Effect of DAG on Milk Fat TAG Crystallization

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ABSTRACT: The effect of milk fat and standard DAG on the crystallization behavior of milk fat TAG (MF-TAG) was investigated. When milk fat DAG were added to MF-TAG at the 0.1 wt% level, crystallization was delayed. Racemic purity was shown to be an important factor in the ability of DAG to influence TAG crystallization. Only *sn*-1,2 isomers of blends of MF-TAG with 0.1 wt% of the racemic mixtures of dipalmitin and diolein increased the activation free energy barrier to MF-TAG nucleation (ΔG_c) and delayed the subsequent crystallization process by increasing the crystallization induction time (τ_{SFC}) determined from solid fat content–time measurements. Although crystallization kinetics were affected, the properties of the resulting network structures remained unchanged upon addition of milk fat minor components at the 0.1 wt% level

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Minor components in milk fat have previously been shown to have an inhibitory effect on the crystallization of milk fat TAG (MF-TAG) (1). Milk fat DAG, in particular, delayed the onset of crystallization at temperatures above 20°C. It was suggested that structural complementarity between the MF-DAG and crystallizing TAG allowed the DAG to co-crystallize within early seed crystals and subsequently further delay TAG crystallization (1). The ability of DAG to stabilize metastable polymorphs in fats is related to their structure. For example, 1,2-DAG consistently delay polymorphic transitions more effectively than 1,3 isomers (2–5). This difference has been attributed to the fact that 1,2-DAG prefer orthorhombic chain packing, whereas 1,3-DAG prefer the triclinic subcell arrangement (2). The ability of DAG to influence TAG behavior seems to be related to the chemical composition of both the added DAG molecules and TAG. A delay in the α to β′ transition is achieved in cocoa butter when DAG having FA compositions similar to cocoa butter TAG are added (4). Also, maximal delay of the β′ to β transition in hydrogenated rapeseed oil is achieved when the DAG chain length is similar to that of the crystallizing TAG (2).

Reports of the effects of DAG isomerism on the TAG crystallization rate are somewhat variable. In palm olein, the 1,3 isomer of dipalmitin caused rapid crystallization (6), whereas 1,3-DAG were especially effective at reducing the TAG growth rate in trilaurin (7). Therefore, although DAG stereospecificity may be a factor, the effect of DAG on TAG crystallization is also related to composition (5,6,8). The importance of structural complementarity between DAG and TAG was further demonstrated in studies with coconut oil (8), palm oil (6,9), and trilaurin (5,7). In coconut oil TAG, dilauroylglycerol was found to retard nucleation, whereas dioleoylglycerol did not (8). The ability of FFA to influence coconut oil crystallization also depended on the FA chain length and saturation. Oleic and, to a lesser extent, lauric acids increased the crystallization induction time of deodorized coconut oil, whereas palmitic acid decreased it (8).

In palm olein, dipalmitoylglycerol enhanced crystallization, palmitoyloleoylglycerol slowed crystallization, and dioleoylglycerol had no effect (6). The effects of DAG addition were a function of DAG concentration and were much more pronounced at lower degrees of supercooling (9). Smith *et al*. (5) and Smith and Povey (7) investigated the effects of partial glycerides on trilaurin crystallization at levels of 1, 2, and 5 wt%. The presence of dilaurin isomers significantly reduced the growth rate (5). 1,3-Dilaurin was especially effective at slowing crystal growth, with a higher concentration of the additive resulting in greater retardation. Smith and Povey (7) found that the most significant delays in crystallization were observed when the chain length of the additive matched that of trilaurin.

The crystallization behaviors of anhydrous milk fat (AMF), purified MF-TAG, and MF-TAG with added MF-DAG were previously compared (1). In that study, a 1998 batch of milk fat was used. In this paper, we report on the effects of native milk fat DAG as well as racemically pure synthetic DAG addition on the crystallization behavior of MF-TAG isolated from a second batch of milk fat in the year 2000. These two sets of experiments will be referred to throughout the study as the 1998 and 2000 samples. The reader should note that the reported 1998 results are from experiments carried out in 1998.

MATERIALS AND METHODS

Minor components were separated from TAG (MF-TAG) in AMF, and DAG were isolated from the minor components fraction by TLC as described by Wright *et al.* (1). FA composition of the *sn*-1,3 isomeric DAG TLC bands in two years (1998 and 2000) were determined by preparing FAME and using GLC as previously described (1). MF-DAG isolated in 1998 and 2000 were added back to their respective purified MF-TAG at the 0.1 wt% level. The DAG collected in this way were a mixture of DAG isomers, i.e., the TLC bands for both the 1,2/2,3 and 1,3 isomers were recombined. Samples of MF-TAG with 0.1 wt% milk fat DAG are referred to as MF-DAG-1998 and MF-DAG-2000.

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DAG standards of known purity and composition were also obtained and added to the MF-TAG in 2000 at the 0.1 wt% level. 1-Palmitoyl-2-oleoyl-*sn*-glycerol with purity greater than 99% (Avanti Polar Lipids, Alabaster, AL) was the only mixed-acid DAG investigated because of limited availability. Racemic and stereospecific isomers of dipalmitin and diolein were also obtained. These included 1,2-dipalmitoyl-*sn*-glycerol (greater than 99% pure), dipalmitin (approximately 99% pure; 50% *sn*-1,2 and 50% *sn*-1,3), 1,2 dioleoyl-*sn*-glycerol (greater than 98% pure), and diolein (approximately 99% pure; 85% *sn*-1,3 and 15% *sn*-1,2/2,3) (Sigma-Aldrich, Oakville, Ontario, Canada). The DAG standards were stored unopened at −18°C before use and subsequently at −18°C under nitrogen. Blends of MF-TAG with 0.1 wt% of the racemic mixtures of diplamitin and diolein are referred to as PP and OO, respectively. Blends of the stereoisomers of dipalmitin and diolein and 1-palmitoyl-2-oleoyl-*sn*-glycerol are referred to as 1,2PP, 1,2OO, and 1P,2O, respectively.

The crystallization behaviors of AMF, MF-TAG, MF-DAG, and MF-TAG with the DAG standards were compared. Mettler dropping temperatures (MDT), equilibrium solid fat contents (SFC), and onset temperatures of crystallization were determined as previously described (1). Increases in SFC during crystallization at 25°C were monitored by pulsed NMR (pNMR) with a Bruker PC20 Series NMR analyzer (Bruker, Milton, Ontario, Canada). Crystallization induction times (τ_{SFC}) were determined from the SFC vs. time curves by extrapolating the initial linearly increasing portion of SFC vs. time curve to the time axis. To quantify the crystallization kinetics, the Avrami equation (10–12) was also fitted to the SFC curves by least squares nonlinear regression. The Avrami equation has the following form:

$$\frac{\mathrm{SFC}(t)}{\mathrm{SFC}(\infty)} = 1 - e^{-kt^n} \qquad [1]$$

where SFC refers to solid fat content, SFC(t) describes the SFC as a function of time (t), SFC(∞) is the limiting SFC as time approaches infinity, k is the Avrami constant, and n is the Avrami exponent.

A phase transition analyzer (Phase Technology, Richmond, British Columbia, Canada) was also used to monitor crystallization. Melted fat (150 μL) was pipetted into the sample chamber. The analyzer was programmed to heat the fat for 20 min at 68°C and then cool it to 25°C at 50°C/min. Because this method was very sensitive to early crystallization events, possibly in the vicinity of nucleation, induction times by this method are referred to as nucleation induction times ($\tau_{nucleation}$) (13). $\tau_{nucleation}$ was defined as the time when two consecutive signal readings of 1.0 were observed. Values for $\tau_{nucleation}$ were used in the determination of nucleation free energies (ΔG_c) using the Fisher–Turnbull equation (Eq. 2). ΔG_c can be calculated according to the Fisher–Turnbull equation (14),

$$J = (NkT/h)\exp(-\Delta G_d/kT)\exp(-\Delta G_c/kT) \qquad [2]$$

where J is the rate of nucleation and is inversely proportional to the induction time (τ) of nucleation, N is the number of molecules per cm^3 in liquid phase, k is the gas constant per molecule, T is the temperature, h is Planck's constant, and ΔG_d is the free energy of diffusion. ΔG_c is the free energy of nucleation and, for a spherical nucleus, is related to the surface free energy of the crystal/liquid melt interface and the degree of supercooling according to Equation 3:

$$\Delta G_c = (16/3)\pi\sigma^3 T_m^{\,2}/(\Delta H)^2(\Delta T)^2 \qquad [3]$$

ΔH is the enthalpy of nucleation. A plot of ln (τT) vs. $1/T(\Delta T)^2$ yields a slope (m) and permits calculation of the free energy of nucleation according to the following equation (Eq. 4):

$$\Delta G_c = mk/(T_m - T)^2 \qquad [4]$$

T_m (K) is the melting point, and T (K) is the crystallization temperature.

The effect of DAG on TAG microstructure was determined by using polarized light microscopy. An Olympus BH polarized light microscope (Olympus, Tokyo, Japan) was used to visualize the samples after crystallization for 24 h at 25°C. Images were acquired using a Sony XC-75 CCD video camera (Sony Corporation. Tokyo, Japan) and LG-2 PCI frame grabber and Scion Image (Scion Corporation, Frederick, MD). An automatic blank field subtraction and averaging of 16 frames per image were performed using the computer software.

DSC in the melting mode was used to investigate differences in polymorphic behavior between the DAG samples. DAG standards (7–10 mg) were sealed in aluminum pans, heated at 80°C for 15 min, and then crystallized at 25°C for 24 h. The endothermic heat flow when the samples were heated at 5°C/min was then monitored in a DuPont 2090 differential scanning calorimeter (TA Instruments, Mississauga, Ontario, Canada). To determine the effect of DAG standards on liquid structure before crystallization, blends of MF-TAG with PP and 1,2PP were also analyzed by X-ray diffraction spectroscopy (XRD) using an Enraf-Nonius KappaCCD diffractometer with a FR590 X-ray generator (Nonius, Delft, The Netherlands). Samples were analyzed after crystallization for 4 and 7 min at 22.5 and 25.0°C, respectively, as previously described (1).

The percentage of FFA (%FFA) in AMF was determined according to the AOCS official method (15). %FFA were calculated on the basis of oleic acid.

RESULTS AND DISCUSSION

Comparison between the 1998 and 2000 studies. Minor components were isolated from AMF in 1998 (1) and 2000, and the crystallization behaviors of the AMF and purified MF-TAG were compared. In both years, minor components were found to have a kinetic, rather than thermodynamic, effect. Removal of the minor lipids did not significantly affect AMF's MDT, equilibrium SFC profile, or onset temperature of crystallization (~20°C). Minor components primarily served to inhibit the early stages of crystallization (nucleation and/or early crystal growth)

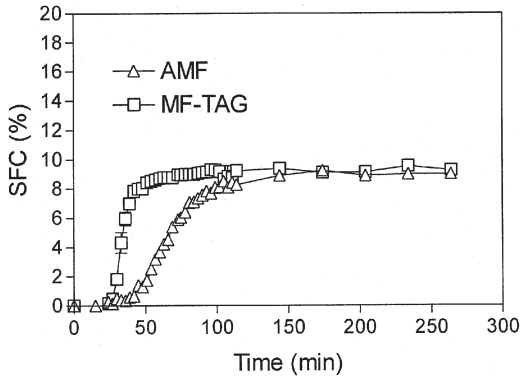

FIG. 1. Solid fat content (SFC) vs. time during static crystallization of anhydrous milk fat (AMF) and milk fat triacylglycerols (MF-TAG) in 2000 at 25.0°C. Symbols represent the average and SE of three replicates. Error bars are smaller than symbols.

at low degrees of supercooling. Above 20°C, minor components increased both τ_{SFC} and $\tau_{nucleation}$. The crystallization curves for AMF and MF-TAG at 25°C are shown in Figure 1.

MF-DAG were isolated in each year and added back to their respective MF-TAG. Although the behaviors of AMF and MF-TAG in 1998 and 2000 were identical, the effect of DAG addition differed between the two years. In 1998, addition of 0.1 wt% milk fat DAG to MF-TAG resulted in a significant delay in crystallization and changes in the Avrami exponent above 20°C (1). The inhibition by milk fat DAG was much weaker in 2000 compared to 1998. Figure 2 shows the crystallization curves for AMF, MF-TAG, MF-DAG-1998, and MF-DAG-2000 at 25°C .

Figure 2 shows that the pronounced delay in crystallization observed for MF-DAG-1998 was not evident in MF-DAG-2000. In 1998, 0.1 wt% DAG significantly increased the MF-TAG induction time. In 2000, this had only a small effect on the MF-TAG crystallization. MF-DAG from 2000 were also added to the MF-TAG in 2000 at a concentration of 1 wt%. Figure 3 shows that this resulted in a greater delay in crystallization than when 0.1 wt% was added. Although this was not investigated further, the effect of DAG on MF-TAG crystallization seems to be concentration dependent.

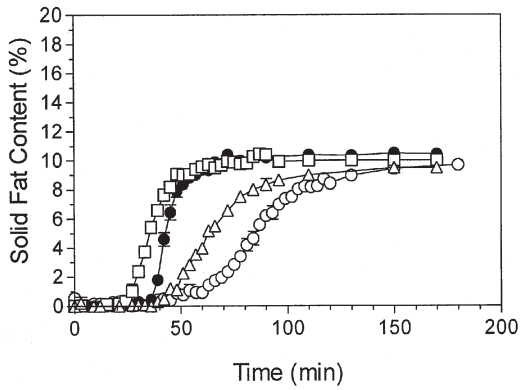

FIG. 2. SFC vs. time during static crystallization of AMF (△), MF-TAG (□), MF-DAG-1998 (MF-TAG with 0.1% milk fat DAG added) (○), and MF-DAG-2000 (MF-TAG with 0.1% milk fat DAG added) (●) at 25.0°C. Symbols represent the average and SE of three replicates. See Figure 1 for other abbreviations.

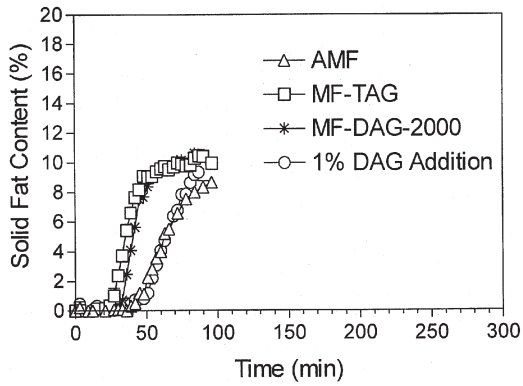

FIG. 3. SFC vs. time during static crystallization of AMF, MF-TAG, MF-DAG-1998 (MF-TAG with 0.1% DAG added), and MF-TAG with 1% DAG added at 25.0°C. Symbols represent the average and SE of three replicates. Error bars are smaller than symbols. For abbreviations see Figures 1 and 2.

When DAG were added to the MF-TAG in 1998, the Avrami exponent, *n,* was significantly increased at temperatures above 20°C. Similarly, DAG addition was also correlated with lower values of *k* (1). The Avrami model was fitted to the MF-DAG-2000 crystallization curves at 25.0°C. Table 1 compares the Avrami parameters of MF-DAG-2000 at 25°C to those of AMF, MF-TAG, and MF-DAG-1998.

Statistical differences were not observed between *k* and *n* for the fats at 25.0°C, although qualitatively, DAG addition did seem to correspond with decreased values of *k* and increased values of *n*. A decrease in *k* reflects the DAG-induced delay in crystallization rate. *k* depends on both the rates of nucleation and crystal growth. Some of the decrease in *k* with DAG addition in 1998 must be related to the effect of DAG on nucleation specifically. The following nucleation times ($\tau_{nucleation}$ ± SD, min) at 25°C were recorded: AMF, 17.1 ± 1.1^{A}; MF-TAG, 10.0 ± 1.0^{B}; MF-DAG-1998, 16.8 ± 1.9^{A}; and MF-DAG-2000, 9.6 ± 0.9^{B}, where different superscript letters indicate significant differences ($P < 0.05$) between values.

The fact that DAG addition in 2000 had no effect on MF-TAG nucleation (see preceding paragraph) but did slightly delay bulk crystallization (evidenced by an increased τ_{SFC} in Fig. 2) indicates that DAG can exert an effect on crystallization beyond nucleation. Therefore, DAG have the ability to inhibit nucleation and also retard crystal growth. Lower values of *k* could also indicate a decrease in the crystal growth rate. The trends in the values of *n* (Table 1) also support the statement that DAG can exert an effect on both crystal nucleation and growth. Values of *n* for MF-DAG-1998 and MF-DAG-

TABLE 1
Avrami Constants, *k*, and Exponents, *n*, for AMF, MF-TAG, MF-DAG-1998, and MF- DAG-2000 at 25°C[a]

	Avrami constant, *k*	Avrami exponent, *n*
AMF	$2.5 \times 10^{-6} \pm 2.5 \times 10^{-6A}$	3.0 ± 0.2^{A}
MF-TAG	$1.6 \times 10^{-7} \pm 1.2 \times 10^{-7A}$	3.9 ± 0.2^{B}
MF-DAG-1998	$2.6 \times 10^{-9} \pm 1.8 \times 10^{-9A}$	4.5 ± 0.2^{B}
MF-DAG-2000	$6.6 \times 10^{-9} \pm 6.5 \times 10^{-9A}$	6.5 ± 1.4^{B}

[a]Mean value of three replicates with SEM. Different roman superscript letters (A,B) indicate significant differences ($P < 0.05$) within column. AMF, anhydrous milk fat; MF, milk fat.

2000 are qualitatively ($P > 0.05$) higher than those for MF-TAG. An increase in *n* could be indicative of sporadic, as opposed to instantaneous, nucleation. In 1998, addition of MF-DAG to MF-TAG delayed nucleation, whereas in 2000, no such effect was observed. The DAG in 2000 did inhibit the overall crystallization process as monitored by pNMR (Fig. 2), but their influence was not evident at the nucleation level. In contrast, the delay in crystallization caused by the MF-DAG in 1998 involved the inhibition of nucleation. The fact that $\tau_{nucleation}$ for MF-DAG-2000 was not significantly higher than that of MF- TAG but *n* increased suggests that the TAG growth mechanism is also affected by the DAG. A change in the dimensionality of growth, rather than an effect on nucleation, may account for the higher (although not statistically significant, $P > 0.05$) values of *n* in the MF-DAG-2000.

Compositional differences between 1998 and 2000 milk fat DAG. Stereospecific analysis of the 1998 and 2000 DAG revealed differences in the FA positional distribution between the two years, which may explain their differing effects on crystallization. Table 2 shows the FA composition of the *sn*-1,3 DAG in the 1998 and 2000 MF-DAG. This is compared with the FA composition of milk fat's high melting fraction (HMF).

The *sn*-1,3 positions of the 1998 DAG were enriched in palmitic acid (16:0), whereas the primary positions of the 2000 DAG were enriched in oleic acid (18:1). In 1998, the primary positions of the DAG contained nearly 50% palmitic acid. Palmitic acid is also the most prominent FA in HMF. Whereas oleic acid represented less than 20% of the FA in the 1998 DAG *sn*-1 and -3 positions, it accounted for nearly 42% of the FA found in these positions in the 2000 DAG. Oleic acid accounts for less than 10% of the FA in HMF.

The different effects of the 1998 and 2000 DAG may be explained by molecular complementarity arguments. When milk fat is cooled, molecules in the HMF are the first to experience undercooling. These TAG species crystallize out first and subsequently act as seeding agents and growth sites for other TAG molecules as they become sufficiently undercooled. The FA composition of the 1,3-DAG in 1998 resembles that of the HMF molecules more closely than does the profile for the 1,3 isomers of the 2000 DAG. This may allow the 1998 DAG to co-crystallize with the early HMF crystals. As the DAG become incorporated in the solids, they would create irregularities in the growing crystal because of the hydroxyl group they contain in place of a FA chain. This polar region, or the structural vacancy created in the lattice, may hinder the incorporation of TAG molecules into the crystal and subsequent crystallization.

In the 2000 DAG, the oleic acid-enriched *sn*-1 and -3 positions could lead to incompatibilities with the HMF molecules. As a result, these DAG might be prevented from co-crystallizing with HMF TAG and subsequently delaying crystallization. Although MF-DAG nucleation was not delayed in 2000, some inhibitory effect on crystallization, probably at the growth level, still was observed. Although these DAG would be excluded from early milk fat crystals, it is reasonable to think that they would become incorporated into the solids over time along with other TAG molecules. At this point, they may be able to exert their effect on crystallization. Milk fat contains a myriad of TAG species and forms mixed crystals quite readily. Therefore, once crystallization is under way, the DAG inhibitory effect will be less significant. There will be many more potential growth sites available, and also more TAG species will be sufficiently undercooled to crystallize.

The compositional differences between the 1998 and 2000 DAG may also affect nucleation by influencing the lamellae layering or liquid structure in the MF-TAG melt before crystallization. (The influence of DAG on structure in the melt during cooling and just before crystallization is discussed shortly.) The different DAG behaviors could also be related to their melting temperatures. Unfortunately, melting points were not determined at the time of the 1998 study and cannot be determined now because of insufficient sample. In both years the DAG that were collected and added back to the MF-TAG were plastic-like, i.e., neither completely liquid nor completely solid at room temperature (~22°C). Table 3 shows the FA composition of the combined DAG samples (both TLC bands).

Based on the higher proportion of stearic acid and lower proportion of oleic acid in the 1998 DAG, Table 3 suggests that these DAG would have a higher melting temperature than the 2000 DAG. This should result in a similar mechanism as described for molecular complementarity; the higher-melting DAG (1998) would become incorporated earlier than the lower-melting species (2000 DAG). The importance of DAG melting temperature on MF-TAG crystallization is discussed later.

TABLE 2
FA Composition (wt%) of 1998 and 2000 Milk Fat *sn*-1,3 DAG and Milk Fat's High Melting Fraction (HMF)[a]

FA	1998 *sn*-1,3-DAG	2000 *sn*-1,3-DAG	HMF[b]
Caproic	0.2 ± 0.3	2.1 ± 0.1	0.2 ± 0.2
Caprylic	0.1 ± 0.2	1.3 ± 0.1	0.3 ± 0.2
Capric	1.5 ± 0.0	3.0 ± 0.2	1.8 ± 0.8
Lauric	3.8 ± 0.3	4.0 ± 0.4	3.8 ± 1.0
Myristic	15.0 ± 0.1	10.8 ± 0.7	16.5 ± 1.7
Palmitic	48.2 ± 0.1	29.5 ± 2.5	45.4 ± 1.0
Stearic	13.5 ± 0.0	7.3 ± 0.5	22.0 ± 2.8
Oleic	17.7 ± 0.1	42.1 ± 3.2	9.9 ± 1.6

[a]Mean value of three replicates with SD.
[b]Adapted from Wright *et al.* (16).

TABLE 3
FA Composition (mol%) of the Combined Milk Fat DAG from 1998 and 2000 Anhydrous Milk Fat

FA	1998 DAG[a]	2000 DAG[b]
Caproic	2.88 ± 0.26	3.40 ± 0.29
Caprylic	2.05 ± 0.15	2.01 ± 0.02
Capric	3.83 ± 0.10	4.58 ± 0.09
Lauric	5.20 ± 0.08	6.17 ± 0.28
Myristic	15.79 ± 0.05	13.55 ± 0.20
Palmitic	41.82 ± 0.28	27.61 ± 0.18
Stearic	11.56 ± 0.13	8.72 ± 0.42
Oleic	16.85 ± 0.23	33.90 ± 0.51

[a]Mean value of three replicates and SD.
[b]Mean value of two replicates and SD.

Effect of DAG standards on MF-TAG crystallization. To further investigate the effect of DAG composition on MF-TAG crystallization, racemic and *sn*-1,2-specific DAG of palmitic and oleic acid were added to the 2000 MF-TAG at the 0.1 wt% level. These included racemic mixtures of PP and OO, 1,2PP, *sn*-1,2 diolein (1,2OO), and *sn*-1-palmitic, 2-oleic (1P,2O). Addition of the DAG standards did not significantly ($P > 0.05$) affect the MDT (°C), as shown by the following data (mean ± SD): MF-TAG, 34.8 ± 0.1; PP, 34.9 ± 0.1; 1,2PP, 34.7 ± 0.2; OO, 34.7 ± 0.1; 1,2OO, 34.8 ± 0.1; 1P,2O, 34.8 ± 0.1.

Figure 4 shows that addition of the *sn*-1,2 DAG standards (1,2PP, 1,2OO, and 1P,2O) delayed the crystallization of MF-TAG at 25.0°C. In contrast, the racemic mixtures of DAG (PP and OO) slightly enhanced MF-TAG crystallization.

Nucleation induction times and free energies of nucleation ($\tau_{nucleation}$ and ΔG_c, respectively) for MF-TAG alone and MF-TAG with the standard DAG are shown in Table 4.

The monoacid 1,2 specific isomers (1,2PP and 1,2OO) increased ΔG_c, suggesting that they inhibit TAG nucleation. Although 1P,2O delayed the development of solid fat (Fig. 4), it did not significantly affect ΔG_c (Table 4). Therefore, 1P,2O delayed crystal growth but not by exerting an effect on nucleation. This is similar to the action of the MF-DAG in MF-DAG-2000. Values of the Avrami parameters determined for the crystallization curves in Figure 4 are shown in Table 5.

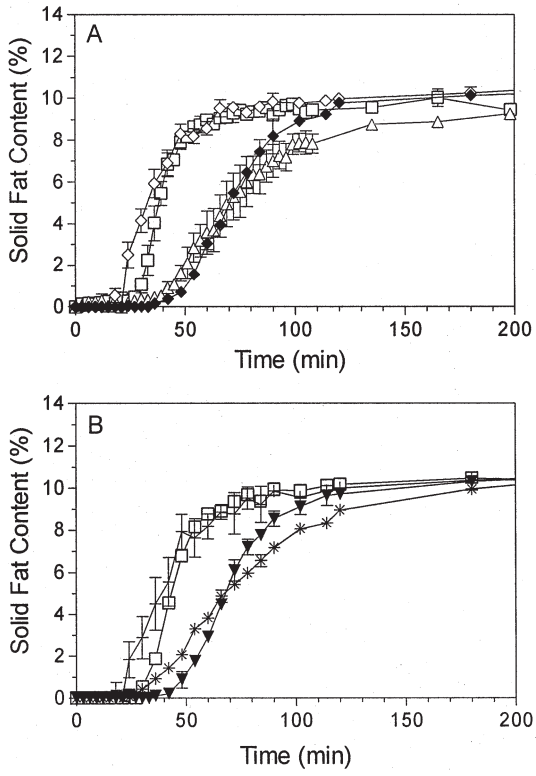

FIG. 4. SFC vs. time during static crystallization of AMF (△), MF-TAG (□), and MF-TAG with 0.1% standard DAG added [OO (◇) and 1,2OO (◆)] at 25.0°C (A). SFC vs. time during static crystallization of MF-TAG (□), and MF-TAG with 0.1% standard DAG added [PP (+), 1,2PP (✳), and 1P,2O (▼)] at 25.0°C (B). Symbols represent the average and SE of three replicates. PP, dipalmitin; 1,2PP, 1,2-dipalmitoyl-*sn*-glycerol; 1P,2O, 1-oleoyl,2-palmitoyl-*sn*-glycerol; OO, diolein; 1,2OO, 1,2-dioleoyl-*sn*-glycerol; for other abbreviations see Figures 1 and 2.

TABLE 4
Nucleation Induction Times and Nucleation Free Energies for MF-TAG and MF-TAG with 0.1 wt% DAG Standards (PP, OO, 1,2PP, 1,2OO, 1P,2O) at 25.0°C[a]

	$\tau_{nucleation}$ (min)	Nucleation free energy (kJ/mol)
MF-TAG	10.04 ± 1.02^{A}	$1.67 \pm 0.33^{B,C}$
PP	9.47 ± 0.91^{A}	$1.89 \pm 0.35^{B,C}$
OO	9.80 ± 0.17^{A}	1.33 ± 0.18^{C}
1,2PP	9.23 ± 1.22^{A}	$2.45 \pm 0.23^{A,B}$
1,2OO	8.37 ± 0.38^{B}	2.88 ± 0.23^{A}
1P,2O	7.84 ± 1.68^{B}	1.40 ± 0.37^{C}

[a]Mean value of three replicates with SD. Different superscript letters (A–C) indicate significant differences ($P < 0.05$). MF, milk fat; PP, dipalmitin; OO, diolein; 1,2PP, 1,2-dipalmitoyl-*sn*-glycerol; 1,2OO, 1,2-dioleoyl-*sn*-glycerol; 1P,2O, 1-palmitic,2-oleic-*sn*-glycerol.

The only statistically significant differences observed between the Avrami parameters for the DAG/MF-TAG blends involved 1,2PP. The value of *n* for 1,2PP was significantly lower than those for 1P,2O and 1,2OO ($P < 0.05$). The value of *k* determined for 1,2PP was also statistically different from those determined for 1,2PO, 1,2OO, and OO ($P < 0.05$). This reflects the fact that the shape of the crystallization curve for 1,2PP is different from the others—it is less sigmoidal (Fig. 4). Addition of 1,2PP increased the energy barrier to nucleation at 25°C (ΔG_c), although this was not reflected in a significant increase in $\tau_{nucleation}$ at 25°C (Table 4). This was the same trend observed for 1,2OO. Both monoacid 1,2-specific DAG significantly increased ΔG_c. The palmitic and oleic stereoisomers (*sn*-1,2) seemed to affect the growth process differently, however. Figure 4 shows that the SFC crystallization curve for 1,2OO was more sigmoidal than the curve for 1,2PP. Of the five DAG standards, only 1,2OO resulted in as substantial an increase in *n* as the native MF-DAG (Table 4).

Figure 5 shows that the microstructures of MF-TAG with 0.1 wt% of the standard DAG are similar after crystallization at 25°C for 24 h. Although MF-TAG crystallization was influenced by the DAG standards differently, these differences are not reflected in the resulting microstructures. PP and 1,2PP accelerated and delayed crystallization, respectively, but these DAG/MF-TAG blends had similar microstructures. MF-TAG containing these DAG qualitatively possessed the same microstructures. The very dissimilar values of *n* for

TABLE 5
Values of *k* and *n* Obtained When the Avrami Model Was Fitted to the 25°C Crystallization Curves of MF-TAG Containing the Standard DAG[a]

	Avrami constant, *k*	Avrami exponent, *n*
PP	$9.27 \times 10^{-6} \pm 1.11 \times 10^{-6}$	3.54 ± 0.61
1,2PP	$3.06 \times 10^{-5} \pm 1.73 \times 10^{-5}$	2.63 ± 0.21
OO	$3.60 \times 10^{-6} \pm 3.42 \times 10^{-6}$	3.84 ± 0.45
1,2OO	$1.23 \times 10^{-8} \pm 1.66 \times 10^{-8}$	4.50 ± 0.65
1P,2O	$1.87 \times 10^{-7} \pm 1.93 \times 10^{-7}$	3.94 ± 0.27

[a]Mean value of three replicates with SD. For abbreviations see Table 4.

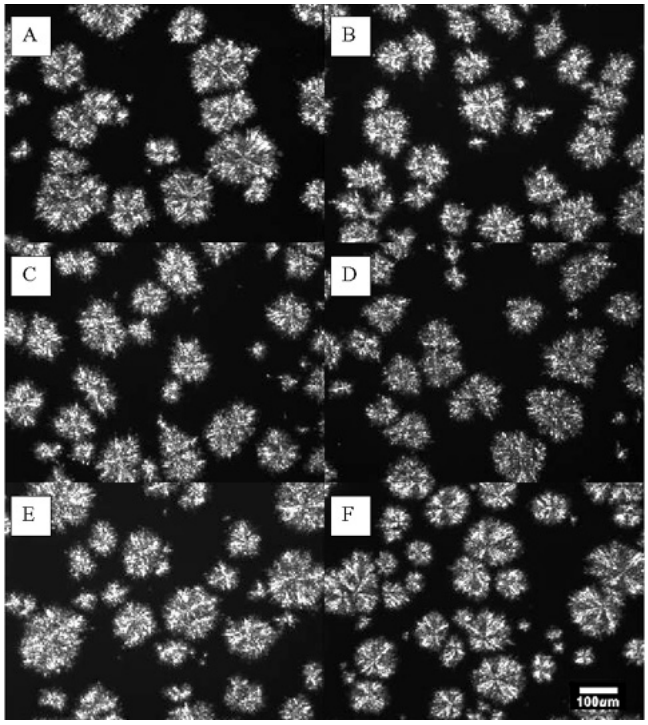

FIG. 5. Polarized light micrographs of MF-TAG (A) and MF-TAG with 0.1 wt% standard DAG [1P,2O (B), OO (C), 1,2OO (D), 1,2PP (E), and PP (F)] at 10× magnification after crystallization at 25°C for 24 h. For other abbreviations see Figures 2 and 4.

1,2PP and 1,2OO (2.63 and 4.50, respectively) did not correlate with different resultant network structures. The Avrami exponent *n* describes the shape of the crystallization curve, which is related to the crystallization mechanism, but it is not a good indicator of the resultant network structure. The polarized light micrographs for each sample show spherulitic clusters of crystals of between approximately 50 and 150 µm in diameter (Fig. 5). Even though fat microstructure seems to be unaffected by the DAG, it was thought that differences might be observed. In previous studies, minor components have been found to affect crystal habit (i.e., size and shape) by acting as crystal structure modifiers (5,17,18). When impurities are incorporated into a crystal they usually change the growth of certain faces preferentially, which results in changes in morphology. These differences were not seen, but they might not be obvious in a polycrystalline system of large spherulites and when aggregation is a consideration

The differences observed between the crystallization behaviors of the DAG/MF-TAG blends cannot be attributed to differences in DAG melting temperatures. Table 6 shows that both PP and 1,2PP, for example, have peak melting temperatures of approximately 50 and 65°C, although they affected MF-TAG crystallization very differently.

Based on their high melting temperatures, we might expect both dipalmitin standards to act as seeds to MF-TAG crystallization. However, only the racemic mixture, PP, had this effect. Figure 4 shows that the MF-TAG with PP added crystallize before the pure MF-TAG. 1,2PP had the same inhibitory effect on crystallization as 1,2OO, despite the fact that at 25°C we would expect 1,2PP to be in the solid and 1,2OO to be in the liquid state. The ability of the DAG standards to delay milk fat crystallization is at least partly related to the racemic purity of the DAG. Only the *sn*-1,2-DAG delayed crystallization. 1,2 (2,3)-DAG isomers are generally more effective than 1,3-DAG at delaying the velocity of phase transformations in fats (19). This may be related to known differences between *sn*-1,2- and *sn*-1,3-DAG. Shannon *et al.* (20) confirmed that 1,2- and 1,3-DAG have different crystal structures and polymorphism. The α-polymorph has been identified in *sn*-1,2-DAG but not in *sn*-1,3-DAG (21). When racemic monoacid 1,2-DAG are crystallized, the α-polymorph separates from the melt and then transforms to the stable β′-form (22). In saturated symmetrical DAG, two β-forms (β_1 and β_2) that have fairly similar melting temperatures are observed during crystallization (23). The melting points of the β_1- and β_2-forms in pure *sn*-1,3 dipalmitin are 71.5 and 72.5°C, respectively, whereas the α- and β-polymorphs in *sn*-1,2 dipalmitin have melting temperatures of 50.0 and 63.5°C, respectively (as reported by Gunstone *et al*., 23). The experimental sample of *sn*-1,2PP had peak melting temperatures of 50 and 67°C by DSC (Table 6), which agrees fairly well with this literature value.

The directionality of the FA chains in the crystalline state also differs between DAG isomers. In *sn*-1,3-DAG, the FA chains extend in opposite directions and the molecules tend to pack in monolayers. Conversely, the FA chains in *sn*-1,2-DAG extend in the same direction (24). The shape of these molecules is more reminiscent of the tuning fork arrangement that describes TAG conformation. Of course, this describes DAG packing in a crystal composed entirely of the partial glycerides. This description may bear no relevance when the DAG are present in minute quantities in a mixture of TAG. These observations do, however, illustrate the fact that differences exist between DAG stereoisomers.

The influence of the racemic and *sn*-1,2-DAG on TAG crystallization may be related to different polymorphic behaviors between the DAG, although no differences were observed in the polymorphism of the pure DAG standards as monitored by DSC (Table 6). The melting temperatures of PP and 1,2PP reported in Table 6 refer to DAG in isolation. DAG behavior in a mixture of TAG could be different.

TABLE 6
Peak Melting Temperatures (°C) by DSC for Standard DAG in Isolation (PP, 1,2PP, OO, 1,2OO, and 1P,2O) After 24 h at 5°C[a]

	Peak 1 (°C)	Peak 2 (°C)
PP	50	65
1,2PP	50	67
OO	24	—
1,2OO	7	—
1P,2O	7	15

[a]For abbreviations see Table 4.

The effect of PP and 1,2PP on MF-TAG liquid structure and polymorphic behavior during crystallization at 25°C was investigated using DSC and XRD to determine if the racemic and stereospecific DAG affected the ordering of the liquid structure or early polymorphism differently. Unfortunately, insufficient solids were present in the DAG/MF-TAG blends for adequate signal by DSC until after a relatively long time at 25°C (70 min for PP and 5 h for 1,2PP). At these times, each DAG-MF-TAG blend demonstrated a melting peak minimum of approximately 37°C.

Orientation in polymer melts is known to affect the rate of crystallization (25). We have previously observed liquid-state structure in blends of milk fat fractions by XRD (16). Differences in the crystallization behavior of samples were postulated to relate to differences in liquid ordering. Minor components may influence liquid ordering or TAG orientation in the melt and subsequently delay TAG nucleation. PP-MF-TAG and 1,2PP- MF-TAG were the systems chosen to investigate this. XRD analysis was performed on the PP-MF-TAG and 1,2PP-MF-TAG blends early during the crystallization in order to explore differences in the liquid ordering between the two samples. Table 7 shows the short- and long-spacings observed by XRD in samples of MF-TAG with 0.1 wt% PP and 1,2PP after crystallization for 7 min at 25.0°C and 4 min at 22.5°C. These times were chosen because they are shortly before the induction times observed using the phase transition analyzer ($\tau_{nucleation}$). Isothermal data collection for 5 min was, however, required.

A short-spacing of 3.82 Å is consistent with the presence of the β′-polymorph. A second spacing at approximately 4.2 Å is also expected for the β′-form, although the only other short-spacing observed in PP and 1,2PP at 22.5°C is at approximately 4.44 Å. The ring in this vicinity is quite broad and diffuse, however, and probably includes the reflections for both the second α spacing (4.2 Å) and the liquid oil (4.53 Å), which are not distinguishable from each other. The same pattern was observed in PP after 7 min at 25.0°C. After crystallization of 1,2PP for 7 min there is only evidence of liquid oil at 25.0°C.

After 4–9 min at 22.5°C, both PP and 1,2PP contained crystalline material in the β′-polymorph. At 25.0°C, PP crystallized before 1,2PP. This observation is consistent with the results from the other methods and points to differences in the rates of crystallization between the DAG samples. Differences in the liquid ordering of the samples, however, were not found. Each sample had a long-spacing at approximately 23 Å, indicating the presence of some weak structure, probably lamella layering, in the melt. Perhaps no differences exist, but this observation could be related to the fact that the data collection period also was relatively long (5 min) considering the short crystallization times being investigated (4 and 7 min). Changes occurred in these samples during the analysis, and differences between the samples could have been averaged out over time. Time-resolved synchrotron XRD would be required to resolve this further by monitoring the earliest appearance of reflections in the long-spacing region.

The different crystallization behaviors observed between MF-DAG-1998 and MF-DAG-2000 are not completely accounted for. Differences may be related to differing interactions between the DAG and MF-TAG molecules because of varying degrees of complementarity between the glycerides. Unfortunately, the complex FA composition of milk fat makes it difficult to speculate on molecular interactions and packing arrangements.

Our study using standard DAG showed that the effect on MF-TAG crystallization was more related to DAG racemic purity than to DAG FA composition. The MF-DAG added to the MF-TAG were a mixture of 1,3 and 1,2/2,3 isomers. Although DAG from both TLC DAG bands were combined and added back to the MF-TAG, the relative proportion of each isomer in 1998 and 2000 is unknown. Based on the importance of racemic purity in the DAG standards study, we speculate that the greater delay observed in MF-DAG-1998 over MF-DAG-2000 points to a higher proportion of *sn*-1,2-DAG in 1998. *sn*-1,2-DAG are present in milk fat because of incomplete synthesis and also because lipases and phospholipases in milk, in particular lipoprotein lipase, preferentially cleave FA from the *sn*-3 position. To determine the relative degree of lipolysis in the AMF samples, the %FFA was determined by titration. This analysis might have indicated that the 1998 AMF had undergone a greater degree of lipolysis and therefore would contain a higher proportion of *sn*-1,2-DAG than the 2000 AMF. However, no significant difference was found between the %FFA in the 1998 and 2000 samples of AMF ($P > 0.05$). The %FFA in the 1998 and 2000 batches of AMF were 0.40 ± 0.08 and 0.40 ± 0.01%, respectively.

The likelihood of differences in the racemic purity of the DAG between 1998 and 2000 is minimized by the fact that at least some acyl migration surely occurred in the DAG during the preparative work and storage. DAG undergo rapid isomerization, particularly in polar solvents (26). In the liquid state, a pure DAG will form an equilibrium mixture of about equal proportions of the 1,2- and 1,3-DAG isomers within only a few hours (21).

TABLE 7
***d*-Spacings (Å) Determined by Powder X-Ray Diffraction for PP and 1,2PP (MF-TAG with 0.1 wt% DAG standard) After Crystallization at 22.5 and 25.0°C for 4 and 7 min, Respectively[a]**

Temperature (°C)	PP	1,2PP
22.5	3.82, 4.42, 23.18	3.82, 4.46, 21.32
25	3.82, 4.46, 23.18	4.53, 23.18

[a]For abbreviations see Table 4.

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