LETTER TO THE EDITOR

Practical Considerations in Nucleation Studies: The Polarized Light Microscopy Technique

Sir:

The induction time of crystallization (τ) is a kinetic parameter that is usually defined as the interval between the moment crystallization temperature is reached and the start of crystallization (1). The induction time of crystallization is usually related to the activation free energy of nucleation, ΔG_c, by the Fisher–Turnbull equation (2,3):

$$J = (NkT/h) \exp(-\Delta G_d/kT) \exp(-\Delta G_c/kT) \qquad [1]$$

where J is the rate of nucleation; ΔG_d, activation free energy of diffusion; k, gas constant per molecule; T, temperature; N, number of molecules per cm^3 in the liquid phase; and h, Planck's constant. J can be taken as being proportional to the inverse of the induction time of nucleation (τ). For a spherical nucleus, the activation free energy of nucleation is related to the surface free energy of the crystal/melt interface, σ, and the supercooling (melting point − crystallization temperature) $\Delta T = (T_m - T_c)$ by

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

with ΔH being the enthalpy of nucleation.

For a TAG system, the main barrier to diffusion is the molecular conformation; therefore, the first exponential in Equation 1 is equal to

$$-\alpha\Delta S/R \qquad [3]$$

where α is the fraction of molecules that should be in the right conformation for incorporation in a nucleus, ΔS is the decrease of entropy on crystallization of one mole of TAG, and R is the gas constant. Combining Equations 1, 2, and 3 and rearranging them gives the following equation:

$$\tau T = h/Nk \exp(\alpha\Delta S/k) \exp[(16/3)\,\pi\,\sigma^3\, T_m^{\,2}/kT(\Delta H)^2(\Delta T)^2] \qquad [4]$$

From a plot of ln τT vs. $1/T(\Delta T)^2$, a slope (s) can be evaluated, which allows calculation of the activation free energy of nucleation from

$$\Delta G_c = sk/(T_m - T_c)^2 \qquad [5]$$

where T_m is the melting temperature and T_c is the crystallization temperature. It is clear from Equation 5 that the activation free energy is a function of supercooling. At high supercooling, this energy barrier tends to zero (4). Experimentally, crystallization temperature selection should take into account the melting temperature of the fat system, since when a very low temperature is selected, there is no induction period; therefore, the fat system crystallizes before it reaches crystallization temperature. In general, when enough supercooling is generated in a fat system, it can remain as a liquid for a time interval at temperatures no more than 10°C below the melting point. In a recent study, it was reported that light microscopy is a good technique for studies of early crystallization in fat systems (5). However, by employing this technique it is very easy to make the mistake of measuring an induction time at a temperature where there is no induction time for nucleation. To help operators decide the right range of temperatures for induction time measurements in fat systems, we report here some practical considerations. Figure 1 shows an image taken 10 s after a 20–80% sunflower oil (SFO)–high-melting fraction (HMF) milk fat blend was placed on a microscope slide at 15°C. The blend was previously melted at 80°C and kept at that temperature for 30 min. The temperature of the blend was followed with a copper–constantine thermocouple. This photograph was taken at the exact moment the sample reached crystallization temperature, that is, zero time for the induction time measurement. If at this temperature there was an induction time for nucleation, no crystals should appear at zero time. As can be observed, many crystals with sizes from 0.2 to 20 μm covered the field. It is evident from Figure 1 that crystallization started before the system reached crystallization temperature. The analysis of 1000 photographs taken with our system showed that crystals must reach a minimum size of 0.2 μm to be detected with the microscope. Thus, based on crystal size, not only nucleation has occurred before the sample reached crystallization temperature, but also growth. Crystals of 20 μm can be considered growth crystals, taking into account the detection limit of the method. Figure 2 shows images of the same blend crystallized with the same thermal treatments but with a crystallization temperature of 35°C. When a fat is cooled to a temperature at which there is an induction time for crystallization, only a few crystals are observed per field and there are also many empty fields. Early crystals appear as dark spots, and sometimes it is difficult to decide whether they are crystals (Fig. 2A). As practical advice, it is helpful to follow crystallization for at least half an hour after the first spots appear. The induction time measured by microscopy for the images

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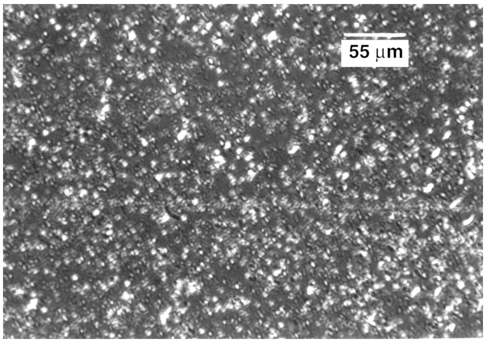

FIG. 1. Image of a 20–80% sunflower oil–high-melting fraction milk fat blend taken 10 s after it was placed on a microscope slide at 15°C. Scale as shown in photograph.

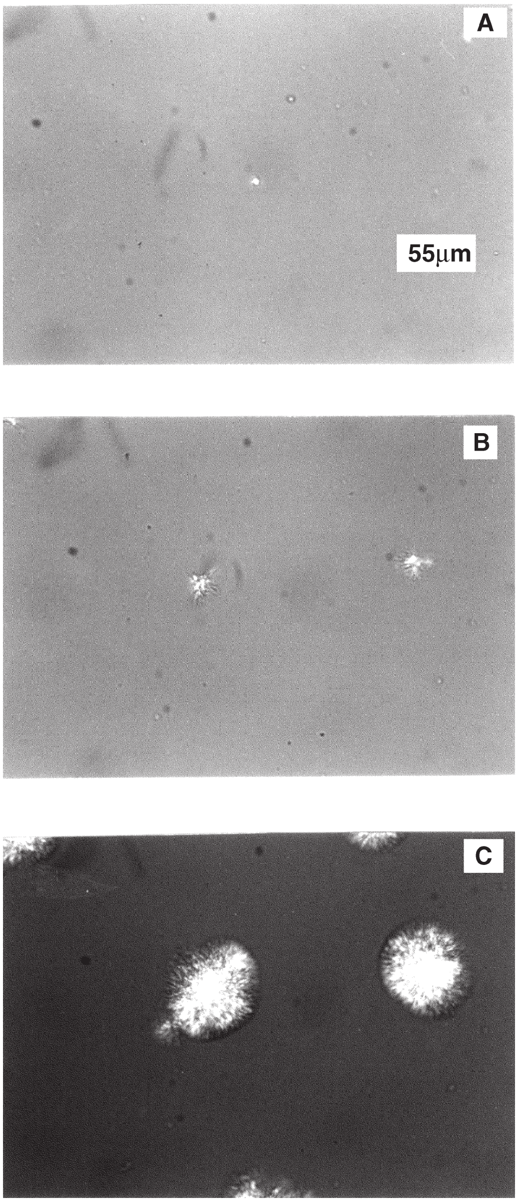

FIG. 2. Images of a 20–80% SFO–HMF blend crystallized at 35°C. (A) 8 min, (B) 16 min and, (C) 25 min. For scale and abbreviations, see Figure 1.

in Figure 2 was 10.5 min. Figure 2B was taken 16 min after the sample was placed at crystallization temperature. When these crystals grow, they show a very different morphology from those obtained at lower crystallization temperatures (Fig. 2C). In general, when crystallizing a fat, if an image similar to Figure 1 is obtained, it is most likely that there is no induction time for nucleation at this temperature. An image like the one shown in Figure 2 usually corresponds to a temperature at which there is an induction time. As the SD is higher than that obtained by indirect methods such as laser polarized turbidimetry (6), results should be the average of at least five runs, especially if the calculation of activation free energies of nucleation is intended.

Fat systems do not undergo glass transition. Therefore, nucleation behavior is not a diffusion control-led process, as happens in sugars and polymers in which viscosity undergoes dramatic changes with temperature, especially close to the glass transition temperature. In fat systems, the higher the supercooling, the higher the nucleation rate. For high supercoolings there is no activation energy for nucleation, as predicted by Equation 5. This means that for high supercoolings, fat systems crystallize immediately. To perform nucleation studies by polarized light microscopy, one should take into account that there is a limited range of temperatures at which there is an induction time for nucleation. In addition, the sample must be at crystallization temperature to start measuring the time. No matter at what cooling rate the sample is crystallized (that is, how long it takes to cool the sample from 80°C to crystallization temperature), zero time is always the time at which the sample reaches crystallization temperature.

REFERENCES

1. Sato, K., Crystallization of Fats and Fatty Acids, in *Crystallization and Polymorphism of Fats and Fatty Acids*, edited by N. Garti and K. Sato, Marcel Dekker, New York, 1988, pp. 255–256.
2. Strickland-Constable, R.F., Nucleation of Solids, in *Kinetics and Mechanism of Crystallization*, Academic Press, London, 1968, pp. 74–129.
3. Ng, W.L., A Study of the Kinetics of Nucleation in a Palm Oil Melt, *J. Am. Oil Chem. Soc. 67*:879–882 (1990).
4. Boistelle, R., Fundamentals of Nucleation and Crystal Growth, in *Crystallization and Polymorphism of Fats and Fatty Acids*, edited by N. Garti and K. Sato, Marcel Dekker, New York, 1988, pp. 189–226.
5. Wright, A.J., S.S. Narine, and A.G. Marangoni, Comparison of Experimental Techniques Used in Lipid Crystallization Studies, *J. Am. Oil Chem. Soc. 77*:1239–1242 (2000).
6. Herrera, M.L., C. Falabella, M. Melgarejo, and M.C. Añón, Isothermal Crystallization of Hydrogenated Sunflower Oil: I–Nucleation, *Ibid. 75*:1273–1280 (1998).

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