Polymorphic Transitions of Mixed Triglycerides, SOS, in the Presence of Sorbitan Monostearate

Olga Jacome Guth[a], **Judith Aronhime**[b], and **Nissim Garti**[b]*

[a]Mexico City, Mexico, and [b]Casali Institute of Applied Chemistry, School of Applied Science and Technology, Hebrew University of Jerusalem, Israel.

The polymorphic behavior of 1,3-distearoyl-2-oleo glycerol (SOS) has been investigated in the presence of a selected food emulsifier, sorbitan monostearate. Five polymorphs, named α, γ, pseudo-β', β_2, and β_1 were crystallized and identified by both x-rays and DSC. Each polymorph was treated using two selected protocols—the "screen cycle" and the "aging cycle." It has been demonstrated that sorbitan monostearate, when present in the molten fat, will significantly retard the α to γ transition during both the screen cycle and the aging cycle. A retardation effect was not detected for the γ to pseudo-β' transition (during the screen or the aging cycle). The emulsifier that is inserted into the fat behaves as a conventional impurity, causing significant reduction in the melting temperatures of each polymorph. As a result, the fat tends to melt prior to its transition. The behavior of SOS in the presence of the sorbitan monostearate is therefore different than that of SSS (tristearin), and may explain, in part, the absence of the strong blooming transition to high melting polymorph that occurs when the fat is rich in SOS. This behavior can also shed some light on the behavior of cocoa butter in the presence of sorbitan esters.

INTRODUCTION

The polymorphic behavior of mono-saturated acid triglycerides is well understood (1-9). The polymorphism of triglycerides with unsaturated acyl chains is less clear, and the literature involves many contradictions. This is manifested even in the most simple and representative mixed acid triglyceride, SOS (symmetric 1,3-di-stearoyl-2-oleoyl glycerol). Daubert and Clarke (10) reported three forms, and Lutton (11) proposed three forms with a triple length structure in SOS. Malkin and Wilson found four forms (12,13). Gibon et al. (14) reported six polymorphs. There is much confusion related to the nomenclature of these forms, as well as with their identification. Wang has recently studied the polymorphism of a series of sat-O-sat triglycerides and detected a new β_1 form (15). Sato (16-18) was the first to describe the polymorphism of SOS using a 99.9% pure sample. The x-ray and DSC studies helped to identify the various polymorphs and to observe the polymorph transitions, melt crystallization, and solvent crystallization.

Larsson's (19) and Hernqvist's (20) methods have been commonly employed (based on subcell packing) for the nomenclature of the polymorphs. α, having the hexagonal packing (H), β', the orthorhombic perpendicular packing ($O\perp$), and β, the triclinic, paralled ($T_{//}$). Therefore, β'_1 and β'_2 are modifications having the same subcell structure (subscript 1 represents the more stable form). Sato's nomenclature also employs the packing concepts, but adds the structural characteristics to it. Therefore, new polymorphs nomenclature such as γ and pseudo-β' are

introduced. We have decided to use Sato's concept in this study.

Cocoa butter (CB) is the most important natural confectionary fat with multiple polymorphs (21-28). CB consists of 17% POP and 26% SOS. Six polymorphs have been identified. some of them have similar XRD short spacing spectra to the SOS polymorphs. α and pseudo-β' forms in SOS correspond to those of Forms II and IV of cocoa butter. The short spacing of β_2 and β_1 in SOS are almost identical to those of Forms V and VI in cocoa butter.

Two models for the mechanism of bloom in CB have been suggested. The first concerns polymorphic transformation from the metastable form to the most stable form VI (24-28). Another one assumes self-fractionation of the fat components in high-melting fat, from those involving lower-melting fat fraction in cocoa butter (29). Sato (30) has demonstrated that in experiments carried out on mixtures of triglycerides that are similar in composition to cocoa butter, β_1-form, which corresponds to Form VI of CB, was obtained from β_2, which corresponds to Form V via the solid-state polymorphic transformation. Therefore, Sato concludes that the fat bloom of CB is essentially caused by the polymorphic transformation from Form V to Form VI.

If we assume that such a mechanism is feasible, it is important to study the effect of several food emulsifiers, known to be crystal structure modifiers and polymorphic retarding agents, on each of constituents of CB, POP, SOS, POS, and to compare their effect to the one obtained with saturated monoacid triglycerides (SSS and PPP), as well as with CB itself.

Several selected food emulsifiers, such as 1,2 and 1,3-diglyceride stearate and sorbitan esters (sorbitan mono and tristearate) have been shown to retard polymorphic transitions of saturated monoacid triglycerides (SSS) and CB (31,32). Sorbitan monostearate is incorporated into the molten fat and cocrystallizes with the less stable polymorph because of its good chemical structural affinity with the fat. Its existence in the fat helps to retard the polymorphic transitions into the more stable form.

Therefore, the aim of this work is to confirm Sato's findings on the characterization of the various SOS polymorphs and to study the effect of sorbitan monostearate on the rate of transformation of each of the polymorphs. Such a study will allow us to comprehend the SOS behavior to SSS and to CB, and will also help to clarify the bloom phenomenon in CB. Special effort was required in this study to isolate and characterize each of the five polymorphs of SOS—α, γ, pseudo-β', β_2 andβ_1—by careful cooling profiles under a very controlled scheme.

The aging cycle—aging at a temperature just below the melting of each obtained polymorph—is one of the techniques used in this study to evaluate the effect of the emulsifier. A special focus was put on the $\alpha \to \gamma \to$ pseudo-β' transitions, which corresponded to the Form II to Form IV transitions in CB. In addition, the possible transition of β_2 to β_1 is very interesting since it corresponds to the

transformation of Form V to Form VI in CB, which is believed to be the main cause for the blooming.

EXPERIMENTAL

Materials. 1,3-distearoyl, 2-oleo glycerol (SOS), was purchased from Larodan Fine Chemicals and was 99% pure. The emulsifier tested was sorbitan monostearate (Span 60 or SMS) from Grindsted Products, Denmark. The emulsifier was added at the level of 5 wt% (31,32). The sample was blended in the molten state in order to obtain a homogeneous mixture.

Methods. The thermal measurements were performed on the Mettler Differential Scanning Calorimeter TA 3000, calibrated for temperature readings and calorimetric accuracy with zinc and indium. The weighed samples were sealed in an aluminum pan; a similar empty pan served as reference. The thermal profile of each experiment is given within the figure or the text as presented in the results. The fusion enthalpies (ΔH_1) were automatically computed by the instrument.

RESULTS

A sample of pure SOS from a bottle was aged at room temperature (20°C) for several months and analyzed by DSC and X-rays. The recorded melting points indicated that the γ-form (mp 35.4°C and pseudo β'-form [mp 36.3°C]) were present (Fig. 1).

Transition by the "screen process" (α→γ). The pure α-form was obtained according to Sato's method by fast cooling (50 C/min) of the melt to 10°C and aged at this temperature for a few minutes. Confirmation of the existence of the pure α-form was obtained from the x-ray analysis (single peak at 4.2Å and sharp melt at 23.6°C [heating cycle of 5°C/min]), which was found to be in agreement with Sato's results (16-18). The sample was reheated according to the heating scheme presented in Figure 2. It can be clearly seen that the heating rate affects the transformation rate of the α-form. At a low heating rate (Fig. 2a, 1°C/min) the α-form transforms completely to γ and pseudo-β'-forms (35.0 and 36.0°C, respectively). At faster heating rates the α-form did not transform completely, and therefore both the melting of α (26.6°C) and the crystallization of γ can be detected along with the melting of

the γ and pseudo-β'-forms (Fig. 2b). At relatively high heating rates (10°C/min, Fig. 2d), the α-form only partially melts (24.4°C), and the formation of γ-form can be hardly detected. The melting points of γ and pseudo-β' are fused and occur together (Figs. 2c and 2d).

A sample of SOS was mixed together with 5 wt % of sorbitan monostearate and has been subjected to the same thermal treatment. The effect of the food emulsifier is clearly detected (Fig. 2). Even at slow heating rates the pure α-form does not completely transform into the γ-form and both the melting of α and the formation of γ can be clearly detected. Further heating reveals the melting process of γ and pseudo-β' (34.0 and 35.2°C, Fig. 2c). At high heating rates one can easily observe the retardation effect of the sorbitan ester (Fig. 2d). Comparison of the behavior of the α-form at 10°C/min both in the presence and absence of the emulsifier, shows that while in the absence of the emulsifier the mixture of γ and pseudo-β' is detected, and with the addition of SMS, only the α-form is present in the mixture and it did not transform to the higher forms.

The SMS, during the heating process (the "screen process"), retards the transformation of α-form into the γ-form. The α-form corresponds to what is commonly detected in triglycerides, being unstable polymorph characterized by a single short spacing around 4.2Å that can be easily transformed to the γ or pseudo-β'-forms. The effect of the heating rate and the emulsifier on the ΔH is summarized in Table 1. The Table shows that the melting points of the α-form are higher by a fraction of a degree in each heating rate in the presence of the emulsifier, in comparison to the melting in its absence. Similar increases in the melting points applies for the melting (T_m) of the γ-form or the pseudo-β'-form. The recorded peaks are also always broader in the presence of the impurity than in its absence. The ΔH values that were measured in the presence of the surfactant indicated the formation, as expected, of lower amounts of the high melting form. The emulsifier is supposed to interact with the molecule in the lattice and retard the conformational disordering in the portion of the aliphatic chain.

Transition by the "aging process." Solid-solid transition of α to γ-form was achieved when the α-form was heated to 22°C and aged for 10 or 20 minutes at this temperature (Sato aged the α-form to obtain the γ-form
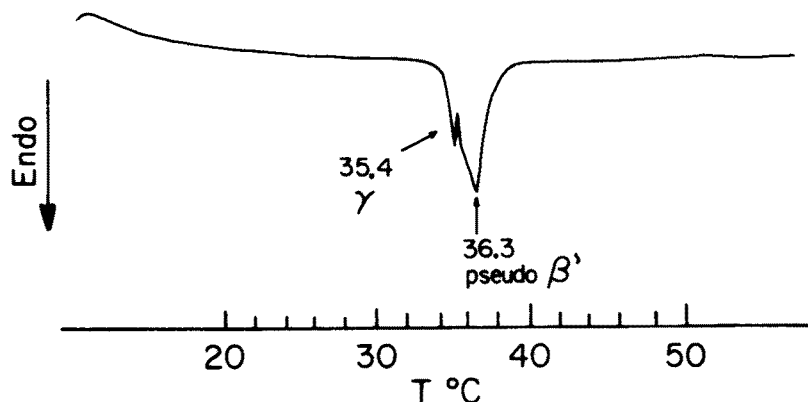

FIG. 1. DSC curves of the thermal behavior of 99% pure SOS from bottle aged for a few months at room temperature. ΔH_m at 35.4°C (γ-form) and 36.3°C (pseudo β').

TABLE 1

Effect of the Heating Rate and SMS Addition on the T_m and ΔHf of SOS

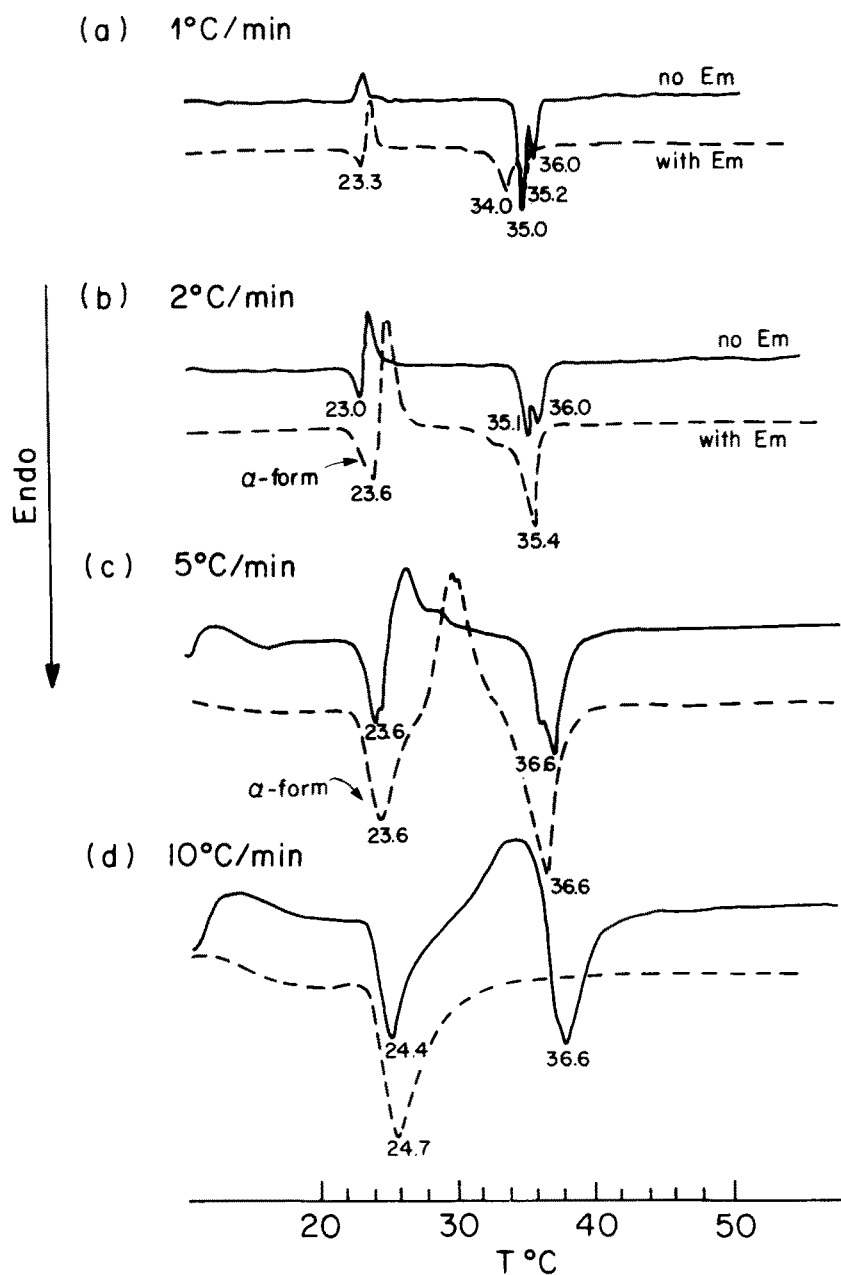| Heating rate fat + SMS	1 (°C/min)		2(°C/min)		5(°C/min)		10(°C/min)	
	SOS	SOS + SMS	SOS	SOS + SMS	SOS	SOS + SMS	SOS	SOS + SMS
Tm	—	23.3	23.0	23.6	23.6	23.9	24.4	24.7
ΔH (J/g)	—	15.12	26.05	51.05	58.42	70.10	72.12	74.13
Tm (°C)	35.0	34.0	35.2	35.4	36.6	35.8	36.6	—
	36.0	35.2						
ΔH (J/g)	115.0	105.9	126.09	97.32	118.47	106.12	90.69	—

FIG. 2. Thermal behavior of pure α-form (obtained from melt by fast cooling to 10°C and aged for 5 min at this temperature) when heated at 1, 2, 5, and 10°C/min, in absence (———) and presence of 5 wt% sorbitan monostearate (- - - -); a) 1°C/min; b) 2°C/min; c) 5°C/min; and d) 10°C/min.
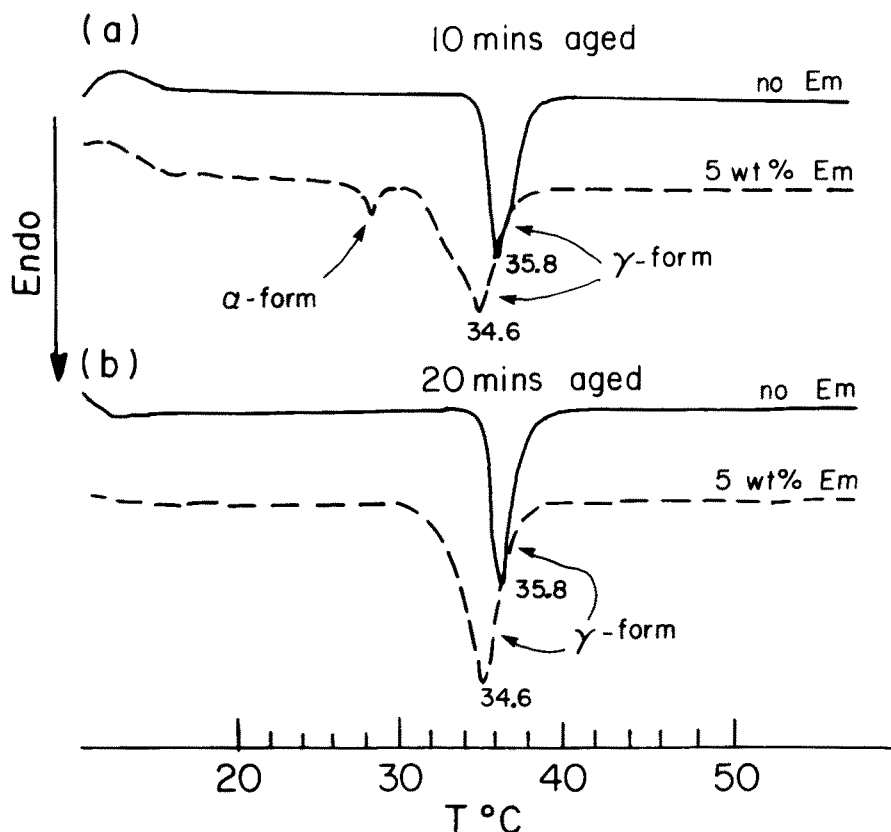
FIG. 3. Thermal behavior of pure α-form after aging at 22°C for a) 10 min and b) 20 min in the absence (———) and presence (- - - -) of 5 wt% sorbitan monostearate (see text for details). The heating rate is 5°C/min for each sample.

at 17°C for 12 hr). This process was repeated with and without the presence of SMS for two aging times (Figs. 3a and 3b). After 10 min of aging in the absence of emulsifier, one can detect the pure γ-form alone (at 35.8°C), and a typical x-ray pattern. After the same aging time at 22°C, in the presence of 5 wt% of SMS, part of the α-form is still present, indicating only a partial transformation into γ; (Fig. 3a), suggesting a minor retardation effect. It should be noted that the retarding effect of the emulsifier was practically not detected after 20 min of aging (Fig. 3b).

Those findings suggest that at temperatures close to the α-melting, α to γ transformation is rather fast and occurs within less than 10 min. The surfactant will be only slightly helpful in retarding such a transformation. After more prolonged periods of aging (20 min) the kinetic retarding effect of the surfactant is very minor, and practically all the α-form was transformed into the γ-form.

Gamma to psuedo-β' transition ($\gamma \rightarrow$ pseudo-β') "Transition by the 'aging process'". The pseudo-β' form melts at only 0.9°C higher than the γ form, and therefore, the two melting peaks tend to fuse. Attempts to obtain pure pseudo-β' from γ-form were made by Sato (1 hr aging at 30°C). Our approach was to follow the heating scheme as described in Figure 4. The obtained sample was heated to 34.8°C (1°C below the melting of γ) and aged for 20, 80, 250, 900 and 1000 minutes. The existence or absence of the transformation was again followed by heating the sample to melt (60°C) at 5°C/min in the DSC. After a short aging (10-80 min), two melting points were detected at

35.3 and 36.8°C, corresponding to γ- and pseudo-β'. This indicates that not all of the γ-form was transformed to the pseudo-β'-form. The more prolonged aging time did not yield pure pseudo-β', but rather a mixture of γ- with the pseudo-β' and β_2-forms (34.9, 36.7, and 40.4°C, Fig. 4b). We were unable to obtain the pure pseudo-β'-form in this technique since it tends to partially transform, at this temperature, into the β_2-form. The rate of transformation of pseudo-β'-form to β_2 is faster than that of γ- to pseudo-β' at this temperature which indicates that the conformational transformation is easier for the more dense pseudo-β'-form. Sato gives the name of pseudo-β' to this form because the short spacing spectrum are similar to the patterns of β' of SSS. In the presence of SMS only one melting peak has been observed for any aging time (20 and 1000 min, Fig. 4d and 4c). The peak has been identified as the melting of pseudo-β', which indicates the surprising acceleration effect that the emulsifier has on this transformation. While in the absence of an emulsifier, even after 1000 min we could not account for the transformation of most γ-form into pseudo-β'-form, and the presence of 5 wt% emulsifier induced the full transformation of γ-form into pseudo-β' within 20 min. On the other hand, no further transformation of pseudo-β' to β_2-form was detected in the presence of the emulsifier in contradiction to the result observed, on this transformation, in the absence of the additive. The surprising, unexpected effect on the transformation rate will be further discussed in view of the behavior of other
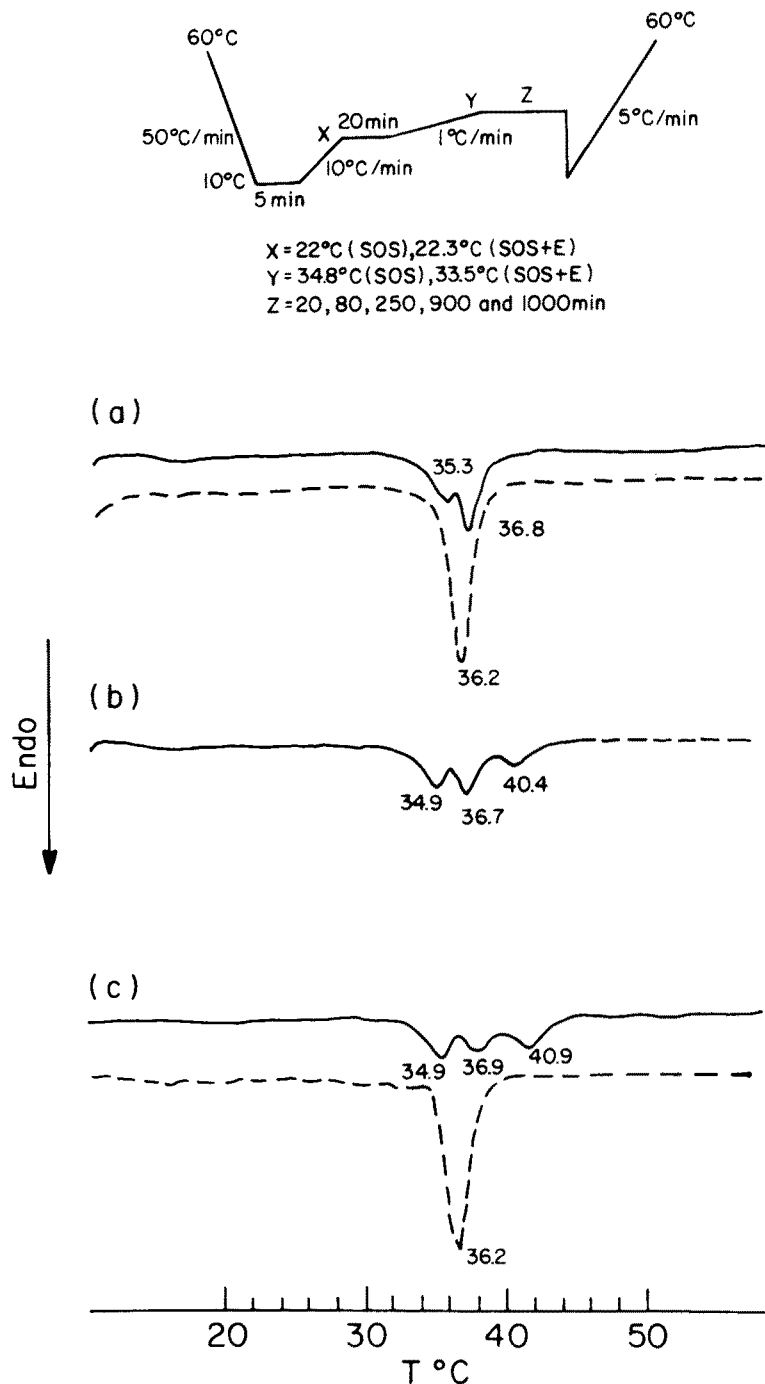
X = 22°C (SOS),22.3°C (SOS+E)
Y = 34.8°C(SOS),33.5°C (SOS+E)
Z = 20,80,250,900 and 1000min

FIG. 4. Thermal behavior of the γ-form (obtained after heating α, see scheme) aged at 34.8°C for a) 20 or 80, b) 250, c) 1000 min in the absence (———) and presence (- - - -) of 5 wt% emulsifier.

polymorphic forms of saturated triglycerides. It should also be noted that the presence of the emulsifier—as in impurity in the crystal lattice of the γ-form—will reduce its melting point slightly. In contrast to its effect in the α-form transition, a melting point of 36.2°C was recorded for pseudo-β'-form, in comparison to the expected 36.5-37.1°C (Figs. 4a and 4c). The x-ray powder diffraction of the pseudo- β'-form was in agreement with Sato's findings.

"*Transition by screening.*" An attempt was made to obtain the pure pseudo-β'-form from the γ-form by gradual heating of pure γ-form to 35.8°C (1°C below the melt of pseudo-β') according to the thermal profile shown in Figure 5. The γ-form was totally transformed into the pseudo- β'-form as expected. A melting of 37.1°C was recorded for this form (which is about 0.6°C above Sato's report and 0.3°C above our recording for aged samples). This comparison can be seen in Figures 4b and 5a.
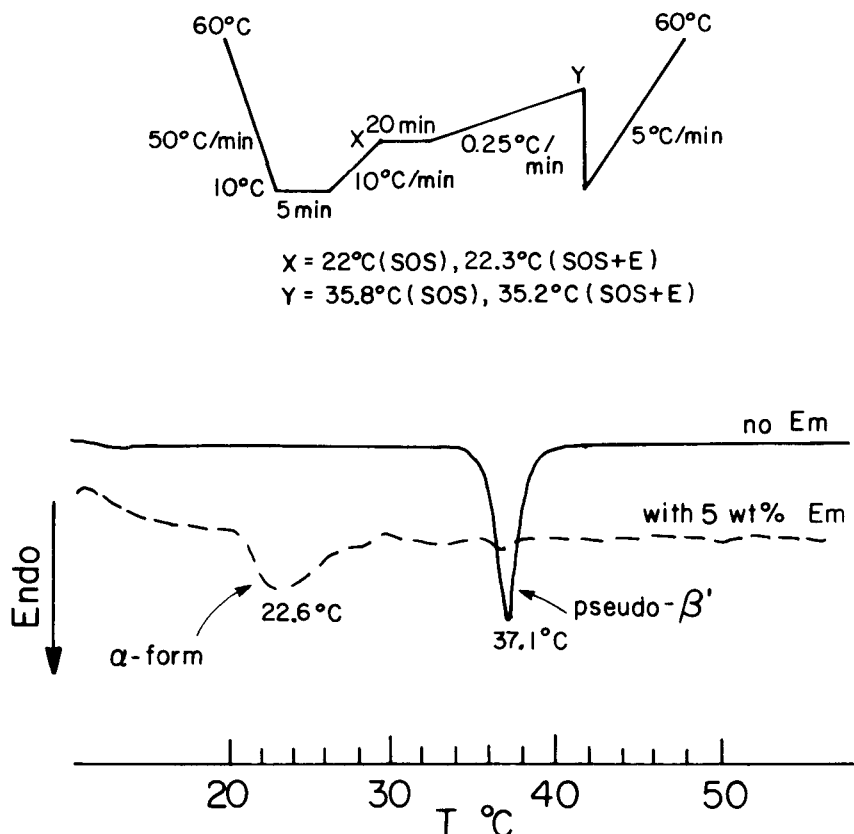
FIG. 5. Thermal behavior of γ-form during a heating cycle with (- - - -) and without (———) the presence of an emulsifier.

Surprisingly, the melting of γ or of pseudo-β'- form could not be detected in the presence of SMS (Fig. 5), and only one peak at 22.6°C was recorded corresponding to the melt of α. This strange behavior was unexpected, but can be explained. The γ-form has been transformed into the pseudo- β'-form, but since its melting is not 1-2°C lower than expected (due to the presence of the impurity), the aging process caused it to melt and re-solidify in the α-form during the cooling sequence. Therefore, no pseudo-β' or γ-forms were detected. Once again, this indicates that the emulsifier speeds up the transition of the "medium melting forms", and that they are not easily detected.

Transition of pseudo-β' to β_2. Transition by the "aging process." The transformation of pseudo-β'-form to β_2 was performed by an aging program according to the complex heating-cooling profile shown in Figure 6. The obtained pseudo-β' was aged for 250-1000 min at 36.1°C for transformation to β_2-form. Figure 6a shows the formation of the β_2-form together with the existence of the pseudo-β'-form, which is still present after 250 min of aging. The solid-solid transformation is quite slow, and 1000 minutes are required to transform the whole pseudo-β'-form into β_2-form (Fig. 6b). Figure 6b shows a single melting endothermic peak at 41.7°C, characteristic to the β_2-form, which indicated that the pseudo-β'-form was transformed completely to the next β_2-form. This transformation was achieved as expected. Sato reports the formation of β_2 from pseudo-β' within 10 hr at 35°C.

The addition of SMS again caused a dramatic effect, since it lowered the melting point of the pseudo-β' (Fig. 6b), and therefore the melting process took place prior to the expected transformation into the β_2-form. The fact that the temperature was maintained at 35.2°C, which is rather high and too close to the melting of the pseudo-β'-form, could be the cause for such preferable melting over the solid-solid transformation. The only one peak detected was the melting of the resolidified α-form obtained via the melt mediated route.

The transition of β_2 to β_1. The transition of the β_2-form into β_1-form is presented in Figure 7.

The β_2-form was aged for 1000 minutes at 40.7°C, which is 1°C below the melting of β_1, and, as can be seen in Figure 7, pure β_1-form was obtained. This process was carried out in the absence of an emulsifier. The additon of the emulsifier was catastrophic, causing fast melt of the obtained form. Therefore, the study of the emulsifier behavior was not possible for this transition since any β_2-form obtained in the previous cycle in the presence of the emulsifier melted before it could be aged for the next β_2 β_1 transitions.

CONCLUSIONS

As expected, five polymorphs of SOS could be detected and characterized when molten SOS was cooled and heated under selected thermal protocol. This reconfirmed Sato's findings.
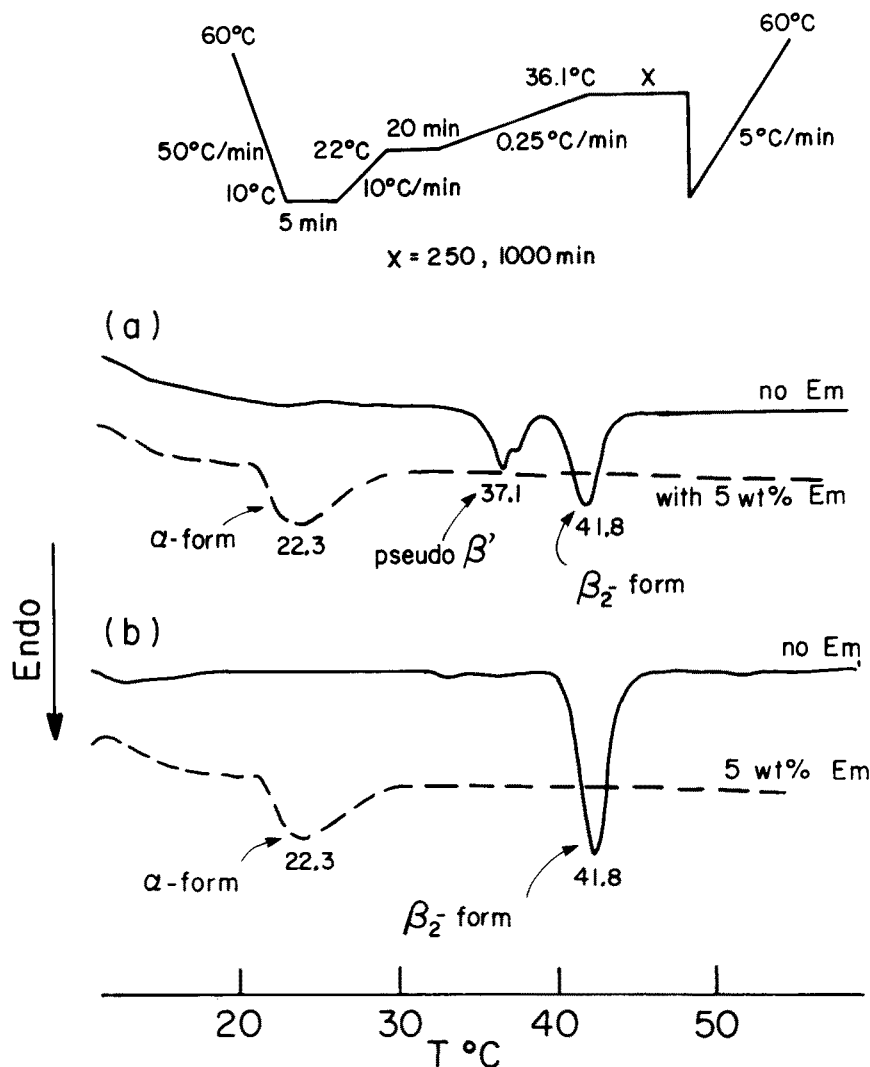
FIG. 6. Transition of pseudo β' to β_2-form by aging after a) 250 min and b) 100 min in the absence (———) and presence of the emulsifier (- - - -).
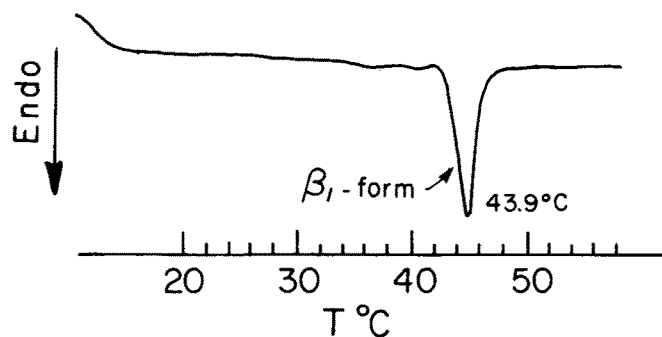

FIG. 7. Transition of the β_2 form into β_1 form by aging the sample at 40.7°C (1°C below the melting of β_2-form) for 1000 min without the emulsifiers (———).

Sorbitan monostearate has significantly retarded the transition of α to γ or pseudo-β' during the heating cycle and, to some extent, during the aging process as well. The fact that pseudo-β'-form is the most thermodynamically stable form explains these findings: The emulsifiers that have good "structure compatibility" with the fat tend to slow down the solid-solid transition as explained in our previous studies (27). Low melting SOS polymorphs will behave in a similar trend as SSS. The less stable modifications of SOS will transform to the more stable ones at a reduced rate in the presence of the emulsifier.

The transitions of the "high melting forms" are not retarded by the addition of the emulsifier, but accelerated instead. The melting points of any polymorph were reduced in its presence, at any aging or heating cycle, causing accelerated transitions, which were followed by "early melting" of each polymorph. The surfactant induced the conformational distortion of the lattice and caused faster and early melting. The emulsifier did not

behave as lattice structural impurity as it does probably during the transition of low into high melting forms of tristearin. It should be noted that the "low melting forms" of SOS behave similarly to the α-form of SSS and their transition is retarded by the food emulsifier. On the other hand, the "high melting forms" behave similarly to CB and their transformations are accelerated by "impurities" (25,27).

The study of POP in the presence of sorbitan esters, followed by a study of the behavior of mechanical blend of the most important constituents of CB is in progress.

REFERENCES

1. Small, D.M., in *Handbook of Lipid Research*, edited by D.J. Hanahan, Vol. 4, Plenum Press, NY, 1986, Chapter 10.
2. Malkin, T., *Progress in the Chemistry of Fats and Other Lipids*, Vol. 2, Academic Press, NY, 1954.
3. Hagemann, J.W., *Crystallization and Polymorphism of Fats and Fatty Acids*, edited by N. Garti and K. Sato, M. Dekker, NY, 1988, Chapter 2.
4. Hagemann, J.W., and J.A. Rothfus, *J. Am. Oil Chem. Soc. 60*:1123 (1983).
5. Chapman, D., *Chem. Rev. 62*:433 (1962).
6. Larsson, K., *Chemica Scripta 1*:21 (1971).
7. Lutton, E.S., and A.J. Fehl, *Lipids 5*:90 (1970).
8. Hoerr, C.W., *J. Am. Oil Chem. Soc. 37*:539 (1960).
9. Hernqvist, L., and K. Larsson, *Fette Seifen Anstrichm. 84*:349 (1982).
10. Daubert, B.F., and T.H. Clarke, *J. Am. Chem. Soc. 66*:690 (1944).
11. Lutton, E.S., *Ibid. 68*:676 (1946).
12. Malkin, T., and B.R. Wilson, *J. Chem. Soc. 369* (1949).
13. Lutton, E.S., and F.L. Jackson, *J. Am. Chem. Soc. 72*:3254 (1950).
14. Gibon, V., F. Durant, and C. Deroanne, *Ibid. 63*:1047 (1986).
15. Wang, Z.H., K. Sato, N. Sagi, T. Izumi, and H. Mori, *J. Japan Oil Chem. Soc. 36*:671 (1987).
16. Sato, K., and T. Kuroda, *J. Am. Oil Chem. Soc. 64*:124 (1987).
17. Sato, K., *Food Microstructure 6*:151 (1987).
18. Koyano, T., I. Hachiya, T. Arishima, K. Sato, and N. Sagi, *J. Am. Oil Chem. Soc., 66*:675 (1989).
19. Larsson, K., *Acta Chem. Scand. 20*:2255 (1966).
20. Hernqvist, L., B. Herslof, and M. Herslof, *Fette Seifen Anstrichm. 86*:393 (1984).
21. Willie, R.L., and E.S. Lutton, *J. Am. Oil Chem. Soc. 43*:491 (1966).
22. Chapman, G.M., E.E. Akehurst, and W.B. Wright, *Ibid. 48*:824 (1971).
23. Merken, G.V., and S.V. Vaeck, *Lebensm.-Wiss. U. Technol. 13*:314 (1980).
24. Garti, N., J. Schlichter, and S. Sarig, *Thermochimica Acta 85*:517 (1985).
25. Garti, N., J. Schlichter, and S. Sarig, *J. Am. Oil Chem. Soc. 63*:230 (1986).
26. Schlichter-Aronhime, J., S. Sarig, and N. Garti, *Ibid. 65*:1140 (1988).
27. Schlichter-Aronhime, J., S. Sarig, and N. Garti, *Ibid. 65*:1144 (1988).
28. Schlichter, J., and N. Garti, Solidification and Polymorphism in Cocoa Butter and the Blooming Problems. *Crystallization and Polymorphism of Fats and Fatty Acids*, edited by N. Garti, M. Dekker, NY, 1988, p. 263.
29. Manning, D.M., and P.S. Dimick, *Food Microstructure 4*:249 (1985).
30. Arishima, T., Ph.D. thesis, Hiroshima University, 1989.
31. Garti, N., in *Crystallization and Polymorphism of Fats and Fatty Acids*, edited by N. Garti, M. Dekker, NY, Chapter 7, pp. 267–305.
32. Garti, N., and K. Sato, *J. Am. Oil Chem. Soc. 63*:236 (1986).