Polymorphism of Stabilized and Nonstabilized Tristearin, Pure and in the Presence of Food Emulsifiers[1]

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ABSTRACT: The polymorphism of tristearin (SSS) was studied by means of differential scanning calorimetry and powder X-ray diffraction. The influence of 5% in weight of different food emulsifiers—i.e., 1-monostearin, sorbitan tristearate, and sugar monostearate—was also studied. Because polymorphism is sensitive to thermal treatment, two thermal conditionings were applied. According to the dynamic process (melting, quenching, and heating at 5°C/min), SSS showed three polymorphic forms: α, β', and β_1. The presence of the emulsifiers hindered the $\beta' \rightarrow \beta$ transformation, and a destructured β_2 form was recorded. According to the stabilization process (stabilization at 57°C for various periods of time), SSS showed two β' forms: β'_2 and β'_1. Three hours of stabilization were necessary to recover the whole triglyceride under the β form. The emulsifiers slowed down the polymorphic transition rates. Indeed, after six hours of stabilization, mixtures of β' and β were observed. Sugar monostearate seemed to have the most powerful effect on the transition kinetics because large amounts of α form were detected.

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KEY WORDS: DSC, dynamic process, food emulsifiers, polymorphism, powder X-ray diffraction, stabilized systems, tristearin.

Like other molecules with long hydrocarbon chains, triglycerides can crystallize in different crystal modifications, corresponding to a different packing of the hydrocarbon chains. This phenomenon is called polymorphism (1). Basically, triglycerides present three different polymorphic forms, denoted α, β', and β, according to nomenclature defined by Larsson (2). In some cases, a sub-α form is observed (3). The sequence sub-α, α, β', and β corresponds to an increasing order of thermal stability. The different polymorphic forms are characterized by their powder X-ray diffraction patterns. The short spacing values (15–30° 2θ) represent the distances between chains. The long spacing values (1–10° 2θ) correspond to the longitudinal packing and allow the observer to determine if the triglycerides crystallize in double- or in triple-chain packing.

Food emulsifiers are known to influence the crystallization rate of fats and oils. Therefore, they are often used to improve shelf-life stability of finished products. Indeed, several studies have shown that even little amounts of those amphiphilic molecules, added to fatty phases, influence the polymorphic transitions of triglycerides (4–10).

Different approaches can be used to determine the behavior of triglyceride/emulsifier systems. For example, nonstabilized systems can be investigated. This method provides useful information on the short-term behavior of fats, such as addition of emulsifiers to a triglyceridic lattice after mixing, melting, and cooling. This essential information allows the determination, for example, of the amount of free emulsifier (portion of emulsifier excluded from the triglyceridic lattice),which might interact with water and therefore stabilize an emulsion. On the other hand, depending on the triglyceride composition, the kinetics of phase transition can be slow. That is why it is useful to determine the long-term behavior of these systems and get an idea of the shelf-life stability of finished products. Let us mention the case of cocoa butter, where fat bloom is associated with the development of the most stable polymorphic form (11,12). This is achieved by tempering the different mixtures.

The aim of this work is to compare the polymorphism of pure tristearin (SSS) with that of SSS in the presence of 5% food emulsifiers, e.g., 1-monostearin (1-S), sorbitan tristearate (STS), and sugar monostearate (MES). Both types of thermal conditioning have been applied.

EXPERIMENTAL PROCEDURES

Triglyceride. SSS ($C_{18:0}$) was purchased from Sigma Chemical Co. (St Louis, MO). Its purity, checked by gas–liquid chromatography, was superior to 99%.

Emulsifiers. 1-S, STS, and MES were obtained from Rijken (Rijken Vitamin Co., Tokyo, Japan), Grindsted Products (Brabrand, Denmark), and Ryoto (Ryoto Co., Tokyo, Japan), respectively. The hydrophilic moiety is made of a molecule of glycerol, sorbitol, or sucrose, respectively. The lipophilic portion is mainly composed of palmitic and stearic acids (Table 1). The esterification positions are not known but are probably random.

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TABLE 1
Fatty Acid Composition of the Emulsifiers (wt%)[a]

	1-S	STS	MES
$C_{14:0}$	1.7	1.4	1.7
$C_{16:0}$	28.2	43.7	27.6
$C_{18:0}$	65.3	53.1	66.3
$C_{20:0}$	2.1	0.5	1.8
Others	2.7	1.3	2.6

[a] 1-S, 1-monostearin; STS, sorbitan tristearate; MES, sugar monostearate.

Thermal measurements. Determination of melting and transition points, as well as enthalpies of fusion (ΔH), were performed on a Perkin-Elmer DSC-7 (Norwalk, CT), assisted by an IBM PS/2 computer and coupled to a TAC 7/3 control instrument (Perkin-Elmer). An intracooler allowed measurements at temperatures lower than 0°C. Samples of 3–5 mg were sealed into aluminum pans; a similar empty pan served as reference.

Crystallographic measurements. Powder X-ray diffraction patterns were recorded on a Philips PW 1710 (Eindhoven, The Netherlands) diffractometer (Cu tube, $\lambda = 1.54178$ Å) composed of a camera, equipped with a thermostatization unit (HUBER HS-60, Offenburg-Elgersweier, Germany; TTK-ANTON PAAR, Graz, Austria), and a temperature control system (Pt 100 probe) connected to a heating device (20 to 300°C) and to a cooling unit, allowing a minimum temperature of –40°C. Gaseous nitrogen flow prevented condensation of water during measurements at low temperatures. The diffractometer was coupled to a Digital Micro-Vax II system (Digital Equipment Corporation, Maynard, MA) that allowed data treatment.

Thermal conditioning. The samples were conditioned according to different methods. Short-term behavior was pointed out after a dynamic process and long-term behavior according to two different stabilization processes. The dynamic process is schematically shown in Figure 1A. To allow homogenization of the mixtures (5% by wt) and to eliminate crystals that might influence recrystallization, the samples were first heated at 100°C for 5 min, then quenched at a cooling rate of 25°C/min and kept at –40°C for 5 min. The differential scanning calorimetry (DSC) curves were recorded at a scanning rate of 5°C/min. Both metastable and stable polymorphic forms were identified by this thermal process. As far as the stabilization process is concerned, two methods were developed. The first one is described in Figure 1B. After being kept for 5 min at 100°C, the mixtures were rapidly cooled at 57°C and held at that temperature during different periods of time. In this way, the mixtures stabilized at a temperature higher than the melting point of pure SSS α form [T_m (SSS_α) = 55.6°C]. Then, the temperature was quickly reduced to 45°C (25°C/min), and the DSC curves were recorded at a scanning rate of 5°C/min. The second method (Fig. 1C) consisted of melting the samples at 100°C for 5 min and then, by removing them from the oven of the DSC, keeping them at room temperature (around 20°C) for 5 min as well. Afterwards, the temperature of the oven was increased to 57°C, and the samples were put back in the oven and kept at that temperature during various periods of time. Before recording the DSC curves, the samples were cooled to 45°C (25°C/min) and kept at that temperature for 2 min.

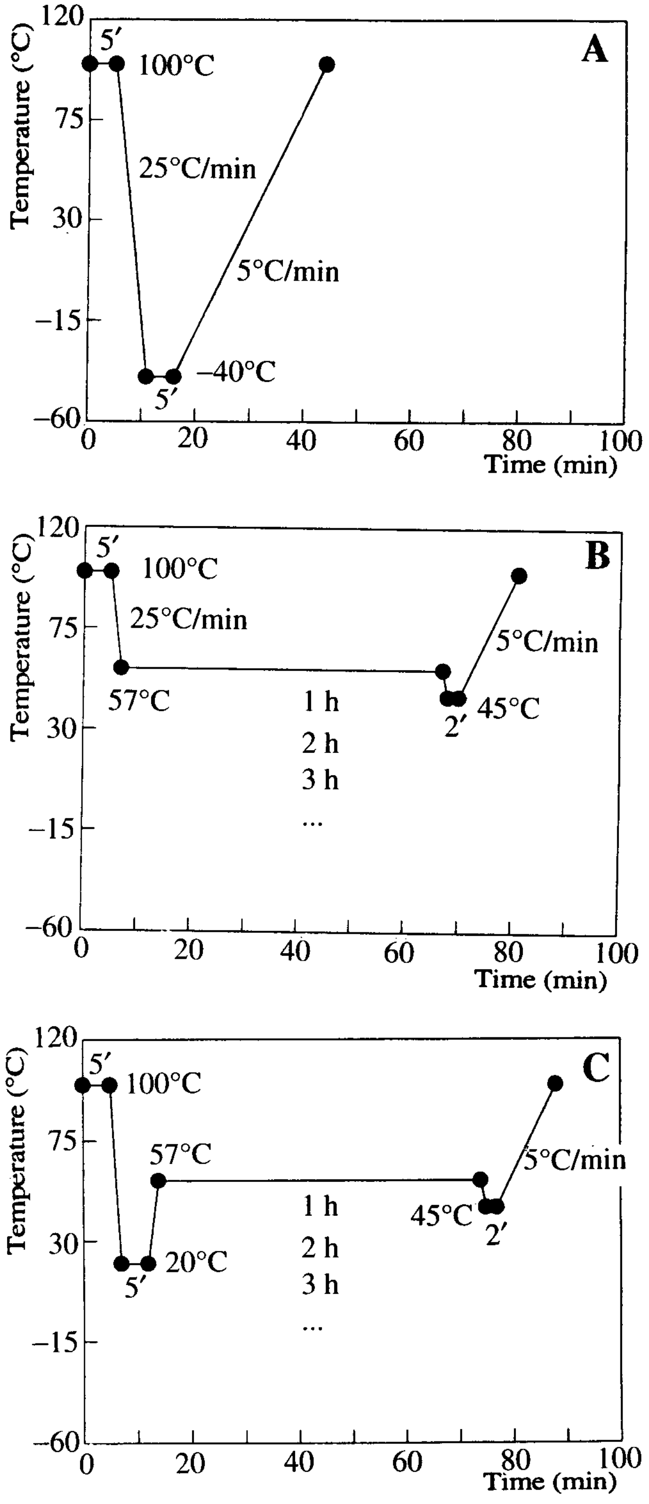

FIG. 1. Schematic representation of the thermal conditionings used in this study—dynamic process (A) and stabilization processes (B) and (C).

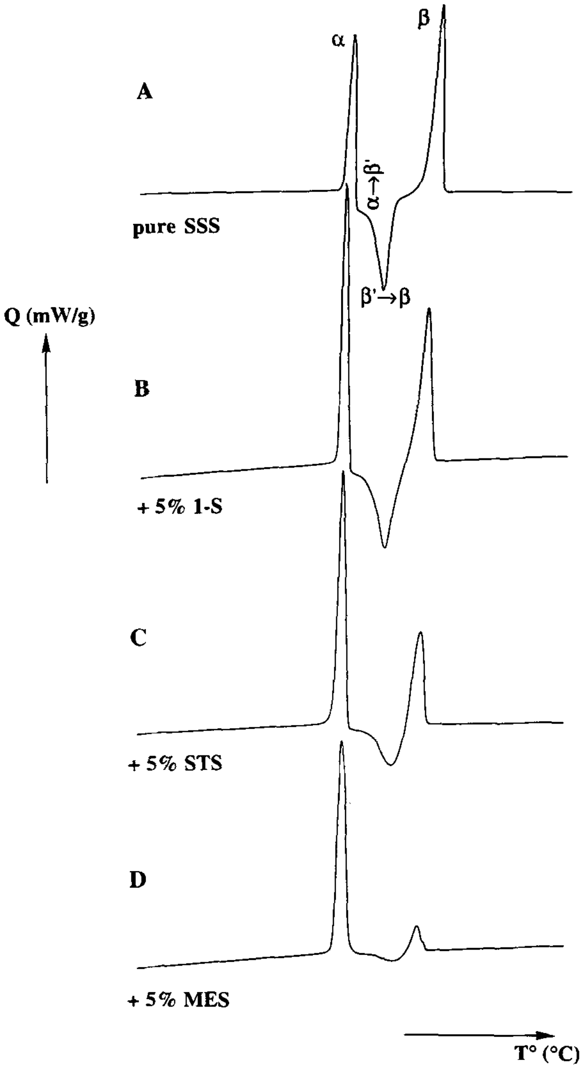

FIG. 2. Differential scanning calorimetry curves of pure tristearin (SSS) (A) and SSS in the presence of 5% of emulsifiers (B, C, D) recorded according to the dynamic process; 1-S, 1-monostearin; STS, sorbitan tristearate; MES, sugar monostearate.

TABLE 2
Melting and Transition Temperatures (°C) and ΔH of Fusion ($J.g^{-1}$ - esd: ± 5 $J.g^{-1}$) of SSS[a]

	SSS	SSS/1-S	SSS/STS	SSS/MES
Melting				
α	55.6	55.8	55.5	56.1
β	72.1 (β_1)	70.6 (β_2)	70.8 (β_2)	70.0 (β_2)
Transition				
α → β′	± 60	± 60	± 60	± 60
β′ → β	64.0	65.3	65.0	65.2
ΔH fusion (α)	113	119	120	110
ΔH fusion (β)	174	127	74	23

[a] Tristearin (SSS) both pure and in the presence of 5% of 1-S, STS, and MES (wt%) after melting, rapid cooling, and heating at constant rate (5°C/min); esd, estimated standard duration. Other abbreviations as in Table 1.

RESULTS AND DISCUSSION

Dynamic process. The DSC curve of pure SSS recorded under the dynamic process is shown in Figure 2A. After melting and rapid cooling, SSS crystallizes in the sub-α form. During heating at controlled rate (5°C/min), it successively evolves toward the α and β′ forms before melting in the β form. All those polymorphic forms crystallize in a double chainlength structure. A dramatic change in the DSC curve occurs when 5% of each emulsifier is added to the triglyceride (Fig. 2B, C, and D). The presence of the emulsifiers—1-S, STS, and MES—reveals a broadened exotherm for the β′ → β transition and a decrease of the endotherm representative of the β-form melting. The changes in melting points and heats of fusion of the β form of SSS indicate that 1-S, but especially STS and MES, modify the degree of organization of this form. On the other hand, as far as the α form is concerned, no modification seems to be induced by the presence of the emulsifiers. All thermal data are summarized in Table 2. As no splitting of the diffraction lines, corresponding to the long spacing values, is observed, one can assume that the emulsifiers are incorporated into the crystal lattice of SSS. Powder X-ray diffraction patterns that characterize the transversal packing of the hydrocarbon chains have been recorded. The influence of the emulsifiers is only visible at the level of the β′ → β transition where a less organized β form is obtained. Therefore, we differentiate a well-organized β_1 form—characterized by the three specific diffraction lines of the β form at 4.6, 3.9, and 3.7Å—from a less structured β_2 form, represented by two broad lines located around 4.6 and 3.8Å. The β_2 form is observed when the emulsifiers are added (Fig. 3).

Stabilization process. According to the first stabilization process (described in Fig. 1B), the DSC thermograms recorded for SSS after different stabilization periods of time are shown in Figure 4. After one hour at 57°C, SSS crystallizes as a mixture of α and β′ forms. The α form stems from the liquid portion that crystallizes during the cooling at 45°C. The β′ form corresponds to a $\beta'_2 + \beta'_1$ mixture, taking into account that a shoulder is observed on the β′ melting endotherm. It is well known that long hydrocarbon chain compounds present this phenomenon (13–15). After two hours at 57°C, most of the fat crystallizes in the β form. However, small amounts of the β'_2 and β'_1 forms are still present. After three hours at 57°C, SSS is completely crystallized in the β form. This form is thermodynamically the most stable, and no further crystalline evolution is possible. The same experiments were carried out on mixtures that contained 95% in weight of SSS and 5% emulsifier. DSC curves were recorded after each hour, up to six hours of stabilization (Fig. 5). Table 3 summarizes the changes in melting and transition temperatures and heats of fusion throughout conditioning for six hours. We can see from the DSC curves that each emulsifier influences the crystalline evolution of SSS and delays the establishment of the β form. Indeed, after six hours of stabilization, that is to say after a time twice as long as the time required to obtain total crystallization in the β form of pure SSS, each system contains a mixture of β′ and β crystals, and even a large amount of α phase as far as the SSS–MES mix-

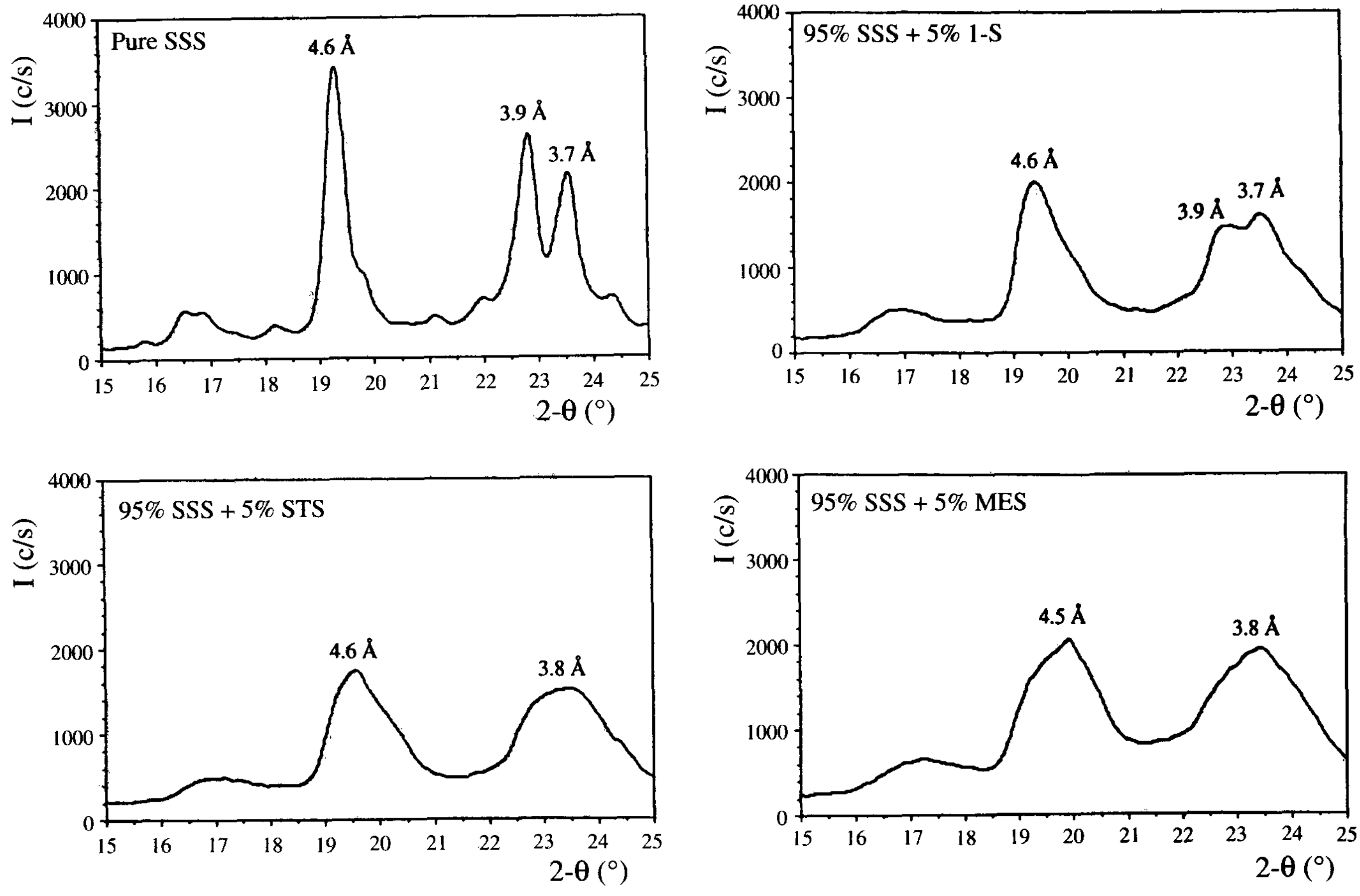

FIG. 3. X-ray diffraction patterns of the β form of SSS, pure and in the presence of 5% of emulsifiers. Abbreviations as in Figure 2.

ture is concerned. The emulsifier that seems to have the slightest influence on the polymorphism of SSS is 1-S. This emulsifier delays less the polymorphic transitions, and the same polymorphic forms as in pure SSS are observed. As far as the slowing down of transition kinetics is concerned, STS seems to induce an intermediate behavior. The SSS–STS mixture only exhibits one β′ form, as opposed to the pure triglyceride. The strongest effect is obtained in the presence of MES. After six hours of stabilization at 57°C, a very intense α melting peak is observed on the DSC curve, whereas the β′ and β melting endotherms are still weak, meaning that this emulsifier prevents—or at least strongly delays—the crystallization of the triglyceride at that temperature. In general, it appears that the emulsifier is never excluded from the triglyceridic lattice after six hours of stabilization at 57°C. Indeed, no endotherms corresponding to the emulsifier melting points were observed.

As shown in Figure 5, the influence of the emulsifiers on the polymorphic transitions of SSS seems to be correlated to the stabilizing effect that they have on the α form. From the ΔH of melting of the α form, we can see that 1-S has the least stabilizing effect and MES the strongest [1-S: $\Delta H_f(\alpha) = 30$ $J.g^{-1}$; STS: $\Delta H_f(\alpha) = 48$ $J.g^{-1}$; MES: $\Delta H_f(\alpha) = 118$ $J.g^{-1}$]. The appearance of two β′ forms is also correlated to the α form stability—the less stable α form (obtained in the presence of 5% of 1-S) gives rise to two β′ forms (β'_2 and β'_1), whereas in the presence of STS and MES, only one β′ form is detected on the DSC curves. On the other hand, we also observe that the more stable the α form is, the more the subsequent polymorphic transitions occur *via* the liquid state. Indeed, after six hours of stabilization at 57°C (Fig. 5), we can see that the β′ → β transition takes place in the solid state (presence of an exotherm of crystallization) in the presence of 1-S, this phenomenon disappears in the presence of STS and especially in the presence of MES; in this case, the transition *via* the liquid state is promoted.

This method is slow and time-consuming, and we have searched for another stabilization process, which would accelerate the polymorphic transitions. This second stabilization process is decribed in Figure 1C. To eliminate the α form coming from the portion of fat still liquid at 57°C after several hours of stabilization, the mixture is no longer cooled to 45°C; instead, we cool it rapidly to room temperature. Consequently, the fat directly crystallizes in the α form, and when the temperature is increased to 57°C, the α form directly transforms into the β′ form. Therefore, we only observe the β′ → β transformation. DSC curves have been recorded after 15 and 30 min, and 1, 2, and 3 h of stabilization. According to this method, only one hour is required to recover all SSS in the β form, and the mixtures containing 5% emulsifier are al-

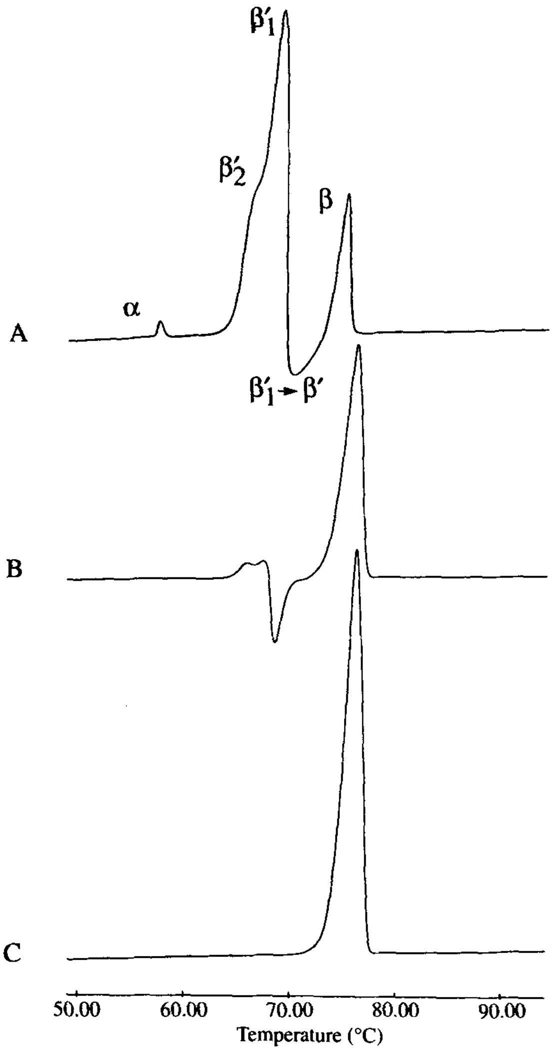

FIG. 4. Differential scanning calorimetry curves of tristearin recorded after (A) one hour, (B) two hours, and (C) three hours of stabilization at 57°C.

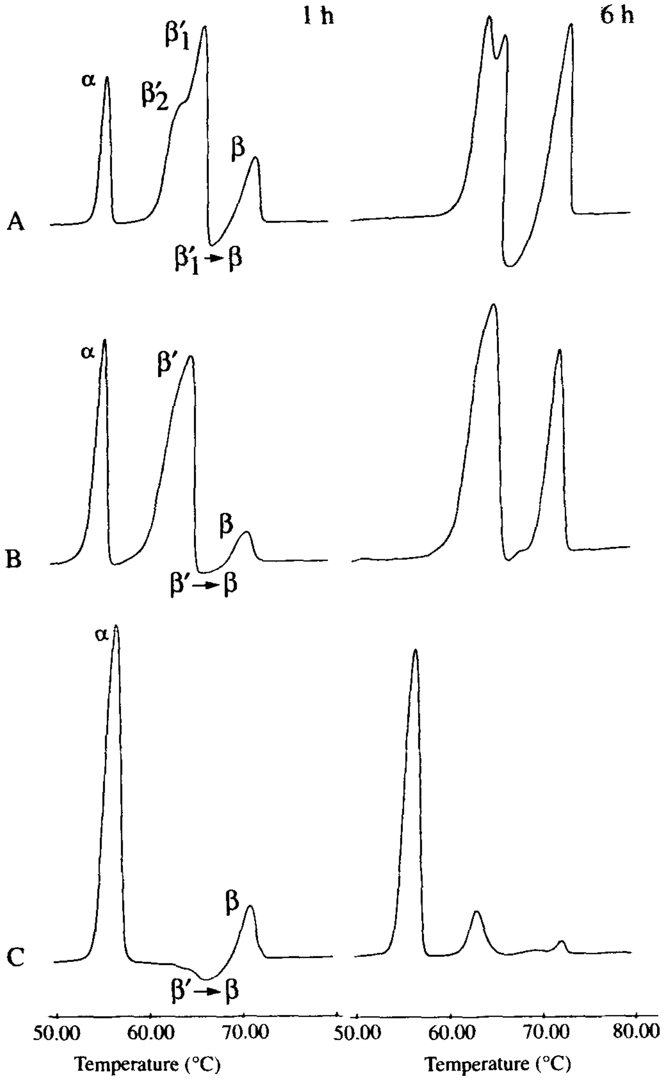

FIG. 5. Differential scanning calorimetry curves of the mixtures of SSS containing 5% of 1-S (A), STS (B), and MES (C) recorded after one and six hours of stabilization at 57°C. Abbreviations as in Figure 2.

most completely transformed into the β form after three hours. However, small traces of the β' form are detected in the presence of STS and MES. Even in this stabilization process, MES is the emulsifier that seems to influence in the most important way the polymorphic transitions of SSS, in particular α → β'. Indeed, after 15 min of stabilization, no α form is recorded on the DSC curves of the mixtures that contain 5% of 1-S or STS, but a strong endotherm representative of the α form is found in the presence of 5% MES. On the other hand, because of the increase of the transformation rate, it is no longer possible to detect two β' forms as before.

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Table 3
Melting and Transition Temperatures (°C) and DH of Fusion (J.g^{-1}) of SSS[a]

		1 h	2 h	3 h	4 h	5 h	6 h
SSS	Melting α	55.0	—	—			
	Melting β'_2	63.6	63.3	—			
	Melting β'_1	66.1	64.8	—			
	Melting β	72.6	73.7	73.9			
	Transition $\beta'_1 \rightarrow \beta$	67.9	66.0	—			
	ΔH fusion (α)	2	—	—			
	ΔH fusion (β')	136	22	—			
	ΔH fusion (β)	29	199	203			
95% SSS + 5% 1-S	Melting α	55.5	54.9	55.3	(55)	(55)	—
	Melting β'_2	63.6	63.0	63.0	63.0	63.3	63.4
	Melting β'_1	65.8	65.4	65.6	64.8	65.1	65.1
	Melting β	71.7	72.7	72.9	72.3	72.2	72.1
	Transition $\beta'_1 \rightarrow \beta$	67.3	67.0	67.2	66.9	67.0	67.0
	ΔH fusion (α)	30	12	5	—	—	—
	ΔH fusion (β')	113	96	106	114	133	130
	ΔH fusion (β)	21	88	85	77	74	75
95% SSS + 5% STS	Melting α	55.3	54.4	52.8	54.9	(51.5)	(51.2)
	Melting β'	64.6	64.8	65.0	65.0	64.9	64.9
	Melting β	71.2	70.1	71.8	71.8	71.8	72.0
	Transition $\beta' \rightarrow \beta$	66.5	67.0	66.7	67.0	66.8	66.7
	ΔH fusion (α)	48	12	2	0.5	—	—
	ΔH fusion (β')	97	148	153	143	135	—[b]
	ΔH fusion (β)	8	7	15	26	37	—[b]
95% SSS +5% MES	Melting α	56.2	56.0	55.8	56.1	56.0	55.9
	Melting β'	—	63.4	63.1	62.8	63.2	62.9
	Melting β	71.0	71.8	71.4	70.7 (β_2) 72.2 (β_1)	70.8 (β_2) 72.2 (β_1)	72.1
	Transition $\beta' \rightarrow \beta$	66.3	66.7	66.1	—	—	—
	ΔH fusion (α)	118	79	58	112	93	104
	ΔH fusion (β')	—	39	34	8	26	17
	ΔH fusion (β)	17	14	57	9	8	2

[a] SSS both pure and in the presence of 5% wt of the emulsifiers, recorded according to the first stabilization process. Abbreviations as in Tables 1 and 2.
[b] Data not available.

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