Demulsification of Oil-in-Water Emulsion Under Freezing Conditions: Effect of Crystal Structure Modifier

Takuya Harada* and Kazuhisa Yokomizo

Food R&D Laboratories, Ajinomoto Co., Inc.,
Kawasaki-shi, 210-8681, Japan

ABSTRACT: The demulsification of oil-in-water (O/W) emulsions under freezing conditions is connected to fat crystallization in the oil droplet. Therefore, demulsification can be prevented by the use of oil with a low melting point, and also by lowering the O/W ratio. However, an oil with a low melting point, such as sunflower, is rather expensive, and the O/W ratio has a significant effect on the texture of emulsions. We searched for an oil that is suitable for the production of a freeze-stable emulsion and found that soybean oil has unique characteristics. Normally, emulsions are more unstable at lower temperatures; soybean oil emulsion is unstable at −10°C and stable at −20°C. This unique characteristic results from the following two reasons. First, the solid fat content of soybean oil is almost the same at −10 and −20°C. Second, small crystals form a larger network over a period of time, and the higher temperature promotes faster restructuring. This structure formation was microscopically observed with the use of a thermostated stage. Structure formation was suppressed with the addition of a crystal structure modifier, polyglycerol fatty acid full ester, which also suppressed coalescence.

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KEY WORDS: Crystal structure modifier, demulsification, fat crystal network, O/W emulsion, polyglycerol fatty acid full ester, SFC, soybean oil, thermostated stage.

The presence of fat crystals in the oil phase can cause a considerable decrease in the stability of oil-in-water (O/W) emulsions (1). The reason is that fat crystals may stick out into the water phase. If such a crystal is present in the film between approaching globules, it may pierce the film and cause the globules to coalesce. This process is called partial coalescence, and the temperature cycling of cooling, holding, and thawing greatly enhances it (2). Unfortunately, many food emulsions are often stored under such conditions and ultimately separate into oil and water. If the fat crystals act as bridges, it is natural to assume that the emulsion with fewer or smaller crystals is more stable. The amount of crystals depends mainly on the composition of the oil and the temperature. The size of the crystals is often associated with the crystal form; β form tends to produce granular crystals (3,4) and β′ is recommended for the production of small crystals. There are a number of reports (5–7) on the effect of the emulsifier on the crystal form. Johansson and Bergenstahl reported the effect of sintering on the formation of the crystal network (8). Emulsifiers such as lecithin adsorb to the crystal interface and slow the sintering process. Also, the incorporated emulsifier acts as an impurity and lowers the melting temperature (9). However, these findings are often based on bulk oil and are not always applicable to emulsions. Therefore, it was our purpose to categorize the basic vegetable oils and to determine the most suitable set of oil and crystal structure modifier for the production of freeze-stable O/W emulsions.

EXPERIMENTAL PROCEDURES

Materials. Four kind of oils were compared: soybean oil, canola oil, corn oil (Ajinomoto Co., Inc., Tokyo, Japan), and sesame oil (Takemoto Oil Co. Ltd., Gamagori, Japan). They were of food grade, and no further purification was applied.

The emulsifier was commercial-grade polyglycerol fatty acid ester (MSW-750; hydrophilic-lipophilic balance 13.4) purchased from Sakamoto Yakuhin (Sakamoto Yakuhin Kogyo Co. Ltd., Osaka, Japan).

The crystal structure modifier was commercial-grade polyglycerol fatty acid full ester (THL-15), donated by Sakamoto Yakuhin.

O/W emulsions were made with the composition of O/W = 70:30 (wt%). The concentration of emulsifier was 1% to oil weight. Sodium chloride (5%) was added to the water phase to reduce the effect of ice crystals. The oil and water phases were heated to 60°C separately, and the water phase was stirred with an UltraTurrax T-25 homogenizer (IKA Werke GmbH, Staufen, Germany) during agitation at 11,000 rpm for 10 min. The oil phase was added dropwise to the water phase. The particle distribution was monomodal, and the mean particle diameter ($d_{3,2}$) was 7 μm.

Analytical methods. Solid fat content (SFC) was measured with pulsed nuclear magnetic resonance (NMR) measurement equipment SFC-900 (Praxis Corp, San Antonio, Texas). Bulk oil samples (5 mL) were maintained at 60°C for 10 min to destroy crystal history, stabilized at 20°C for 5 min, and maintained at a predetermined temperature. SFC of emulsions were not taken because of the complexity in distinguishing

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*To whom correspondence should be addressed at Food R&D Laboratories, Ajinomoto Co., Inc., 1-1, Suzuki-cho, Kawasaki-ku, Kawasaki-shi, 210-8681, Japan. E-mail: takuya_harada@ajinomoto.com

ice/water and solid fat/liquid oil signals. Canola oil was selected as a control because it showed no turbidity at –30°C for more than 1 h, and no crystallization was detected in the differential scanning calorimetry experiment (30 to –30°C, 5°C/min). Canola oil also did not show a change in pulsed NMR signal amplitude. The control was cooled 5 min prior to measurement.

Size distribution of the O/W emulsions was measured by the laser scattering method with an LA-910 (Horiba Ltd., Kyoto, Japan). All samples were diluted in 1% nonionic surfactant Triton X-100 solution and gently stirred for 5 min prior to measurement.

The polymorphic structures of the fat crystals from bulk oils were determined by x-ray diffractometry (Rigaku, Tokyo, Japan) (Cu Kα: $\gamma = 0.1542$ nm).

The image of the fat crystal network was obtained with a microscope equipped with a thermostated stage (Collet Kogyo, Tokyo, Japan). Sample (100 µL) was put on the stage preheated to 60°C, and the temperature was dropped to −10°C immediately with liquid nitrogen gas. The image was recorded at intervals with a Sony DKR-700 (Sony Co., Tokyo, Japan) and later processed with the public domain NIH Image program ver. 1.61 (National Institutes of Health, Bethesda, MD) on the Macintosh platform. "Enhance Contrast" without parameters was performed for the reinforcement of the image.

RESULTS AND DISCUSSION

Characteristics of vegetable oils. Figure 1 (A–D) summarizes the temperature dependence with time of SFC. SFC of soybean oil and sesame oil rise almost immediately after the immersion, and canola oil and corn oil rise after 2 to 3 h. This means the induction times of soybean and sesame oils are short and those of canola and corn oils are long. Furthermore, soybean oil partially crystallizes even at −10°C, but the SFC does not differ much at –30°C. Sesame oil also partially crystallizes at −10 and –20°C, but the SFC rises to almost 90% at –30°C after 24 h. Canola oil does not crystallize at −10°C for 24 h, but the SFC rises to 75% at –20°C after 15 h, and to 90% at –30°C after 3 h. Corn oil also does not crystallize at −10°C for 24 h, partially crystallizes at –20°C, and 90% crystallizes at –30°C after 24 h. These results show that the SFC of canola oil is highly dependent on temperature, whereas that of soybean oil is not so dependent. The SFC of sesame and corn oils are intermediate.

These vegetable oils can therefore be categorized into three groups. The first is soybean oil, which crystallizes rapidly, but the SFC is low. The second is canola oil, which crystallizes slowly, but the SFC is high. The third group is sesame oil and corn oil, which are intermediate between the other two groups. Soybean oil is sometimes subject to the customer complaints due to the apparent degradation that occurs in the refrigerator. In fact, it is the crystallization of the high-melting fraction, but it differs from the other situations in that the whole oil becomes gel-like, and spherulitic fat crystals are produced after a while. Thus, soybean oil partially crystallizes even at 5°C after a long time.

Stability of soybean oil and canola oil emulsions. With the differences in the bulk oil characteristics, we anticipated that the emulsions of these oils would also have different characteristics. It is usually considered that the emulsion is more unstable at lower temperatures, because the emulsion stability is greatly decreased by the presence of fat crystals. However, crystallization in bulk oil and in an emulsion does not necessarily occur in the same way. Zhao and Reid (10) investigated crystallization kinetics in bulk and emulsified samples of pure

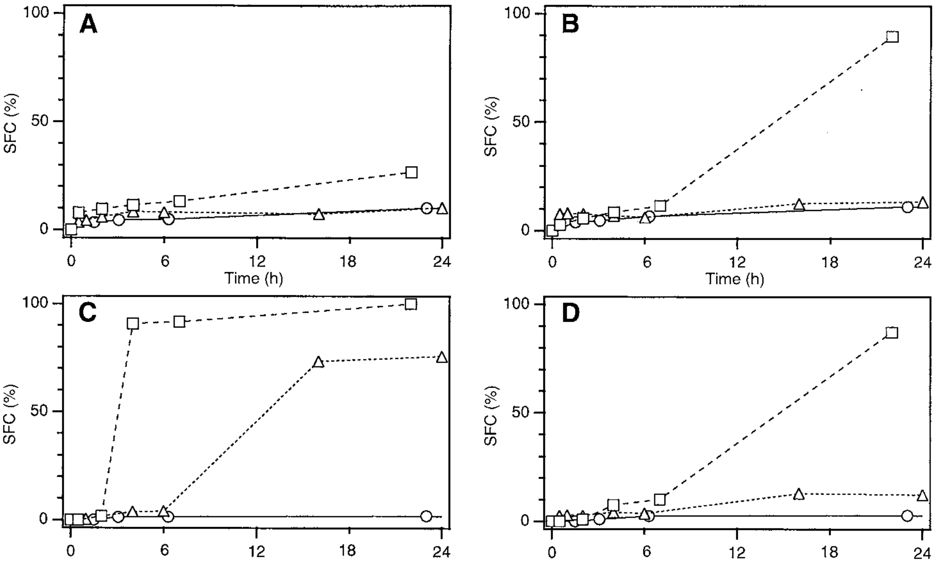

FIG. 1. Temperature dependence of solid fat content (SFC) of vegetable oils: (A) soybean oil, (B) sesame oil, (C) canola oil, and (D) corn oil. Three temperatures are shown: (○) −10°C; (△) −20°C; (□) −30°C.

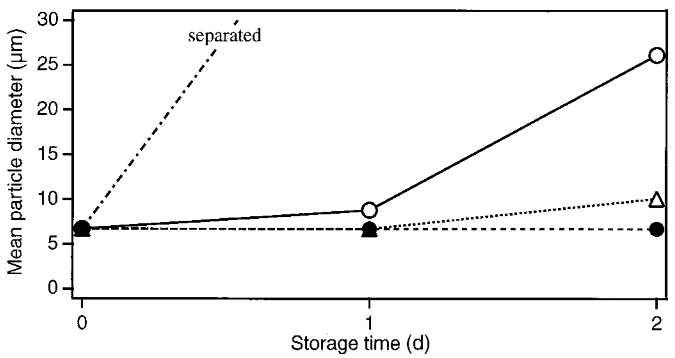

FIG. 2. Stability of soybean oil and canola oil emulsion. Canola oil emulsion stored at −20°C completely separates after 1 d. Soybean oil emulsion at −10°C (○) and at −20°C (△). Canola oil emulsion at −10°C (●) and at −20°C (▲).

TABLE 2
Stability Evaluation of Soybean Oil Emulsion Associated with SFC and Crystal Form of Oil[a]

Time (h)	m.d. (μm)	SFC (%)	Crystal form
0	6.7	0	—
24	6.7	10	—
48	26.1	10	—
72	—	10	—

[a]See Table 1 for abbreviations.

triglyceride and milkfat. They concluded that, although emulsified pure triglyceride shows greater supercooling compared to bulk samples, both emulsified and bulk milkfat crystallize similarly, owing to the influence of the active nuclei. Therefore, we assumed crystallization in bulk oil and in an emulsion occurs similarly. Soybean oil and canola oil were selected. Emulsions (20 g) were stored in laboratory dishes (ϕ 5 cm), cooled, and thawed at room temperature. Figure 2 summarizes the temperature dependence of soybean and canola oil emulsions.

As can be seen in Figure 1C, canola oil does not crystallize at −10°C for 1 d, and 70% crystallizes at −20°C. Accordingly, the canola oil emulsion is stable at −10°C and unstable at −20°C. However, although soybean oil has almost the same amount of crystals at −10 and −20°C, the soybean oil emulsion is unstable at −10°C and stable at −20°C.

To further investigate this difference, we combined the information of mean particle diameter, SFC, and x-ray diffraction (Table 1).

The canola oil emulsion was stored at −30°C and thawed at room temperature after a predetermined period of time. After about 6 h, the emulsion completely separated. The canola oil was also stored at −30°C, and SFC and x-ray diffraction were measured. Because SFC and x-ray diffraction were measured in the bulk oil, not in the emulsion, there is a discrepancy in the state of the oil. However, the changes in SFC and crystal form suggest that this separation was caused by the fat crystal protrusion from the O/W interface.

The soybean oil emulsion (Table 2) shows a different pattern. Although there is almost no change in SFC or crystal form, the soybean oil emulsion gradually coalesces. This means there should be a fat crystal network that somehow connects the globules (Fig. 3). And with the result from Figure 1A, there is a possibility that the soybean oil emulsion becomes once more unstable at −30°C or lower temperatures, due to the increase of SFC (Harada, T., and K. Yokomizo, unpublished data).

Evolution of the fat crystal network. To evaluate this hypothesis, we used a microscope equipped with a thermostated stage. Digitized images (Fig. 4) show the evolution of the fat crystal network. With a combination of SFC and the digitized image (Fig. 5), it can be said that the soybean oil emulsion separates due to the reconfiguration of the fat crystal network. There are two possible connections of globules in this kind of network. One is direct protrusion, which means the aggregated crystals act as a group, bind to the interface, and protrude from the interface. The other is indirect protrusion, which means the network cannot follow the deformation of the oil droplet and is left to protrude from the interface.

Suppression of the fat crystal network. With the assumption that the fat crystal network is responsible for the separation of the soybean oil emulsion, the soybean oil can be used to produce freeze-stable O/W emulsion if the network formation is suppressed. Normally, the emulsifier is applied to alter the crystal form. However, no apparent crystal form was observed in the soybean oil stored at −10°C for 72 h. Also, the emulsifier naturally adsorbs to the interface and alters the stability of the interface. Therefore, we focused on the effect of crystal structure modifier THL-15, which acts as an analog of triacylglycerol and has two effects. One effect is to lower the SFC, and the other is to act as an impurity on the crystal interface. Both effects can suppress the network

TABLE 1
Stability Evaluation of Canola Oil Emulsion Associated with SFC and the Crystal Form of Oil[a]

Time (h)	m.d. (μm)	SFC (%)	Crystal form
0	6.7	0	—
4	6.7	90	—
5	6.7	90	—
6	—	90	β
7	—	90	β

[a]m.d., mean particle diameter; SFC, solid fat content.

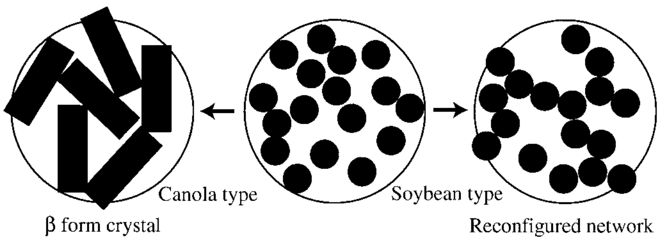

FIG. 3. Schematic representation of fat crystal protrusion from the interface.

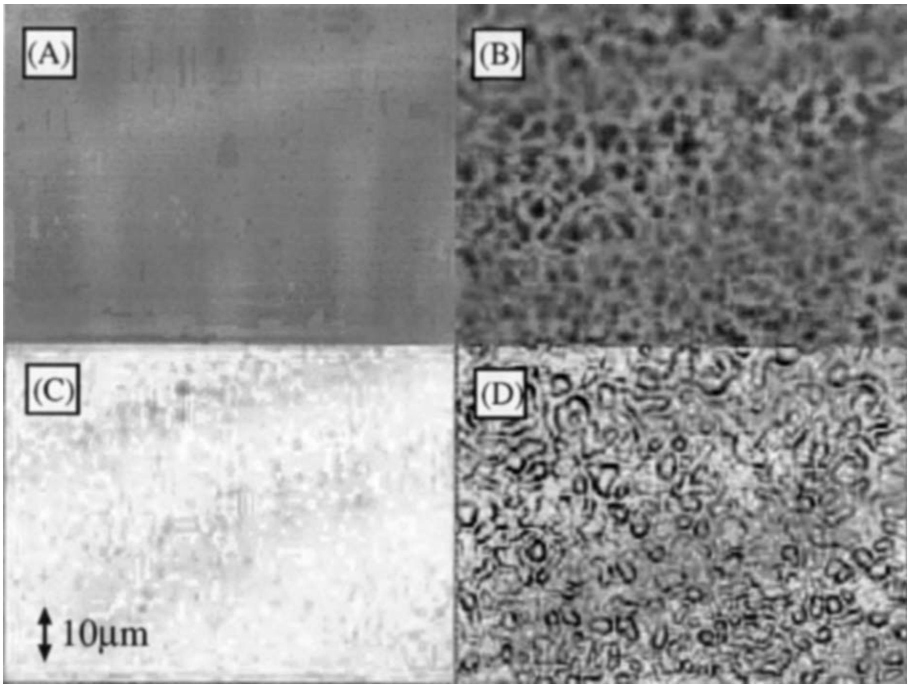

FIG. 4. Digitized image of soybean oil stored at −10°C. (A): 0 h, (B): 24 h. (A) and (B) are enhanced for contrast. (C) is processed image of (A); (D) is processed image of (B).

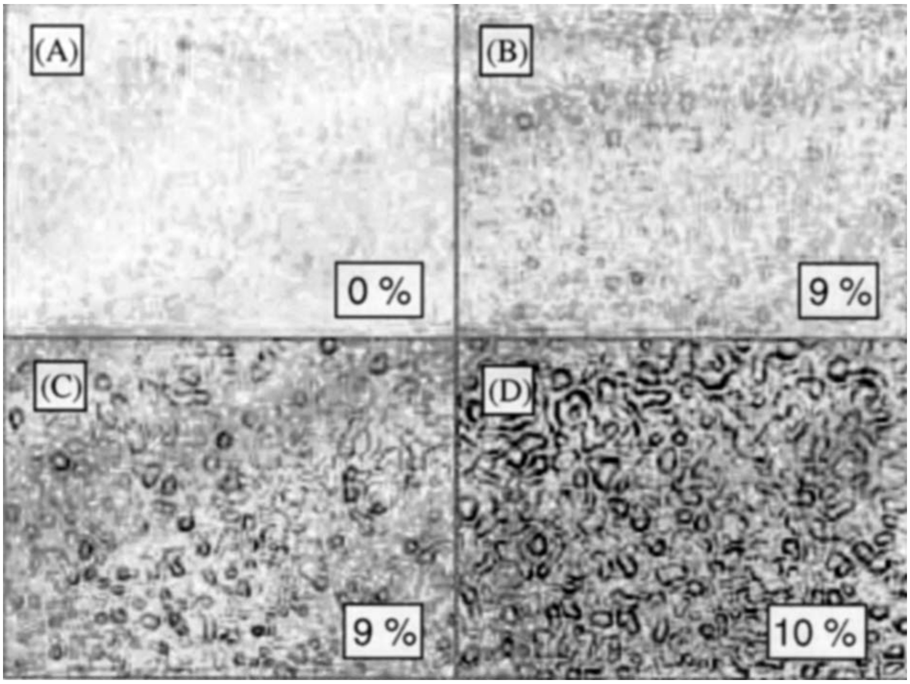

FIG. 5. Evolution of soybean oil crystal network stored at −10°C. (A): 0 h, (B): 14 h, (C): 18 h, and (D): 24 h. Corresponding SFC is shown on the bottom right. For abbreviation see Figure 1.

formation.

After 24 h of storage at −10°C, the oil with 1% THL-15 had a slightly lower SFC, and the fat crystal network was not detected (Fig. 6). It is not yet clear if smaller crystals form the fat crystal network.

Freeze-stable O/W emulsion. The effect of the suppression of the visible fat crystal network was examined in the soybean oil with 1% THL-15 as the oil phase. Stored at −10°C,

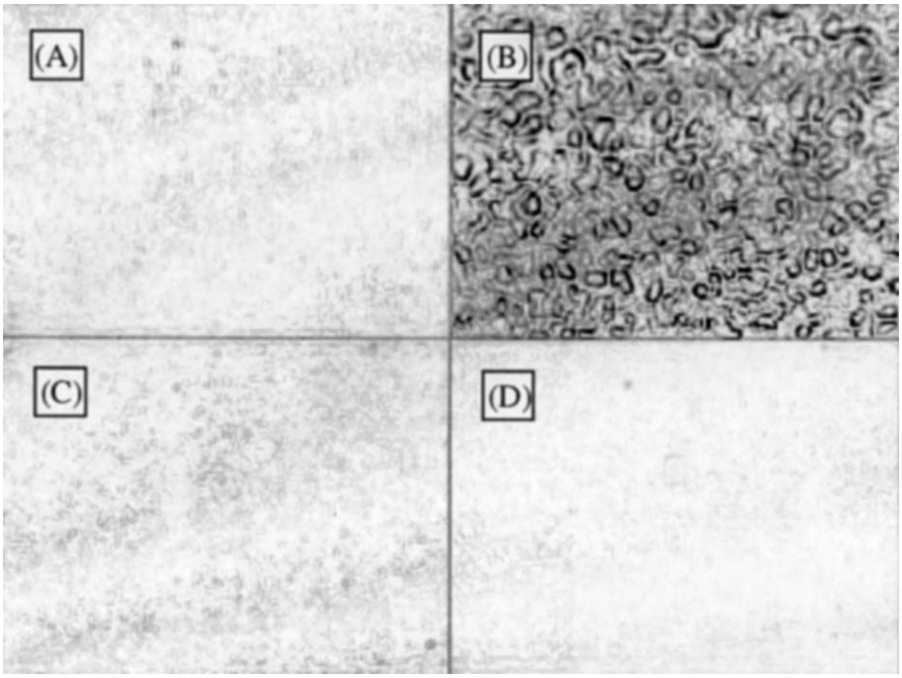

FIG. 6. Effect of the addition of THL-15 (polyglycerol fatty acid ester; Sakamoto Yakuhin Kogyo Co. Ltd., Osaka, Japan) on the crystal structure of soybean oil stored at −10°C. (A): 0 h, (B): 24 h, (C): 0 h with 1% THL-15, and (D): 24 h with 1% THL-15.

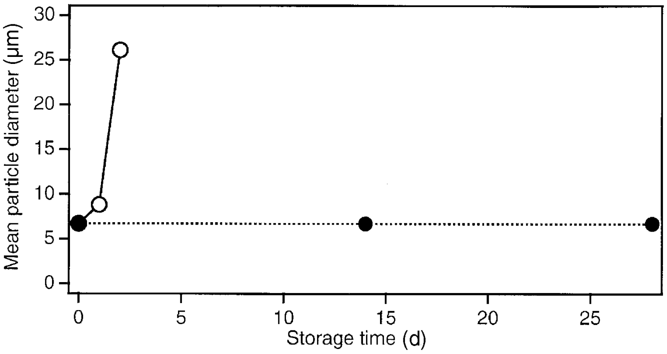

FIG. 7. Effect of the addition of crystal structure modifier THL-15 on the stability of soybean oil emulsion stored at −10°C. Soybean oil (○) and soybean oil with 1% THL-15 (●). For manufacturer see Figure 6.

the emulsion with 1% THL-15 was more than 10 times more stable than the control (Fig. 7).

REFERENCES

1. Boekel, M.A.J.S.V., and P. Walstra, Stability of Oil-in-Water Emulsions with Crystals in the Disperse Phase, *Colloids Surf. 3*:109–118 (1981).
2. Boode, K., C. Bisperink, and P. Walstra, Destabilization of O/W Emulsions Containing Fat Crystals by Temperature Cycling, *Ibid. 61*:55–74 (1991).
3. Kawamura, K., The DSC Thermal Analysis of Crystallization Behavior in Palm Oil, *J. Am. Oil Chem. Soc. 56*:753–758 (1979).
4. Kawamura, K., The DSC Thermal Analysis of Crystallization Behavior in Palm Oil, II, *Ibid. 57*:48–52 (1980).
5. Garti, N., E. Wellner, and S. Sarig, Effect of Surfactants on Crystal Structure Modification of Stearic Acid, *J. Crystal Growth 57*:577–584 (1982).
6. Garti, N., J. Schlichter, and S. Sarig, Effect of Food Emulsifiers on Polymorphic Transitions of Cocoa Butter, *J. Am. Oil Chem. Soc. 63*:230–236 (1986).
7. Garti, N., and K. Sato, Effects of Surfactants on Transition Kinetics of Stearic Acid Polymorphs, *Ibid. 63*:236–239 (1986).
8. Johansson, D., and B. Bergenstahl, Sintering of Fat Crystal Networks in Oil During Post-Crystallization Processes, *Ibid. 72*:911–919 (1995).
9. Guth, O.J., J. Aronhime, and N. Garti, Polymorphic Transitions of Mixed Triglycerides, SOS, in the Presence of Sorbitan Monostearate, *Ibid. 66*:1606–1613 (1989).
10. Zhao, J., and D.S. Reid, Thermal Studies on the Crystallization Kinetics of Triglycerides and Milkfat by DSC, *Thermochim. Acta 246*:405–416 (1994).

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