and appears to be a combined effect of selective crystallization of triglycerides and polymorphism.

Paper no. J9079 in *JAOCS 76,* 1031–1039 (September 1999).

KEY WORDS: Crystallization, differential scanning calorimetry, milk fat, polymorphism, X-ray diffraction.

Milk fat is one of the main constituents of milk and determines the specific properties of butter and cream. It is also an important ingredient in many bakery and confectionery industry applications. The various applications require different properties of milk fat, which in turn requires improved functionality control. The functional properties of milk fat are strongly related to the amount and type of milk-fat crystals at the temperature of application. The crystalline part of the fat determines to a large extent the firmness of products in which fat is present as the continuous phase, such as butter and butter oil, and the stability of products containing an emulsion of milk fat, such as cream. Milk fat has a broad melting range due to a large number of triglycerides with a wide range of chain lengths and degrees of saturation. Moreover, the phase behavior is complicated because of the polymorphism of the solid phase.

Polymorphism of the crystallized phase is a general feature of triglycerides. The different polymorphic forms can be identified by X-ray diffraction (XRD). The polymorphic forms are characterized by the *d*-spacings (short-spacings) of the crystal lattices (typically between 3 and 6 Å) as observed in XRD patterns, which correspond to the distances associated with the lateral packing of the fatty acid hydrocarbon chains. Polymorphs with similar packing of the fatty acid hydrocarbon chains were found for pure tristearin (1), and for natural oils and fats (2), including milk fat (3). The *d*-spacings are characteristic for the type of polymorph, and this has led to the nomenclature given by Larsson (4) that is now widely accepted. Table 1 lists the *d*-spacings of the polymorphs of triglycerides (2). In general, the stable polymorph of triglycerides is either a β′- or a β-crystal form. The density of the β-crystal form is higher than that of the β′-crystal form, and this leads to more severe packing constraints for the first form as compared with the latter (5). As a result, asymmetrical triglycerides, i.e., triglycerides of the SSU or UUS type, in which the single unsaturated (U) or saturated (S) fatty acid resides in either the *sn*-1 or *sn*-3 position, or triglycerides of fatty acids with differences between the chain lengths larger than two carbon atoms, are usually stable in the β′-polymorph (5,6). For β-stable triglycerides, the β′-form is often found as a metastable polymorph. The α-modification is the least stable of the three polymorphs α, β′, and β, and is found as a metastable form for all triglycerides. A fourth polymorph that is found is the γ-form. This form has also been named sub-α, because it has a lower melting point than the α-form, and also β'_2, as it is structurally related to the β′-form. In this paper the term γ is used in correspondence with the nomenclature of Vaeck (7).

TABLE 1
Nomenclature and Assignment of Polymorphs in Triglycerides[a]

Polymorph	Strongest X-ray short spacings (Å)	Subcell form
α	4.15	Hexagonal H
β′	3.8 and 4.2 or 3.71, 3.97, and 4.27	Orthorhombic $O_\perp$
β	3.5–4.0 and 4.6	Triclinic $T_\parallel$
γ (This is a low-melting β′-modification.)	3.7 and 4.2	Orthorhombic $O_\perp$

[a]See Reference 2.

---

*To whom correspondence should be addressed at NIZO food research, Department of Technology, P.O. Box 20, 6710 BA Ede, The Netherlands.
E-mail: aken@nizo.nl

Copyright © 1999 by AOCS Press

Mulder (8) reported a double-melting behavior for milk fat, and ascribed this behavior to the occurrence of polymorphism. The polymorphic forms of milk fat were studied using XRD (3,9–13). Some studies have focused on the differences between the final diffraction pattern obtained after rapid and slow cooling (9,10). It was found that the final diffraction pattern of milk fat showed mainly β′-modification with traces of β-crystals in the samples that were slowly cooled. Van Beresteijn (11) reported the formation of α- and β′-crystals. It was suggested that larger amounts of β-crystals were formed above 30°C after heating from 11°C; however, the diffraction peak at 4.6 Å, typical for the β-form, was not observed. In a study by Schaap *et al.* (12), only the α- and β′-form were found after slow cooling (0.1°C/min) to 28°C and after storing the sample at 28°C for 24 h. These results were confirmed by Timms (3), who found a very weak intensity of the characteristic β-diffraction peaks in slowly cooled milk fat. He also used differential scanning calorimetry (DSC) to measure the heat effects related to melting and crystallization during milk-fat heating. A pattern characteristic for milk fat, with three endothermic peaks (h_1, h_2, and h_3), was observed (Fig. 1). An exothermic heat effect at 16°C, observed between h_1 and h_2, was attributed to a polymorphic transition from the α- to the β′-form. In a recent study by Metin and Hartel (13), DSC and XRD were used to investigate the isothermal crystallization of milk fat. They demonstrated that milk fat crystallizes in the β′-form after 3 h of solidification at 17°C. Timms (14) stated that partial transformation of milk-fat crystals to the β-form can only occur if a considerable amount of milk fat is still liquid.

In some studies (15,16), the three melting peaks (h_1–h_3) in the DSC curves were interpreted in terms of fractions of milk fat. These fractions were called low-, middle-, and high-melting, on the basis of their melting points. The independent melting of these three fractions was assumed to be the reason for the three endothermic peaks in the heating curve of milk fat. In these studies, polymorphism was not considered as a cause for the particular shape of the DSC curves.

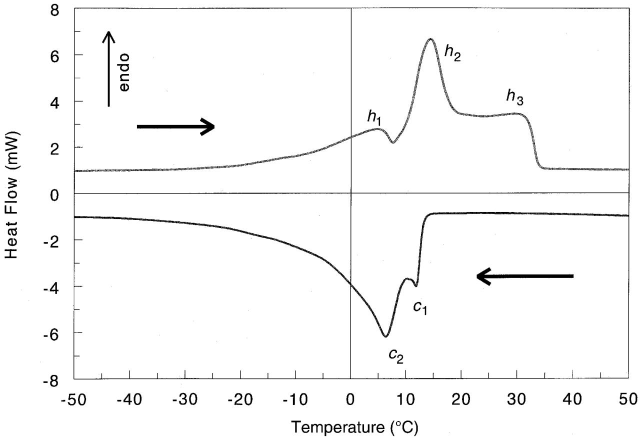


FIG. 1. Typical differential scanning calorimetry (DSC) cooling and heating cycle of milk fat. The two exothermic crystallization peaks during cooling are named c_1 and c_2; the three endothermic peaks during heating are named h_1, h_2, and h_3.

The polymorphic behavior of milk fat is to a large extent determined by the rate of cooling. In previous studies, however, the authors have merely made a distinction between "slow" and "rapid" cooling, often without mentioning the exact cooling rates. With the exception of the studies by Belousov and Vergelesov (17) and by van Beresteijn (11), the polymorphic modifications were usually examined after considerable stabilization periods. For a proper understanding of crystallization behavior, the dynamic processes occurring during cooling and heating should be monitored in an X-ray diffractometer.

In this study, a real-time X-ray powder diffractometer, which was purposely built to follow crystallization as a function of time and temperature (18), and a differential scanning calorimeter were used to investigate the phase behavior of milk fat. By combining the results obtained with these techniques, it is shown that the typical shape of the DSC heating curve for milk fat is caused by a combination of polymorphic transitions and the melting of fractions of triglycerides with different melting points.

MATERIALS AND METHODS

Milk fat. Anhydrous milk fat was obtained from Frico Union de Jong (Noordwijk, Groningen, The Netherlands) and filtered at 70°C before use. The milk fat was separated from milk that was collected in winter 1996. The fatty acid distribution was reported by Van Aken *et al.* (19). The main fatty acids are palmitic acid (16:0), oleic acid (18:1), myristic acid (14:0), and stearic acid (18:0). Milk fat also contains short-chain fatty acids, e.g., butyric acid (4:0) and caproic acid (6:0), in considerable quantities.

XRD. XRD patterns were recorded with an instrument that was initially built to study cocoa butter (18,20). Monochromated CuK_α radiation with a wavelength of 1.5418 Å was used to obtain diffraction patterns in the 2θ range 14.6–29.6°, which corresponds to *d*-spacings from 3.0 to 6.1 Å. The instrument was calibrated with potassium iodide as a reference. The samples were prepared by pressing solid samples into a sample holder with dimensions of $1 \times 10 \times 15$ mm^3, which supported approximately 135 mg of milk fat. The sample holder was positioned in a vacuum measuring chamber. The temperature of the sample holder was measured *via* a Pt-100 element. It was controlled within 0.1°C of preset temperatures by simultaneous water or liquid nitrogen cooling and computer-regulated heating. All XRD patterns are represented as difference patterns with respect to liquid milk fat.

DSC. DSC curves were measured with a Perkin Elmer DSC 7 by cooling or heating the sample at a constant rate and measuring the heat transfer. The instrument was calibrated for all cooling and heating rates applied with gallium and indium. Samples weighed between 5 and 15 mg. An empty sealed pan was used as a reference. Before the actual measurements, the samples were heated to 70°C and held at this temperature for at least 5 min to ensure that all nuclei were eliminated.

Combining XRD and DSC measurements. Both XRD and DSC can be used to monitor crystalline phase transitions. XRD measures the amounts of the different polymorphic forms, and the change in intensity and position of the diffraction peaks is used as a measure of polymorphic changes. However, because different chemical compositions within one polymorphic form lead to approximately the same diffraction pattern, the method is not sensitive for transitions within the same polymorphic form. Within a polymorphic form, the positions of the diffraction peaks shift if the fatty acid chain lengths of the triglycerides are changed. This will lead to a broadening of the diffraction peaks for a mixture of crystals with the same polymorphic structure but with different chemical composition. On the other hand, change of peak width can also be related to a decrease in crystal size, and a shift in peak position can also be related to shrinkage or expansion, usually due to heating or cooling. Therefore, it is difficult to quantify crystalline transitions based solely on XRD patterns.

DSC measures the overall heat flow that is associated with phase transitions. Compared to XRD, DSC has the advantage of less elaborate equipment and enthalpic phase-transition sensitivity, irrespective of whether the transition takes place within the same polymorph or between polymorphs. The main disadvantage of the technique is that it does not measure the presence of a crystal form directly. Moreover, if several processes occur simultaneously, a superposition of heat effects is measured that cannot be easily separated. Another factor that complicates interpretation is that the heat of formation of a polymorph also depends on the triglyceride composition.

To circumvent misinterpretations of the experimental observations solely based on XRD or DSC, these techniques were used in combination. However, we must keep in mind that some differences between XRD and DSC results are expected, due to the differences in sample dimensions and the difference in heat exchange between the sample and the sample holder.

Solidification as a function of cooling rate. Samples were cooled from 70 to −65°C at a cooling rate of dT/dt, varying from 0.5 to 20°C/min, and the crystallization was studied by XRD and DSC.

The XRD patterns were recorded during an exposure time t_E at the start of equally spaced intervals with a length t_I. The temperature at which a polymorphic form first appeared is given by T_s, the rate of change observed in the XRD pattern was largest at temperature T_m, and below a temperature T_e, the XRD pattern had become constant. The polymorphic phases were identified from the XRD patterns, which are different for the γ-, α-, and β′-forms. The diffraction pattern shown by Woodrow and deMan (10) for milk fat crystallized from acetone was taken as the reference pattern for the β-form.

The DSC curves were measured using the appropriate calibration. For the DSC curves, the onset and the maximal temperatures of the peaks were determined using standard analysis routines. After the samples had been kept for 1 min at −65°C, DSC heating curves were recorded by heating to 70°C at a rate of 5°C/min and measuring the heat flow to the sample relative to an empty pan.

Isothermal solidification as a function of temperature. The samples were cooled from 70°C to the final solidification temperature T_f at a cooling rate of −20°C/min and the crystallization was studied using XRD and DSC.

XRD patterns were subsequently recorded for 60 min. The presence of the different polymorphic forms was judged from the XRD patterns. The start of a transition is given by t_s, at t_m the XRD pattern changed the most, and at t_e, the XRD pattern became constant. The heat effects during the isothermal period were recorded by DSC, and after varying periods of isothermal solidification, DSC heating curves were recorded by heating to 70°C at a rate of 5°C/min.

RESULTS

Solidification as a function of cooling rate. The shape of the DSC cooling curve of milk fat depends on the cooling rate (Fig. 2). Generally, two peaks can be distinguished, named c_1 and c_2 (Fig. 1). The temperature at which the milk fat started to crystallize decreased with increasing cooling rate (Table 2). The relative size of the peak c_1 decreased with increasing cooling rate, and at a cooling rate of −20°C/min, it disappeared. The enthalpy of crystallization, corresponding to the total area of the peaks, increased with decreasing cooling rate.

The same temperature profiles were applied to the samples that were measured with XRD. Figure 3 shows the typical diffraction patterns of the γ-, α-, and β′-forms of milk fat. Figure 4 shows consecutively recorded XRD patterns at −5, −30, and −70°C obtained by cooling at a rate of −5°C/min. A typical α-diffraction pattern was observed at −5°C. On further cooling with the same cooling rate to −30 and −70°C, XRD patterns were obtained that showed two peaks with *d*-spacings characteristic for the γ-form (3.7 and 4.2 Å), superimposed on the pattern of the α-diffraction. The polymorphs that formed appeared to depend on the cooling rate, as can be seen from the results of the XRD measurements listed in Table 3.

TABLE 2
Differential Scanning Calorimetry Crystallization Peak Onset, Maximal Temperatures, and Enthalpies

	1st crystallization peak		2nd crystallization peak		
dT/dt (°C/min)	Onset temperature (°C)	Maximal temperature (°C)	Onset temperature (°C)	Maximal temperature (°C)	Δ*H* (J/g)
−20	7.0	−0.2	—	—	−65.5
−10	11.2	9.2	9.5	2.8	−66.5
−5	13.6	11.9	11.0	6.4	−69.8
−2.5	14.6	13.3	11.6	8.2	−70.0
−1	16.7	15.6	12.5	10.6	−71.2
−0.5	17.6	16.8	12.6	11.6	−72.8

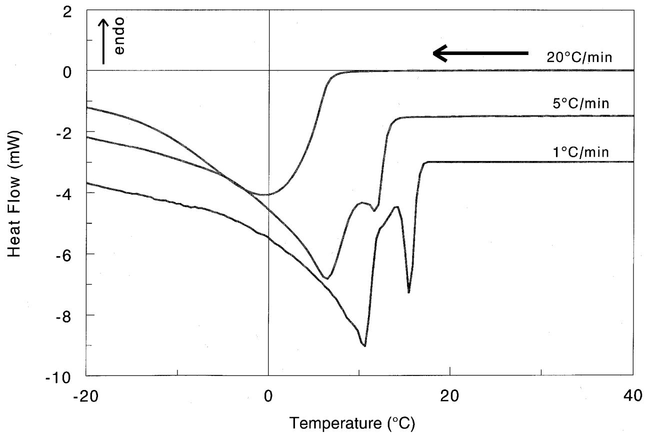


FIG. 2. DSC curves of milk fat cooled at rates varying from 1 to 20°C/min. The heat flows were scaled to the cooling rate of 5°C/min and shifted relative to each other for clarity. See Figure 1 for abbreviation.

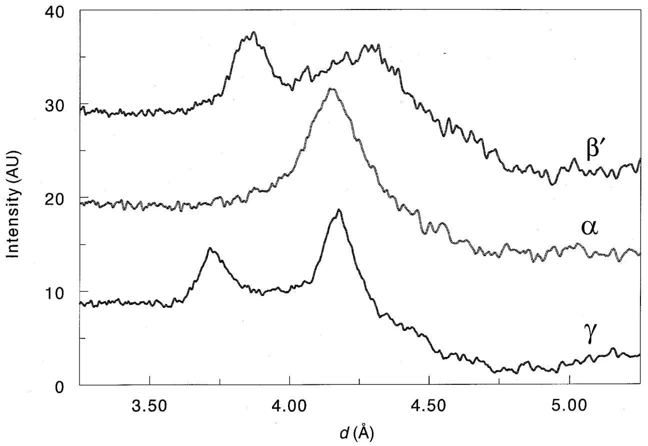


FIG. 3. Characteristic X-ray powder diffraction (XRD) patterns of the three polymorphic forms found in milk fat. The pattern of the γ-modification was obtained at −50°C after cooling at a rate of 20°C/min. The α-diffraction pattern was obtained at −5°C after cooling at a rate of 5°C/min. The β′-diffraction pattern is shown for milk fat kept for 30 min at 20°C. AU, aarbitrary units.

At a cooling rate |*dT*/*dt*| ≥ 2.5°C/min γ-crystals were formed together with α-crystals. At a cooling rate |*dT*/*dt*| of 1.67°C/min, only α-crystals were formed. Cooling rates |*dT*/*dt*| of 1°C/min and below resulted in the formation of β′-crystals together with a small amount of α-crystals (Fig. 5). The amount of α-crystals decreased on decreasing the cooling rate.

DSC heating curves after solidification as a function of cooling rate. DSC curves of milk fat were recorded during heating at a rate of 5°C/min after the milk fat had been cooled at different cooling rates varying from −0.2 to −20°C/min (Fig. 6). The DSC curves of milk fat cooled at a rate of −1, −2.5, −5, and −10°C/min are similar, showing the three distinctive endothermic peaks, h_1–h_3. The first effect (h_1) starts at approximately −40°C and ends at 8°C. The second peak in the curve, h_2, starts at this temperature and is relatively sharp. At 20°C, h_2 ends and the start of a broad shoulder (h_3) is observed that ends abruptly at the clear point of 35°C. Above this temperature the milk fat appeared liquid, although some authors suggest that the triglycerides in the liquid are still ordered in lamellae (21). After cooling at a rate of −20°C/min the dip between h_1 and h_2 was absent in the heating curve. In the heating curve recorded after cooling at rates |*dT*/*dt*| ≤ 0.5°C/min h_1 is absent. Melting started immediately with peak h_2. The broad shoulder (h_3) is also present in this curve, but the clear point has shifted to a slightly higher temperature (by approximately 1°C).

After cooling at a rate of −5°C/min the XRD patterns of milk fat were recorded while heating at a rate of 5°C/min (Fig. 7). After cooling at −5°C/min the milk fat was mainly in the γ-form, which is in agreement with the results shown in Figure 4. Heating led to the disappearance of this form starting at approximately −38°C. The γ-form transformed into the α-form and had disappeared completely at −8°C. β′-Crystals were observed above T = 7°C. The last diffraction of the α-modification disappeared at 20°C. The clear point of the β′-crystals was 35°C. An overview is given in Table 4.

Isothermal solidification as a function of temperature. Isothermal measurements were performed with DSC and

TABLE 3
Crystallization Temperature Ranges of Polymorphic Forms of Milk Fat Determined by XRD, After Cooling from 70°C at Different Cooling Rates[a]

dT/*dt*	t_E	t_I	β′-Crystallization			α-Crystallization			γ-Crystallization		
(°C/min)	(s)	(s)	T_s	T_m	T_e	T_s	T_m	T_e	T_s	T_m	T_e
−20	3	3	—	—	—	(15)	(9)	(3)	−15	−23	−44
−10	6	6	—	—	—	15	10	3	−13	−21	−43
−5	12	12	—	—	—	16	11	5	−12	−20	−35
−3.33	12	18	—	—	—	16	10	4	−9	−16	−30
−2.5	12	24	—	—	—	16	11	6	−9	−13	−17
−1.67	12	36	—	—	—	18	11	5	—	—	—
−1	12	60	17	11	6	(17)	(11)	(6)	—	—	—
−0.5	12	120	20	11	5	—	—	—	—	—	—

[a] Parentheses indicate that the amount of the polymorph formed was very small. T_m, temperature at which rate of change observed in the XRD pattern was largest; T_e, temperature at which XRD pattern became constant; T_s, temperature at which a polymorphic form first appeared; t_E, exposure time; t_T, length of (equal) time intervals; XRD, X-ray powder diffraction.

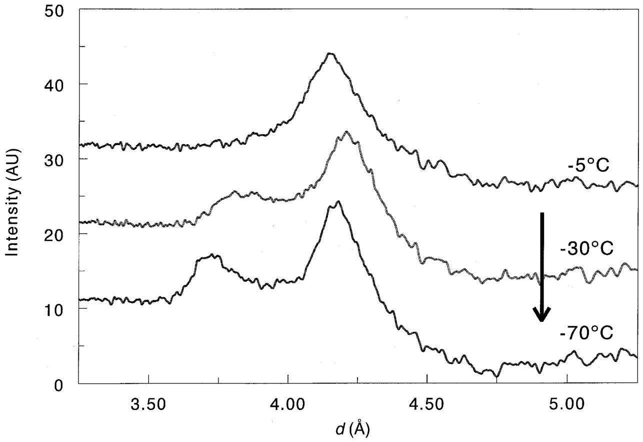


FIG. 4. XRD patterns of milk fat cooled at 5°C/min taken at decreasing temperatures: −5, −30, and −70°C. Arrow indicates order of measurement. See Figure 3 for abbreviations.

XRD to investigate the crystallization process of milk fat at a fixed temperature. The isothermal DSC curves for temperatures of 10, 14, 17, and 20°C are shown in Figure 8. The curves at 10 and 14°C start with a large exothermic transition from time $t = 0$ to approximately 5 min (10°C) and 2 min (14°C), and have a second exothermic transition visible as a peak at later time. The latter transition has its maximal peak at 32 min, $T = 10$°C and at 25 min, $T = 14$°C. In the curve at 17°C, the first exothermic transition is much smaller and clearly visible as a separate peak. The first transition has a maximal peak after $t = 2.5$ min, the second transition after $t = 22$ min. For $T = 20$°C, only one transition was found, which had its maximal peak after $t = 35$ min. Apparently, this peak corresponds to the second transition at lower temperatures, whereas the peak corresponding to the first transition at lower temperatures is absent here. At higher temperatures the position of this transition shifted to longer times.

XRD measurements were performed at six temperatures ranging from −10 to 20°C. The results are summarized in Table 5. At $T = -10$°C and $T = 4$°C, the milk fat crystallized in the α-form during cooling. The intensity of the α-diffraction peak hardly decreased during the first 30 min of isothermal solidification. Only a small amount of β′-crystals was formed during this period. However, after 3 d of isothermal solidification at 4°C the crystals were completely transformed into the β′-form. At $T = 10$°C, the milk fat had crystallized in the α-form during cooling (Fig. 9). After the first 30 min of isothermal solidification, the solid consisted of a mixture of α- and β′-crystals. At $T = 17$°C, only a very weak α-diffraction peak was visible at $t = 0$ min, but it increased to reach its maximal level at $t = 3$ min. Similar polymorphic behavior was found by van Malssen *et al.* (20) for cocoa butter that was kept isothermally at $T = 22$°C. Figure 10 shows the diffraction patterns after 12 min and after 30 min. The solid fraction of milk fat was still completely in the α-form after 12 min,

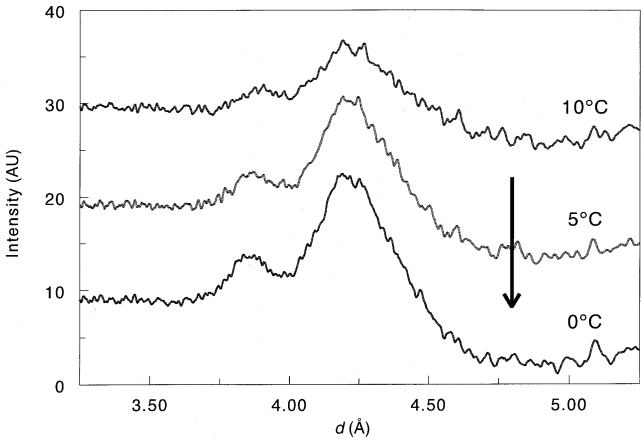


FIG. 5. XRD patterns of milk fat cooled at 1°C/min taken at decreasing temperatures: 10, 5, and 0°C. Arrow indicates order of measurement. See Figure 3 for abbreviations.

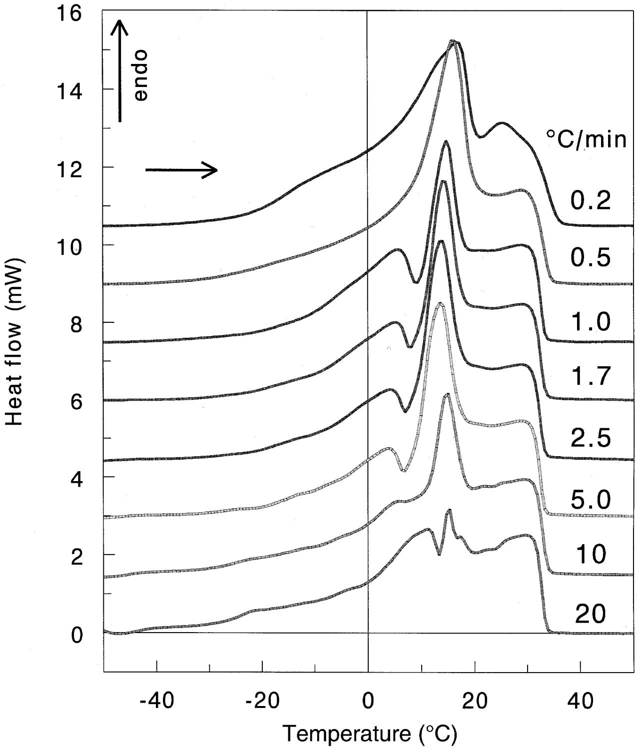


FIG. 6. DSC curves of milk fat heated at 5°C/min after cooling from 70 to −70°C at cooling rates varying from 0.2 to 20°C/min. See Figure 1 for abbreviation.

TABLE 4
Appearance and Disappearance Ranges of Polymorphs of Milk Fat During Heating at a Rate of 5°C/min from −65 to 70°C as Observed by XRD[a]

	Appearance			Disappearance		
Polymorphic form	T_s (°C)	T_m (°C)	T_e (°C)	T_s (°C)	T_m (°C)	T_e (°C)
γ	—[b]	—[b]	—[b]	−38	−18	−8
α	−18	−8	−3	6	11	20
β′	7	12	17	17	22	35

[a] For abbreviations see Table 3.
[b] The γ-form was present after cooling from 70 to −70°C.

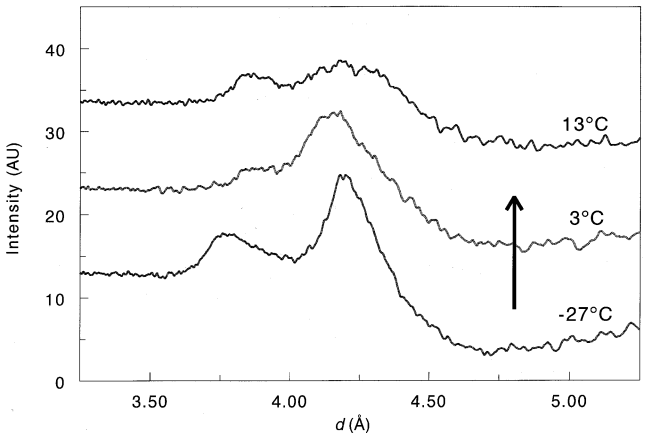


FIG. 7. XRD patterns of milk fat heated at 5°C/min after solidification with a cooling rate of 5°C/min taken at increasing temperatures: –27, 3, and 13°C. Arrow indicates order of measurement. See Figure 3 for abbreviation.

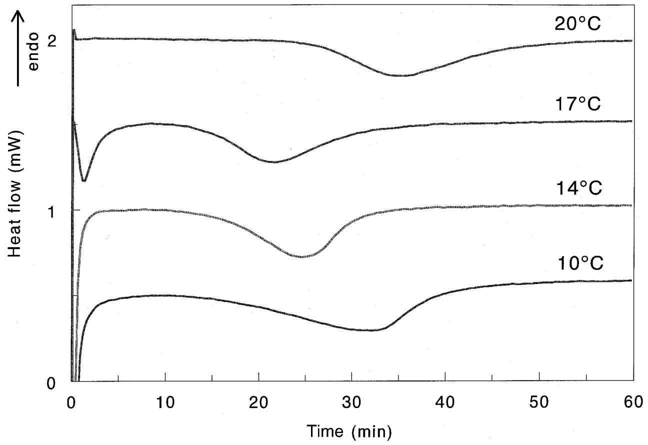


FIG. 8. Isothermal DSC curves of milk fat after cooling from 70°C at a rate of 20°C/min to varying solidification temperatures ranging from 10 to 20°C. See Figure 1 for abbreviation.

but an almost complete transformation to the β′-form occurred during the following 14 min. At $T = 20°C$, no α-crystals were observed. At all temperatures investigated, the β′-diffraction peaks appeared after 9–18 min.

DSC heating curves after solidification as a function of time. DSC heating curves were recorded for milk fat after varying periods between 1 and 60 min of isothermal crystallization at 10°C (Fig. 11). The heating curve after 1 min of isothermal crystallization shows an endothermic effect between 10 and 22°C, followed by an exothermic effect that continues up to 30°C. Between 30 and 35°C a second endothermic effect is observed. After 5, 10, and 20 min at 10°C, the exothermic effect in the heating curve shifted to lower temperatures. Figure 11 shows that the exothermic effect in the DSC heating curve after 20 min at 10°C starts at 19 and ends at 23°C. After 30 min at 10°C, the exothermic effect in the heating curve is much smaller. Finally, the heating curve after 60 min at 10°C has a broad endothermic shoulder between 10 and 35°C, showing relatively little detail.

Similarly, DSC heating curves were recorded after isothermal solidification at 17°C for periods between 1 and 120 min. Figure 12 shows that the heating curves after 1 and after 5 min at 17°C have only a small endothermic effect between 20 and 26°C. Heating after waiting for 10 min or longer resulted in an additional endothermic effect between 26 and 36°C, which increased after longer isothermal storage times. The peak between 20 and 26°C decreased in size after 15 min of isothermal storage.

TABLE 5
Isothermal Crystallization of Milk Fat as Measured by XRD[a]

	α-Formation			β′-Formation		
T_f (°C)	t_s (min)	t_m (min)	t_e (min)	t_s (min)	t_m (min)	t_e (min)
–10	—[b]	—[b]	—[b]	(18)	(24)	(30)
4	—[b]	—[b]	—[b]	(11)	(19)	(27)
10	—[b]	—[b]	—[b]	11	17	23
14	—[b]	—[b]	—[b]	9	17	23
17	(0)	(1)	(3)	13	17	22
20	—	—	—	14	18	24

[a] Parentheses indicate the formation of very small quantities of a polymorph.
[b] Milk fat has crystallized in the α-form during cooling. T_f, final solidification temperature; t_s, start of transition; t_m, time at which the XRD pattern has changed the most; t_e, time at which the XRD pattern became constant; see Table 3 for other abbreviation.

DISCUSSION

The general polymorphic phase scheme that can be derived from the results presented in this study is shown in Scheme 1. With XRD, three different patterns were found for milk fat (Fig. 3). The γ-form is the least stable. It was only observed for high cooling rates ($|dT/dt| \geq 2.5°C/min$) and below a temperature of −8°C, which is probably close to the clear point of the γ-form. Faster cooling resulted in relatively more γ-crystals and relatively less α-crystals. The α-form was ob-

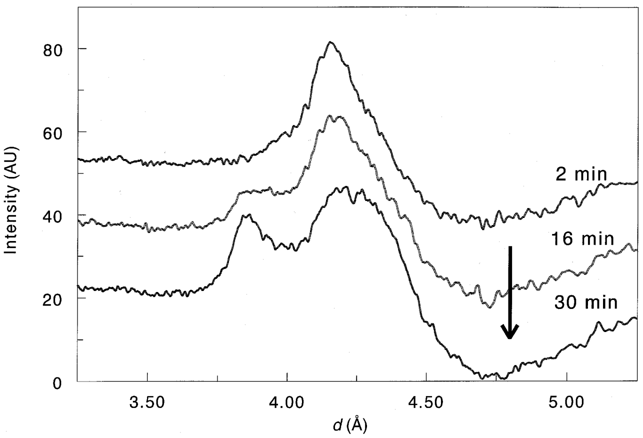


FIG. 9. XRD patterns recorded during isothermal solidification of milk fat after cooling from 70 to 10°C at a rate of 20°C/min. The patterns shown were taken after 2 min, after 16 min, and after 30 min. Arrow indicates order of measurement. See Figure 3 for abbreviations.

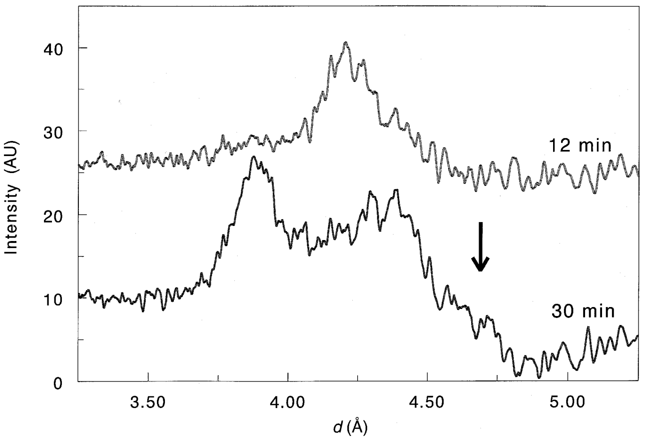


FIG. 10. XRD patterns recorded during isothermal solidification of milk fat after cooling from 70 to 17°C at a rate of 20°C/min. The patterns shown were taken after 12 and after 30 min. Arrow indicates order of measurement. See Figure 3 for abbreviations.

tained at cooling rates ≥ 1°C/min and had a clear-point of approximately 20°C. The amount of under cooling of the α-form was 2–5°C, as determined by XRD. The amount of undercooling increases with increasing cooling rate, which is in agreement with a study by Sato and Kuroda (22) on the occurrence of tripalmitin polymorphs. The β′-form was the most stable form observed in this study. The clear point of this form was approximately 35°C, and under cooling of more than 25°C easily occurred in these experiments.

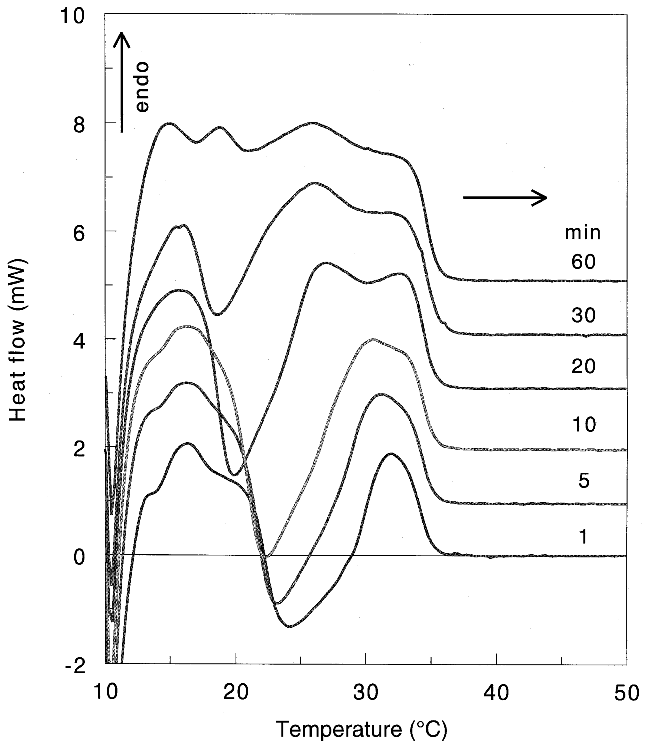


FIG. 11. DSC heating curves (5°C/min) of milk fat after isothermal solidification at 10°C for periods varying from 1 to 60 min. See Figure 1 for abbreviation.

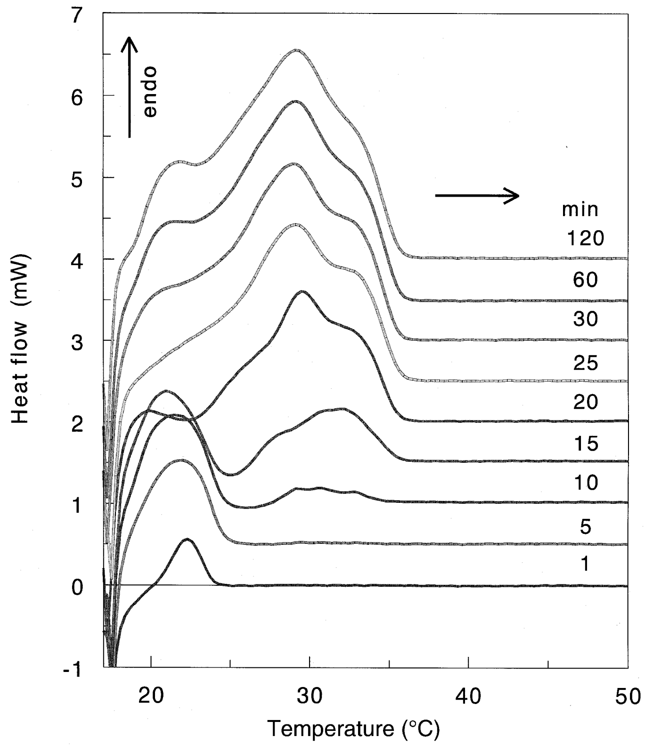
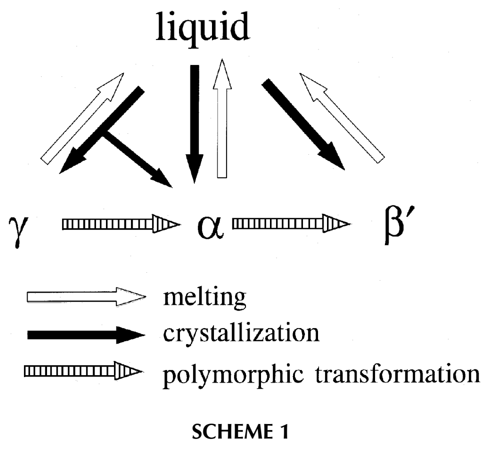


FIG. 12. DSC heating curves (5°C/min) of milk fat after isothermal solidification at 17°C for periods varying from 1 to 120 min. See Figure 1 for abbreviation.

Isothermal solidification experiments show that milk fat is always in the β′-form after sufficiently long waiting times after solidification. In some studies (3,9,10), it was observed that besides β′-crystals, small amounts of β-crystals could occur in milk fat. In this study, the β-form was not observed. A possible explanation for this difference is that milk fat varies in its composition depending on the season and geographical differences. In general, however, the major part of the milk fat always remains in the β′-form, even after prolonged storage (3).

Crystallization of milk fat occurred primarily in the crystal polymorph that is least stable, yet still existent at the temper-



SCHEME 1

ature of crystallization. This is in agreement with the Ostwald rule of stages (23), which states that crystallization starts with less stable polymorphs. This is because the nucleation and growth of less stable polymorphs proceed faster due to the lower surface free energy of the crystals of that polymorph. This explains the sequence observed by both DSC and XRD measurements. Below the clear point of the α-modification (approximately 20°C), milk fat initially showed α-formation, followed by the much slower formation of the β′-modification. The α-modification was unstable with respect to the β′-modification. Therefore, once β′-crystals are formed, triglycerides will dissolve from the α-crystals and crystallize onto the β′-crystals, which effectively leads to the transformation of α-crystals into β′-crystals. From Figure 8, we estimate an induction period (nucleation and growth) for the formation of the α-form of approximately 2.5 min. At later times (after an induction period between 20 and 35 min, depending on the temperature), β′-crystals are formed that grow at the expense of the α-crystals. Both the DSC and XRD measurements showed that this transformation proceeds more slowly at lower temperatures. This phenomenon was also observed in experiments by Sato and Kuroda (22) on tripalmitin. A possible explanation is that the higher stability of the α-form at lower temperatures diminishes the driving-force for transformation into the β′-form. At temperatures between the α-clear point (approximately 20°C) and the β′-clear point (approximately 35°C), crystallization occurs directly in the β′-form. The induction period for the formation of β′-crystals was 35 min at 20°C, increasing for higher temperatures. This is because the formation of nuclei and the rate at which the crystals grow depend on the degree of undercooling, meaning that at higher temperatures, it takes longer to form a substantial amount of β′-crystals.

The results of the isothermal solidification measurements can be used to explain the results from the DSC heating curves after varying crystallization times. It must be noted that the polymorphic behavior of milk fat during heating is even more complex than the crystallization behavior. During cooling only the crystallization of polymorphic forms plays a role, which results in exothermic heat effects. Upon heating, the melting of polymorphic forms gives rise to endothermic heat effects. However, during heating, polymorphic transformations often occur, which are associated with exothermic heat effects. The resulting heat flow is the combination of several heat effects occurring simultaneously. The first endothermic peak in the DSC heating curves after crystallization at 10°C is caused by the melting of α-crystals. At *ca.* 17°C, an exothermic effect starts, which becomes larger than the endothermic effect of the melting of α-crystals at *ca.* 20°C. This effect appears to be caused by the transformation of crystals from the α-form into the β′-form, and the direct formation of β′-crystals from the melt. The second endothermic peak appears to result from the melting of the β′-crystals. It is noteworthy that during heating the transformation of α-crystals into β′-crystals, taking place in the temperature range around 17°C, is completed in about 2 min, whereas it takes more than 15 min for milk fat kept isothermally at 17°C. A probable explanation for this is that in the heating experiment, nuclei of β′-crystals were already present, so that the kinetics were determined by crystal growth only, whereas in the isothermal case, nucleation had to take place first. Apparently the rate-determining step in the formation of β′-crystals is nucleation in the case of isothermal crystallization from the melt, and crystal growth in the heating of milk fat that was precrystallized in the α-form.

Now we will discuss the DSC heating curves that were recorded after isothermal crystallization at 17°C. The α-form crystallizes during the first minute of heating, and this gives rise to an endothermic effect between 18 and 25°C. The relatively high melting point of these α-crystals is probably caused by the selective crystallization of high-melting triglycerides in the α-form. At longer waiting times, the effect of the melting α-crystals becomes smaller, and a peak between 27 and 36°C appears, resulting from β′-crystals. The decrease of the first peak that can be observed in Figure 12 is probably caused by the transformation of α-crystals into β′-crystals.

The influence of the cooling rate on the solidification behavior can be explained as follows. When the melt is solidified by cooling at a constant rate, crystallization will occur immediately in the β′-form when the time span between reaching the β′-clear-point (approximately 35°C, Table 4) and reaching the α-clear point (approximately 20°C, Table 4) exceeds the induction time for crystallization in the β′-form (between 20 and 35 min). This is the case for cooling rates $|dT/dt| < 0.5$°C/min, which corresponds well with the experimental results.

As seen in Figure 1, in the DSC cooling curves, two peaks, (c_1 and c_2) were observed for all cooling rates ranging from 0.2 to 10°C/min. The exception is found for a cooling rate of 20°C/min, where just one peak was observed. The onset temperatures of peak c_1 crystallization are in good agreement with the temperatures that were found with XRD for the start of α-crystallization (for $|dT/dt| \geq 1.67$°C/min) or β′-crystallization (for $|dT/dt| \leq 1$°C/min). The larger undercooling found at high cooling rates for the DSC samples may indicate that cooling is more efficient for the smaller size of the DSC samples relative to the XRD samples. The fact that the first peak (c_1) is related to the appearance of α-crystals would suggest that the second peak (c_2) is related to the appearance of γ-crystals. However, this is not the case. The temperature difference between the onset of c_2, measured with DSC, and the start of γ-crystallization as observed with XRD, is *ca.* 20°C. Moreover, the second peak (c_2) is observed at low cooling rates (≤1.67°C/min), where XRD measurements showed that milk fat crystallizes in the α-form only. A possible explanation is that c_1 and c_2 originate from the separate crystallization of high-melting triglycerides, resulting in c_1, and the crystallization of lower-melting triglycerides, resulting in the second crystallization peak c_2. The absence in the DSC measurements of a recognizable separate peak for the crystallization of liquid milk fat into the γ-form could be caused by the wide temperature range of γ-crystallization (Table 3).

An explanation of the three endothermic peaks (h_1–h_3) observed in the heating part of Figure 1 can be derived by comparing heating curves recorded from –65 to 70°C after cooling at different rates (Fig. 6) and XRD measurements. XRD measurements showed that the γ-form already melts at low temperatures. In the DSC heating curves, this transition is not observed. A reason for this could be that the heat effect accompanying this transition is relatively small and spreads over a considerable temperature range. Moreover, part of the endothermic effect caused by the disappearance of the γ-form coincides with the exothermic effect caused by the formation of α-crystals. The endothermic effect in the DSC heating curves (h_1) can be explained by the melting of the first amount of α-crystals, which are composed of low-/middle-melting triglycerides. Indeed, it is only observed after cooling at higher cooling rates (≥1°C/min). At 7°C, a part of the α-crystals transforms into β′-crystals, which leads to the exothermic effect between peaks h_1 and h_2. The second endothermic peak (h_2), which has a maximum at 14°C, is a result of further melting crystals of higher-melting triglycerides that are in the α-form. The broad shoulder (h_3) in the DSC curves is caused by melting β′-crystals of high-melting triglycerides.

The experiments reported in this study give deeper insight into the crystallization and polymorphic transitions of milk fat. By cooling milk fat at varying cooling rates, and by isothermal solidification of milk fat at varying temperatures, the phase behavior was investigated. It was found that the triglycerides in milk fat can crystallize in three different polymorphic forms: γ, α, and β′. Of these polymorphs, the γ-form is the least stable and the β′-form the most stable. The β-form that is the most stable polymorph for most pure triglycerides was not observed, probably because of the typical milk fat triglyceride composition. Crystallization was shown to obey Ostwald's rule of stages, which means that crystallization first occurs in the least stable polymorph that still exists at the temperature of crystallization. This is due to a delay time of crystallization, which is a sum of the nucleation and growing times, which is longer for the more stable polymorphic forms. This delay time is also responsible for the severe undercooling of the milk fat in the temperature range of 20–35°C.

By comparing the results of the DSC experiments with the temperature-programmed XRD measurements, an explanation is given for the typical shape of the DSC heating curves of milk fat. The heating curves represent a combination of polymorphic transitions and melting of different milk-fat fractions.

ACKNOWLEDGMENTS

The authors wish to acknowledge A.J. van Langevelde of the Institute for Molecular Chemistry for assistance with the XRD equipment. We would like to thank F.D. Zoet and Z.E.H. Otten, both working at NIZO food research, for carrying out many DSC experiments. This research was supported by The Netherlands Ministry of Agriculture, Nature Management, and Fisheries. Finally, EG wishes to thank J.C. van Miltenburg of Utrecht University for discussions on the phase behavior of milk fat.

REFERENCES

1. Lutton, E.S., Review of the Polymorphism of Saturated Even Glycerides, *J. Am. Oil Chem. Soc. 27*:276–281 (1950).
2. D'Souza, V., J.M. deMan, and L. deMan, Short Spacings and Polymorph Forms of Natural and Commercial Solid Fats: A Review, *Ibid. 67*:835–843 (1990).
3. Timms, R.E., The Physical Properties of Blends of Milk Fat with Beef Tallow and Beef Tallow Fractions, *Aust. J. Dairy Technol. 34*:60–65 (1979).
4. Larsson, K., Alternation of Melting Points in Homologous Series of Long-Chain Compounds, *J. Am. Oil Chem. Soc. 43*:559–562 (1966).
5. Wesdorp, L.H., Liquid-Multiple Solid Phase Equilibria in Fats—Theory and Experiments, Ph.D. Thesis, Delft University of Technology, Delft (1990).
6. Timms, R.E., Phase Behaviour of Fats and Their Mixtures, *Prog. Lipid Res. 23*:1–38 (1984).
7. Vaeck, S.V., Cocoa Butter and Fat Bloom, *Manuf. Confect. 40*:35–46, 71–74 (1960).
8. Mulder, H., Melting and Solidification of Milk Fat, *Neth. Milk Dairy J. 7*:149–174 (1953).
9. deMan, J.M., Physical Properties of Milk Fat. II. Some Factors Influencing Crystallization, *J. Dairy Res. 28*:117–122 (1961).
10. Woodrow, I.L., and J.M. deMan, Polymorphism in Milk Fat Shown by X-ray Diffraction and Infrared Spectroscopy, *J. Dairy Sci. 51*:996–1000 (1968).
11. Van Beresteijn, E.C.H., Polymorphism in Milkfat in Relation to the Solid/Liquid Ratio, *Neth. Milk Dairy J. 26*:117–130 (1973).
12. Schaap, J.W., H.T. Badings, D.G. Schmidt, and E. Frede, Differences in Butterfat Crystals Crystallized from Acetone and from the Melt, *Ibid. 29*:242–252 (1975).
13. Metin, S., and R.W. Hartel, Crystallization Behavior of Blends of Cocoa Butter and Milk Fat or Milk Fat Fractions, *J. Therm. Anal. 47*:1527–1544 (1996).
14. Timms, R.E., The Phase Behaviour and Polymorphism of Milk Fat, Milk Fat Fractions and Fully Hardened Milk Fat, *Aust. J. Dairy Technol. 35*:47–53 (1980).
15. Deffense, E., Milk Fat Fractionation Today: A Review, *J. Am. Oil Chem. Soc. 70*:1193–1201 (1993).
16. Lohman, M.H., and R.W. Hartel, Effect of Milk Fat Fractions on Fat Bloomin Dark Chocolate, *Ibid. 71*:267–276 (1994).
17. Belousov, A.P., and V.M. Vergelesov, Polymorphism in Butter Fat, in *Int. Dairy Congr. Proc. 16,* 1962, pp.122–134.
18. van Malssen, K.F., R. Peschar, and H. Schenk, Geometrical Aspects of Real-Time Powder Diffraction Using a Normal Generator and a Linear Diode Array Detector, *J. Appl. Crystallogr. 27*:302–315 (1994).
19. van Aken, G.A., E. ten Grotenhuis, A.J. van Langevelde, and H. Schenk, Composition and Crystallization of Milk Fat Fractions, *J. Am. Oil CHem. Soc.,* in press.
20. van Malssen, K.F., R. Peschar, and H. Schenk, Real-Time X-ray Powder Diffraction Investigations on Cocoa Butter. I. Temperature-Dependent Crystallization Behavior, *J. Am. Oil Chem. Soc. 73*:1209–1215 (1996).
21. Larsson, K., On the Structure of the Liquid State of Triglycerides, *Ibid. 69*:835–836 (1992).
22. Sato, K., and T. Kuroda, Kinetics and Melt Crystallization and Transformation of Tripalmitin Polymorphs, *Ibid. 64*:124–127 (1987).
23. Ostwald, W., Studien über die Bildung und Umwandlung fester Körper, 1. Abhandlung: Übersättigung und Überkaltung, *Z. Physik. Chem. 22*:289–330 (1897).

[Received November 23, 1998; accepted May 21, 1999]