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# Thermal studies on the crystallization kinetics of triglycerides and milkfat by DSC

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**Abstract**

Slow-cooling (1.25 K $min^{-1}$) crystallization experiments were conducted on bulk and emulsified samples of trilaurin, tripalmitin and tristearin. In addition, isothermal crystallization experiments were performed on bulk and emulsified trilaurin. Emulsified samples show greater supercooling, and lower crystallization rates compared to bulk samples. The temperature dependence of the crystallization rates was analyzed.

Slow-cooling crystallization experiments on milkfat samples do not show the same clear differences between bulk and emulsion samples. The temperature dependences of the crystallization rates for bulk and emulsion samples were very similar.

The results, particularly the comparisons between bulk and emulsion samples, and the comparisons between triglyceride and milkfat, suggest that in emulsified triglyceride samples the rate-determining step is nucleation. Both nucleation and propagation contribute to the crystallization rate for bulk triglycerides. For milkfat samples, the results suggest that there may be an unusually high concentration of active nuclei influencing bulk crystallization rates of the low-melting fraction.

*Keywords:* Crystallization; DSC; Emulsion; Isothermal; Kinetics; Milkfat; Non-isothermal; Nucleation; Triglyceride

## 1. Introduction

Most natural fats mainly consist of triglycerides. The physical properties of these fats are mostly determined by the crystallization behavior of the major component

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triglycerides and by the interaction of these triglycerides during the crystallization. In comparison to the water–ice transformation, the crystallization of fats is complicated by their capability of forming different polymorphic forms. The thermodynamic properties of different polymorphic forms of fatty acids have been studied by many researchers, for example, the studies of the polymorphs of tripalmitin and tristearin by Kellens and coworkers [1–4]. However, information on the kinetics of the formation of these polymorphs is far from complete.

Crystallization is the result of two processes, namely nucleation and crystal growth. It is helpful to try to separate out the contributions from these processes. Many nucleation studies have been carried out on water. The fact that ice crystal growth is rapid compared to ice nucleation makes it convenient to study the nucleation kinetics of water using emulsified samples. Wood and Walton, in order to study the homogeneous nucleation kinetics of ice from water developed the mathematics to calculate the nucleation rate of emulsion droplets from the volumetric measurement method of Vonnegut [5]. In later studies, Rasmussen and Loper reported similar nucleation rate determinations for alloys using a differential scanning calorimeter (DSC) [6]. They concluded that the isothermal DSC nucleation rate measurement corresponded to the classical isothermal dilatometric method and the constant-cooling DSC measurement corresponded to the constant-cooling microscopic methods for measuring the temperature dependence of the nucleation rate. Compared to traditional methods, differential scanning calorimetry has the advantage of convenience, rapidity, and accuracy.

In the present study, fat crystallization kinetics was studied using isothermal and constant-rate slow-cooling DSC. Both bulk and emulsified fats were used. The isothermal DSC experiments permit the evaluation of the fraction of unfrozen fat as a function of the isothermal holding time, and the crystallization rate can be calculated from the slope of the logarithm of the height of the DSC signal. The temperature dependence of the nucleation rate is obtained from a series of such experiments performed at different temperatures. In the constant-cooling-rate DSC experiments, the crystallization rate (equated to the fraction which crystallizes over a standard temperature interval) as a function of initial temperature is determined as the sample is cooled at a constant cooling rate. The temperature dependence of the crystallization rate can be obtained by determining this fraction at a series of different temperatures from the same cooling experiment. Comparisons of the crystallization kinetics of bulk and emulsion samples, and also of milkfat and triglycerides were conducted.

## 2. Materials and methods

### *2.1. Materials*

#### *2.1.1. Bulk samples*

Trilaurin (12:0), tripalmitin (16:0) and tristearin (18:0) were purchased from Sigma Chemical Co., St. Louis, Missouri, and had a purity of at least 90%.

Anhydrous butter fat was provided by Dr. J.B. German of the Department of Food Science and Technology, UC Davis.

*2.1.2. Emulsions*

All emulsions were made by dispersing pure liquid fats in distilled water at temperatures above the melting points of the fats using an Ultra-Turrax T25 homogenizer at a speed of 24 000/min for 60 s. 0.5% Tween 80 (Sigma Chemical Co., St Louis, Mo) by volume and 0.2% Keltrol food grade xanthan gum (Kelco, Division of Merck & Co., INC. Rahway, New Jersey, USA) by volume were added as emulsifier and stabilizer. The liquid fat fraction was 40% or 30% in volume.

## 2.2. Methods

For bulk experiments, a solid sample of several milligrams of the lipid was loaded into a DSC pan, and transferred to the calorimeter. The samples were usually loaded and transferred at room temperature (10°C). Then, an initial heating scan was performed, to give a liquid sample, and recorded. For emulsion experiments, around 10 mg of emulsion samples were loaded into preheated DSC pans, and transferred to the calorimeter without allowing cooling. The DSC holder was maintained at a temperature above the lipid melting point during the transfer. Because the emulsion may break down after crystallization, each new crystallization kinetics run required a freshly prepared sample, loaded into the holder in the above way.

To study crystallization kinetics, either constant-rate slow-cooling experiments or isothermal experiments were carried out.

*2.2.1. Isothermal experiments*

To identify the appropriate conditions for the isothermal experiments, a preliminary cooling experiment was conducted to locate the initial crystallization temperature of the emulsified fats in each new system. These temperatures provide the basis for selecting appropriate temperatures for isothermal experiments, which cover a range of a few degrees around the initial crystallization temperature. Separate experiments were performed at each of several selected temperatures within this range. Selected temperatures differ typically by 1–2 K increments. The upper and lower limits were set by measurement limitations. Employing a fresh sample for each experiment, isothermal kinetic experiments were performed as follows. The sample pan was transferred to the DSC holder at a temperature $T_1$, 10 K above the selected temperature of interest, $T_2$, and held to equilibrate for 2 min. Then the temperature was dropped rapidly to $T_2$ and held at $T_2$ for an extended period. The rate of heat evolution was a direct measure of the amount of fat crystallization per unit time. Data were collected until the baseline was steady. The instrumental transients were determined by rewarming the sample to $T_1$ and repeating the experiment with an already frozen sample. Because the temperature $T_1$ was below the melting point of the fat, no further crystallization took place, but the calorimeter still took time to reach thermal equilibrium. By subtracting the second trace

from the initial trace, the results could be corrected for thermal effects not associated with crystallization.

This protocol is similar to that employed in the study of ice crystallization from undercooled water. Fat, however, has a more complicated crystal structure. This can influence interpretation of the experimental results. Two cases should be considered. In the first case, the rate of crystal growth is rapid compared to the rate of nucleation. In this case, the heat evolution from an emulsion will be controlled by the nucleation process, because completion of crystallization within a droplet after initiation happens in a time that is short compared to the nucleation step. In the second case, the crystal growth rate and the nucleation rate are comparable. The heat evolution from such an emulsion will be controlled by both nucleation and crystal growth.

In a uniform emulsion, the same heat is expected to be released from each crystallized droplet; $dq$ is therefore a direct measure of the number of liquid droplets which crystallize, $dN$. The crystallization rate is directly related to the nucleation rate $J$, which can be expressed as the numbers of nuclei formed per unit time per unit volume. If $N$ is the total number of droplets not yet crystallized at time $t$ and each droplet is supposed to crystallize under the influence of a single nucleus, then the number of nuclei that have been active by time $t$ is

$$n = (N_0 - N)$$

so that

$$dn = -dN$$

where $N_0$ is the initial number of droplets. The nucleation rate is therefore

$$J = \frac{1}{V}\frac{dn}{dt} = -\frac{1}{V}\frac{dN}{dt} = -\frac{1}{Nv}\frac{dN}{dt} = -\frac{1}{v}\frac{d\ln N}{dt}$$

where $V$ and $v$ are the total volume of the dispersed phase and the average volume of a single droplet, respectively. If $J$ is assumed to be independent of $t$ then

$$N = N_0 \exp(-Jvt)$$

The amount of heat released is proportional to the number of nuclei formed, namely

$$\frac{dq}{dt} \propto \frac{dn}{dt} = -\frac{dN}{dt}$$

then

$$\ln\left(\frac{dq}{dt}\right)_t = \alpha + \ln(JvN_0) - Jvt = A - Jvt$$

$$\ln\left(\frac{dq}{dt}\right)_0 = A$$

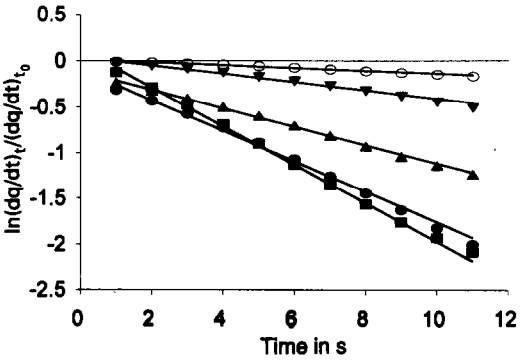


Fig. 1. Plots of the rate of heat evolution as a function of isothermal holding time for trilaurin emulsion: ●, 280; ■, 281; ▲, 282; ▼, 283.5; and ○, 284.5 K.

so that

$$\ln \frac{\left(\frac{dq}{dt}\right)_t}{\left(\frac{dq}{dt}\right)_{t_0}} = -Jvt$$

which, when $J$ is actually independent of $t$, is a straight line passing through the origin of the plane $\{\ln[(dq/dt)_t/(dq/dt)_{t_0}], t\}$, in fair agreement with the initial trend observed for some isothermal runs (see Fig. 1).

Within a bulk phase, both nucleation and propagation contribute to the crystal growth, the rate of which can be represented by the product $V_p \times (dn/dt)$, $V_p$ being the propagation rate. If one correlates the overall thermal effect observed with the rate of crystal growth

$$\frac{dq}{dt} \propto \frac{dN}{dt} VV_p = vN \frac{dN}{dt} V_p$$

it follows that

$$\ln\left(\frac{dq}{dt}\right)_t = \beta + \ln N + \ln\left(\frac{dN}{dt} V_p\right)_t = B - Jvt + \ln\left(\frac{dN}{dt} V_p\right)_t$$

$$\ln\left(\frac{dq}{dt}\right)_{t_0} = B + \ln\left(\frac{dN}{dt} V_p\right)_{t_0}$$

so that

$$\ln \frac{\left(\frac{dq}{dt}\right)_t}{\left(\frac{dq}{dt}\right)_{t_0}} = -Jvt + \ln \frac{\left(\frac{dN}{dt} V_p\right)_t}{\left(\frac{dN}{dt} V_p\right)_{t_0}}$$

which is not necessarily a straight line in the plane $\{\ln[(dq/dt)_{t_0}/(dq/dt)_{t_0}], t\}$, although it will still pass through the origin as shown in Fig. 2.

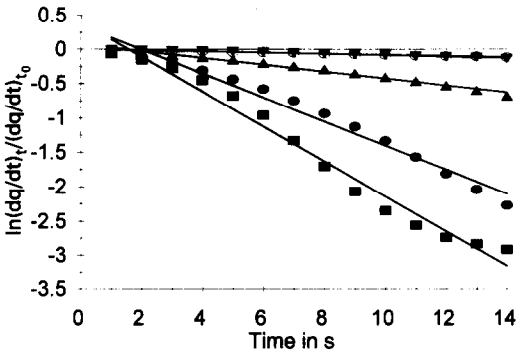


Fig. 2. Plots of the rate of heat evolution as a function of isothermal holding time for bulk trilaurin: ●, 285; ■, 287.5; ▲, 290; ▼, 292.5; and ○, 295 K.

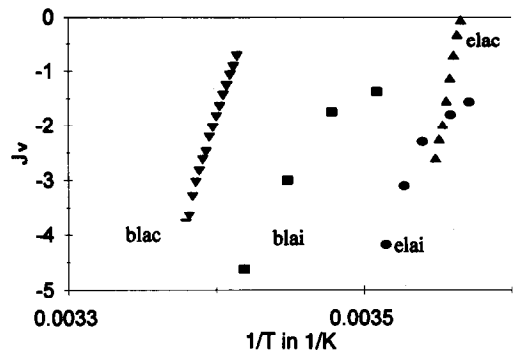


Fig. 3. Temperature dependence of crystallization kinetics in bulk and emulsion trilaurin samples: isothermal (blai and elai); and constant cooling (blac and elac).

The temperature dependence of the crystallization rates was determined, and the results for bulk and emulsion were plotted together, as in Fig. 3.

*2.2.2. Constant cooling experiments*

In a constant cooling experiment, the sample was cooled at a rate of 1.25 or 2.5 K $min^{-1}$. Heat is evolved through the processes of fat crystallization and the pattern of heat evolution was recorded. The total area under the thermogram after any time $t$ is a measure of the frozen fraction, which is directly related to the number of frozen droplets in emulsion. The determination of fat crystallization rate involves the graphical integration of the power–time contour [7]. By using the DSC "partial area" program, the power–time curve was divided into equal time intervals ($\Delta t$) and the corresponding areas under the curve were calculated. For a emulsion, $J$ for a temperature $T$ corresponding to a time $t_1$ can be calculated by the expression

$$Jv = (-1/\Delta t)\ \ln(A_{(t_1+\Delta t)}/A_{t_1})$$

where $A_{t_1}$ and $A_{(t_1+\Delta t)}$ are the areas *remaining* after time $t_1$ and $t_1 + \Delta t$ which are directly proportional to the fractions of fat still to be frozen, and $v$ is the average droplet volume of emulsion. For a bulk sample, the crystallization rate ($JV_p$) can be expressed similarly, using $V_p$ instead of $v$. The temperature dependence of the crystallization rates was determined, and the results for bulk samples and emulsions were plotted together, as in Figs. 3 and 5.

## 3. Results and discussion

### 3.1. Crystallization kinetics of triglycerides

Because the crystallization studies of monoacid saturated triglycerides form the basis for understanding the complex crystallization behavior of milkfat, three monoacid saturated triglycerides (trilaurin (12:0), tripalmitin (16:0) and tristearin (18:0)) were studied first. Trilaurin was studied using both isothermal and constant cooling rate methodologies. The other materials were studied only by the latter technique.

#### 3.1.1. Trilaurin

Before the isothermal experiments were conducted, constant cooling experiments (10 K $min^{-1}$) were employed for bulk and emulsion samples. The results, Fig. 4, show that a crystallization event occurred at a higher temperature in the bulk sample than in the emulsion. Such supercooling for emulsion samples indicated that the initiation of crystallization in the emulsion is much more difficult than in bulk. This is a consequence of the restriction on propagation provided by having the emulsion sample in discrete, non-contacting drops.

*Isothermal experiments.* Cooling scans for bulk and emulsion samples showed the primary crystallization ranges to be 295–285 and 285–280 K, respectively. Based

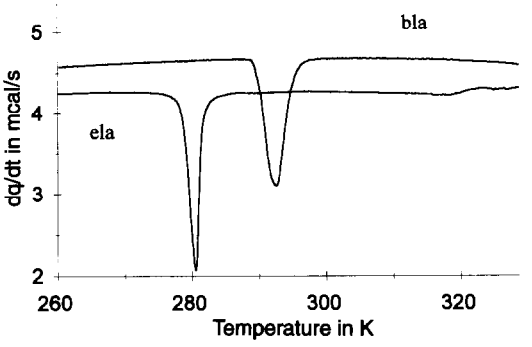


Fig. 4. Comparison of cooling scans at 1.25 K $min^{-1}$ for bulk and emulsion samples of trilaurin (bla and ela).

on this, isothermal experiments on bulk samples were performed at 285, 287.5, 290, 292.5 and 295 K, and isothermal experiments on emulsion samples were performed at 280, 281, 283.5 and 284.5 K.

In isothermal experiments, the rate of heat evolution is a measure of the rate of crystallization at the temperature selected. The rates of heat evolution, $\ln(dq/dt)_t/(dq/dt)_{t_0}$, were plotted as a function of holding time, using the corrected calorimeter trace for each chosen temperature. The linear relationships of the initial portions of these plots confirmed the first-order kinetics of the crystallization processes (Figs. 1 and 2). The rates of crystallization were determined from the slopes of these plots and the relationship between crystallization rate and the temperature for bulk samples and emulsions were also plotted and shown in Fig. 3.

When we compared the crystallization rates in bulk and in emulsion at the same temperature, we found that the crystallization rate in the emulsion was much slower than that in bulk. Because crystallization is the result of two processes, nucleation and crystal growth, the different crystallization rates at the same temperature implied a different relative importance of the two processes (nucleation and crystal growth) in bulk and emulsion. In a bulk sample, a few nuclei are enough to produce growth in the whole sample whereas, in an emulsion, each individual droplet requires separate nucleation, because propagation may not extend beyond the droplet boundary. The much more rapid crystallization rate in bulk shows that crystal growth (propagation) is more rapid than nucleation, and hence a study of the kinetics of the emulsion crystallization should yield information on the nucleation process. Thus, in the emulsion nucleation is the rate-limiting step, and the propagation within the droplet after nucleation is sufficiently rapid to allow for complete crystallization of the seeded droplet in a shorter time period than can be discriminated by the method; in the bulk sample the rate of nucleation and the rate of propagation both contribute significantly to the rate of heat evolution.

*Constant slow-cooling experiments.* In a constant slow-cooling experiment, the calculation of the crystallization rate from the DSC constant slow-cooling trace (Fig. 4) requires the measurement of the areas (per uniform temperature interval) beneath the crystallization peak. The crystallization rate over a time interval $\Delta t$ can be calculated from Eq. (1). The temperature dependence of crystallization rate for bulk and emulsions is shown in Fig. 3, and also in Fig. 5.

*Comparison of isothermal and constant slow-cooling experiments.* The results for trilaurin samples are compared in Fig. 3. The comparison shows that the temperature dependences of crystallization rate obtained from the two methods of bulk crystallization do not coincide. This probably reflects the variable influence of nucleation (because only a few active nuclei are sufficient) on the crystallization of the small bulk samples. Individual bulk samples in constant cooling experiments also gave slightly different results for the same reason. For emulsion crystallization, the results from isothermal and from constant rate cooling experiments are consistent. In the emulsion, droplet size limits the importance of the growth term, and so

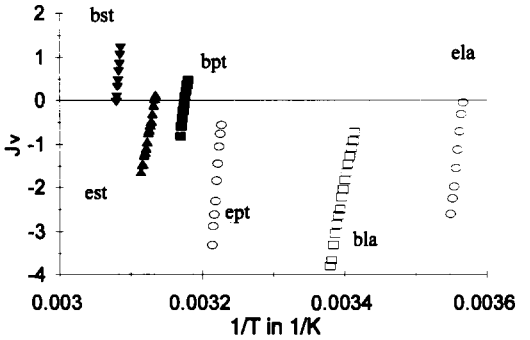


Fig. 5. Temperature dependence of crystallization kinetics from constant cooling rate experiments for bulk and emulsion trilaurin (bla and ela), tripalmitin (bpt and ept) and tristearin (bst and est).

the nucleation process is probably the controlling factor. There are sufficient droplets for the nucleation term to be close to the statistical average.

### *3.1.2. Tripalmitin and tristearin*

Saturated triglycerides, such as tripalmitin and tristearin, play an important role in milkfat crystallization (J.B. German, personal communication). In our studies, both bulk and emulsion tripalmitin and tristearin were used in the constant slow-cooling experiments. A typical comparison of DSC slow-cooling traces for bulk and emulsion triglyceride samples is shown in Fig. 4. The temperature dependence of crystallization rates is shown in Fig. 5.

The results show that trilaurin, tripalmitin and tristearin have similar behaviors. This means that, in comparison to bulk samples, emulsions need further supercooling to crystallize. If compared at the same temperatures, bulk samples always have higher crystallization rates than emulsions. Separate bulk samples yield slightly different results; emulsion samples yield consistent results.

## *3.2. Crystallization kinetics of milkfat*

Constant slow-cooling experiments were conducted using bulk and emulsion milkfat samples. The DSC results are shown in Fig. 6 and the temperature dependence of crystallization rates is shown in Fig. 7.

The results show that the behavior of milkfat is very different from the behavior of the pure triglycerides. There is no marked difference in the crystallization behavior of the emulsion samples and bulk samples: no additional supercooling for the emulsions compared to bulk, and not too much difference in crystallization rate in the middle- and low-melting triglyceride region. Fig. 6 shows that the crystallization process of milkfat can be divided into two regions: the "high-melting triglycerides" region and the "middle- and low-melting triglycerides" region.

In the first region (the high-melting triglycerides), Fig. 7 shows that the crystallization rate of the bulk sample is higher than that of the emulsion although there

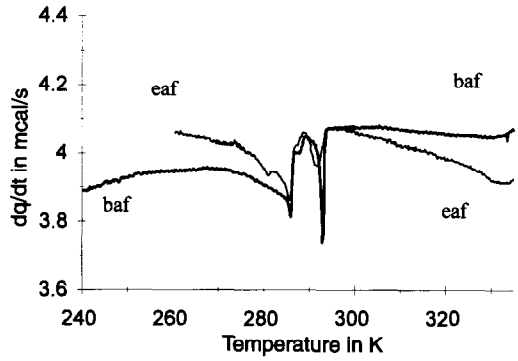


Fig. 6. Comparison of cooling scans at 1.25 K $min^{-1}$ for bulk and emulsion milkfat samples (baf and eaf).

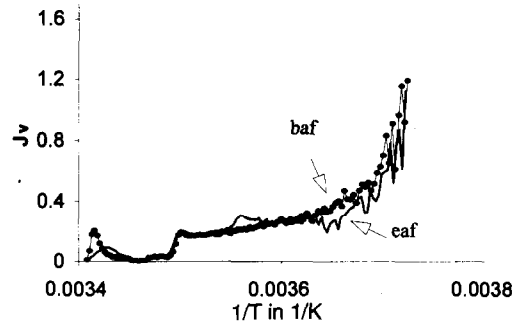


Fig. 7. Temperature dependence of crystallization kinetics for bulk and emulsion milkfat samples (baf and eaf).

is no additional supercooling for the emulsion. In the second region, the two traces are overlapped.

The absence of additional supercooling in the crystallization of the high-melting triglycerides from the emulsion samples compared to bulk might reflect growth inhibition in the bulk sample, or alternatively a significant concentration of active high-temperature nuclei. The second explanation would appear more likely, because growth, i.e. heat evolution, is more rapid in the bulk samples, which suggests that bulk crystals rapidly exceed emulsion droplet size.

In the lower temperature crystallization region, the rates are similar for bulk and emulsion samples. This suggests that the high-melting triglyceride crystals serve as nuclei for the low-melting triglycerides. Crystal growth would be expected to occur from a similar number of growth centers in bulk and emulsion samples, which would be expected to lead to similar kinetics.

This could help explain the difficulty of separating a high-melting fraction from a low-melting fraction in large-scale separations, because it would indicate that the molecules of the low-melting fraction would be able to be incorporated into the high-melting crystal lattice.

### 3.3. Comparisons of crystallization kinetics of emulsion with bulk samples, and of triglycerides with milkfat

As previously indicated, in the pure triglyceride systems both the additional supercooling in emulsion samples and the different crystallization rates of bulk and emulsion samples suggest that nucleation is the rate-limiting step. At the higher temperature, fewer active nuclei exist. In the bulk sample, these are sufficient to initiate crystallization. In the emulsion, the fraction of drops which crystallize is small. Only when the temperature is reduced sufficiently for many active nuclei to be present will a significant fraction of drops freeze. The drop boundaries prevent further propagation of crystals in the emulsion. In bulk, crystal propagation will cease only when contact with another growing crystal occurs. We can assume that the nucleation rate is dependent upon temperature, and is similar in bulk and emulsion samples. The crystallization rate will be a function of nucleation rate and propagation rate, with the growth limit being the droplet surface or the contact zone.

These results can provide the basis for a model for crystallization rate with an initiation term and a propagation term

$$C = P(t)V(t)$$

where $C$ is the crystallization rate, $P(t)$ is a time probability of initiation which is related to nucleation, and $V(t)$ is a volumetric growth factor which accounts for crystal growth. The growth component $V(t)$ will have a volume cut-off for emulsions and a "contact" cut-off for bulk samples. For triglycerides, studies on emulsions can give us the information on the nucleation rate ($P(t)$). Because $V(t)$ is limited by drop size for emulsions, the smaller the emulsion droplets, the greater the influence of the term $P(t)$ on $C$. To get information on crystal growth it will be necessary to derive $P(t)$ from emulsion studies because $P(t)$ should stay the same in emulsion and bulk samples if the external conditions stay the same. $P(t)$ could then be applied in an analysis of bulk sample crystallization rates. This work is now in progress. It will be necessary to perform some of the bulk experiments on samples larger than those typical of small scale DSC, because in these smaller samples, as illustrated by Fig. 3, nucleation has to be treated as a random event. Only in larger samples, with a significant number of separate active nucleation events, can nucleation be treated using a statistical average.

## 4. Conclusions

The crystallization kinetics of triglycerides and milkfat were studied using slow-cooling and isothermal DSC. The results show a difference in crystallization kinetics between bulk and emulsified samples, and also between triglycerides and milkfat. In triglyceride systems, additional supercooling was seen before the crystallization took place in the emulsion. In milkfat, no additional supercooling was noted. This suggests that a large number of nuclei are active in milkfat. In triglycerides, the

rate-limiting step is nucleation, but in milkfat the situation is different. In the "high-melting triglyceride" region, the rate-limiting step is nucleation and the behavior of high-melting triglycerides is similar to that of the pure triglycerides, except that no additional supercooling was observed. In the "middle- and low-melting triglycerides" region, the high-melting triglycerides crystals may serve as nuclei, and crystal growth appears to be the rate-limiting step. By comparing different systems (pure triglycerides or milkfat, emulsion or bulk), nucleation and crystal growth can be studied, and this will help to establish a mathematic model which can be used to describe the crystallization process of various fats.

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