Dynamic Control of Polymorphic Transformation in Triglycerides by Surfactants: The Button Syndrome

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The kinetics of polymorphic transformations in monoacid saturated triglycerides and the influence caused by the presence of certain solid surfactants were investigated. Selected emulsifiers can be incorporated at the level of 10 wt% within the triglyceride, without changing the crystal lattice; on the other hand, their presence affects the heat capacity of the triglyceride and the NMR relaxation time T_1. Following the polymorphic transformation both during aging and during heating in the DSC, it was observed that both the mechanism and rate of transformation of the triglyceride strongly depend on the kinetic conditions and on its own chemical structure. In conjunction with these results it also was observed that the effect of the emulsifier is strongly dependent on the transformation conditions. The kinetic effect of the additive on the solid-solid transformation has been found to be strictly associated with its hydrophilic moiety structure; a model of molecular incorporation has been proposed which describes the arrangement of the surfactant molecules parallel to the triglyceride chains and formation of vacancies. The selectivity of the additive concerning its effect of dynamic controller of polymorphic transformations has been explained by its capacity to create hydrogen bonds with the neighboring triglycerides, which was called the "Button Syndrome." The wide range of different additives and triglycerides used supplied a better understanding of the factors which determine the polymorphic and crystallization behavior in triglycerides.

Crystallization and melting processes of lipids play an important role both in biological systems and industrial products. The transitions liquid ⇌ mesophase ⇌ solid are of primary importance for performance of biological membranes; in the same measure, the phase transitions often determine the quality of industrial products. The crystallization processes in fats are accompanied by the phenomenon of polymorphism, i.e. existence of more than one crystal packing in the same compounds. This occurrence is deleterious most of the time due to the undesired consequences in texture and organoleptic features. In the field of chocolate, margarine, ice cream and cake production it is well known that besides a proper thermal treatment, unwanted polymorphic transformations can be delayed by the addition of low percentages of certain surfactants to the fat.

The main phase transitions in monoacid saturated triglycerides are between the three polymorphs that derive from the three packings feasible in triglycerides: hexagonal (α), orthorhombic (β') and triclinic (β), with respective increase in the thermodynamic stability. A fourth polymorph, sub-α, has been reported in the literature to be obtained through an enantiotropic transformation at very low temperatures (1), not yet known to be relevant to the practical problems involved in polymorphism of plastic fats, e.g., confectionary fats. The three polymorphs constitute the basic picture for more complicated patterns obtained in natural fats. Natural fats are complex mixtures of mixed triglycerides containing considerable levels of unsaturated fatty acids; this feature implies a complicated polymorphic behavior which is beyond the aim of the discussion in this work. Monoacid saturated triglycerides may constitute a basic model for investigating the role of the emulsifier in polymorphic transformations. In spite of the information widely available in the literature concerning detection and characterization of the specific polymorphs (2-8), less emphasis has been placed on the mechanism of transformation. The purpose of the present work is to investigate the performance of the emulsifier as a dynamic controller of polymorphic transformations and to reach a possible comprehension of the phenomenon, which will serve as basic knowledge for more complex systems of fats.

MATERIALS AND METHODS

The different triglycerides were purchased from Sigma Chemical Co., St. Louis, Missouri, and were 99% pure. The additives were commercially available from Grindsted Products of Denmark and other sources. The additives tested were the solid surfactants: sorbitan monostearate (SMS), sorbitan tristearate (STS), glycerol-l-stearate (GMS), citric acid ester of glycerol-l-stearate (CGMS), triglycerol-l-stearate (3GlS), lactic acid ester of glycerol-l-stearate (LGMS), sorbitan monopalmitate (SMP). The liquid surfactants: sorbitan monolaurate (SML), ethoxylated SMS (Tw60), ethoxylated STS (Tw65), ethoxylated sorbitan monooleate (Tw80), sorbitan monooleate (SMO). The nonsurfactant additives: stearic acid, methyl stearate, octadecane, stearoyl alcohol.

The emulsifiers were added at the level of 10 wt%; each sample was blended in the molten state in order to obtain a homogeneous mixture. Thermal measurements were performed on a Mettler Differential Scanning Calorimeter (DSC TA3000), calibrated for temperature reading and calorimetric accuracy with zinc and indium. The weighed samples were sealed in an aluminum pan, while a similar empty pan served as reference.

The α-form solidified in the DSC was obtained by cooling it from 80 to 10 C at 50 C/min and holding the sample at the final temperature for five min. In the case of trilaurin the final temperature was modified to 0 C.

Samples aged at room temperature were prepared in the laboratory, using a hot water bath for melting the samples and an ice-water bath to chill them.

The extent of transformation from α to β form during aging was estimated by the X-ray diffraction powder method.

Effect of surfactants on polymorphic transformations. Most of the experiments were performed on tri-

stearin because stearic acid, together with palmitic and oleic, is the most common fatty acid found in nature.

We followed the polymorphic transformation in tristearin in two ways:

(i) During aging at room temperature. In this way the transformation takes place at a temperature below the melting point of α-form (solid-solid transformation).

(ii) During heating at constant heating rate. In this case the transformation of tristearin occurs through melting of α-form and recrystallization into the β-form.

The DSC technique is very sensitive to enthalpy changes that occur during phase transformations, and therefore supplies worthy information on physical state changes in triglycerides.

PREVIOUS KNOWLEDGE OF SURFACTANTS AS CONTROLLERS OF POLYMORPHIC TRANSFORMATIONS

Addition of sorbitan esters into fats was reported by N. Krog (9) to stabilize the orthorhombic form of fats and prevent the formation of the stable β form, probably due to steric hindrance. Other works reported on the same topic indicated that the presence of selected surfactants, like sorbitan esters or monoglycerides, stabilizes the unstable forms (10-13).

A systematic investigation of the emulsifier's effect on the crystallization processes of stearic acid has been performed recently; it involves kinetic considerations and polymorphic transformations (14-17). It was found that in addition to the nature of the solvent and kinetic conditions, the presence at low percentages of certain surfactants in the solution contributes to the determination of which polymorph solidifies. Moreover, the same additives cause retardation of the enantiotropic polymorphic transformation between forms C and B in stearic acid. In tristearin crystallized directly from the melt, prevention of the β crystallization, during heating in the DSC, has been observed when sorbitan monostearate was added; a similar effect was noted during aging of the unstable α form at room temperature. Further, it was also found that the extent of transformation inhibition is a function of emulsifier concentration, and as little as 2 wt% of sorbitan monostearate apparently decreases the quantity of β crystallization during heating in the DSC (18).

RESULTS AND DISCUSSION

Effect of heating rate on polymorphism. When the α-form of tristearin is heated in the DSC at a constant rate of 5 C/min, the typical thermogram is obtained (19), in which the two endotherms correspond to the fusion enthalpy of the α- and β-forms (ΔH_α and ΔH_β), respectively, while the exotherm corresponds to the transformation enthalpy which includes heat of crystallization of the β-form. The graphs in Figure 1 show the change in ΔH_α and ΔH_β as a function of heating rate. During fast heating the ΔH_β value decreases, indicating that β crystallization is not allowed. On the other hand, the ΔH_α value decreases when the heating rate becomes lower; this suggests that during transformation a larger portion of the fat transforms directly to β without liquefying.

Apparently the polymorphic transformation is constituted by two stages. One is the excitation or liquefaction of the α-form and the other is the crystallization into β. Low heating rate affects the former stage and determines the mechanism of transformation; high heating rate affects the extent of β crystallization. The next step is to clarify whether the emulsifier's effect also depends on heating rate.

Selection of emulsifiers. It was quickly discerned that the presence of any liquid surfactant enhances the polymorphic transformation of tristearin; among the solid surfactants, those with a hydrophilic head small enough to enable a minimal structural compatibility were chosen. The solid surfactants are amphiphilic molecules with long saturated hydrocarbon chains. The presence of the long chain, besides the hydrophilic head, permits the emulsifier to solidify in the temperature range of tristearin, to cocrystallize with the triglyceride and, as will be demonstrated, to interfere with polymorphic transformations.

Effect of emulsifier on polymorphism of tristearin. In order to see if the emulsifier's effect on the polymorphic transformation of tristearin is also dependent on

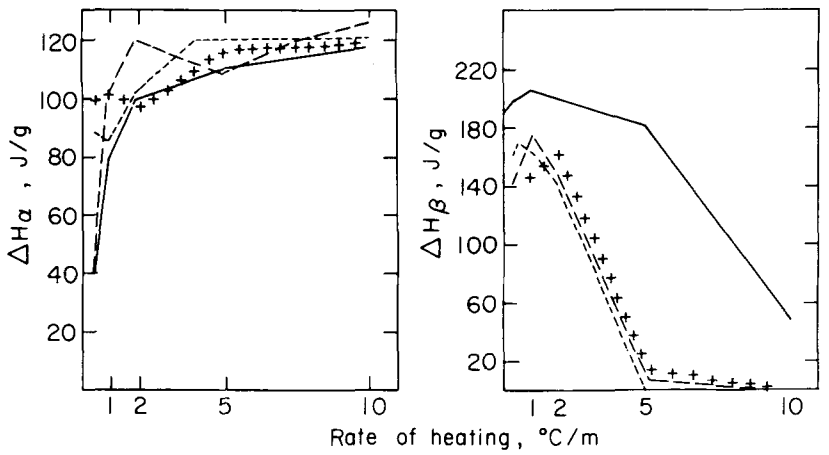

FIG. 1. ΔH_α and ΔH_β values of tristearin (_____) in the presence of SMS (- - - -), CGMS (***) and GMS (— — —).

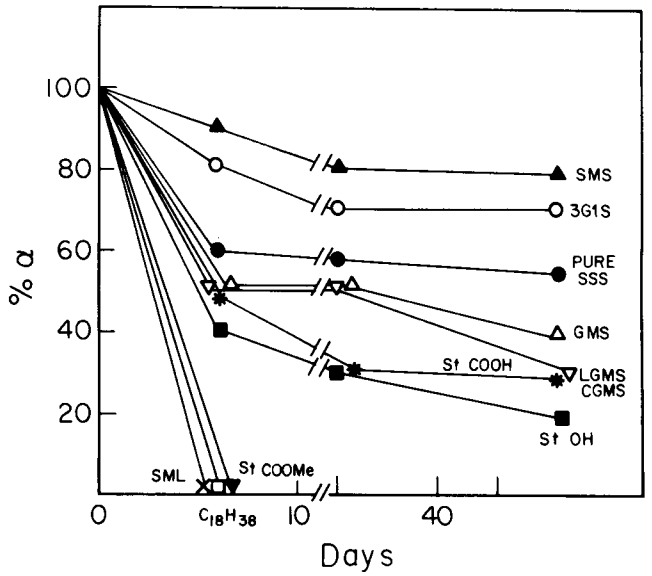

FIG. 2. Aging of tristearin at room temperature in the presence of additives 10 wt%. Neat tristearin (●), tristearin + SMS (▲), tristearin + 3GlS (○), tristearin + GMS (△), tristearin + LGMS (▽), tristearin + stear. ac. (*), tristearin + stearoyl alcohol (■), tristearin + methyl stearate (▼), tristearin + octadecane (□) and tristearin + SML (X).

heating rate, the experiment performed previously on the neat fat was carried out on tristearin after the addition of solid surfactants (Fig. 1). The ΔH_β values in the presence of the additive decrease to zero at 5 C/min, indicating that the β crystallization is completely suppressed by the presence of the emulsifier (19). On the other hand, at lower heating rates the inhibitory effect of the emulsifier is partially neutralized. This points out that the effect of the emulsifier is also dependent on the heating rate; in other words, as stated previously the effect is kinetic rather than thermodynamic and is related to the kinetic conditions of transformation. During aging, in fact, the stabilization of the α form is only temporary and the transformation into β is delayed but not inhibited.

As shown in Figure 1, the effect of all the solid surfactants tested on the α-β transformation is uniform. When the same additives were tested during aging of tristearin at room temperature, the effect of the emulsifiers was no longer uniform (Fig. 2). SMS and 3GlS delay the transformation, while CGMS and GMS enhance it. In the same figure it can be seen that in the presence of SML, which is a liquid emulsifier, the transformation is significantly accelerated. It is evident that the retardation of the polymorphic transformation by the additive through the solid state, besides being associated to the long saturated aliphatic chain, is also connected to the hydrophilic moiety structure. Among the solid surfactants, evidently SMS and 3GlS have in common a particular feature that enables them to stabilize the α form in tristearin.

In the results shown here, only additives which are surface active agents were taken into consideration; in order to test the ability of transformation control by other additives, not exactly surfactants, four additional compounds were investigated: stearic acid, the methyl ester of stearic acid, stearoyl alcohol and octadecane. During aging, these additives do not cause any delay in the transformation, like the solid surfactants shown below, but rather enhance it (Fig. 2). Methyl stearate and octadecane have a drastic effect parallel to that of SML, while stearic acid and stearoyl alcohol accelerate the transformation to a lesser extent, like GMS and CGMS.

The main conclusion drawn from these results is that the ability of the additive to retard the α-β transformation clearly does not depend on its surface activity performance; nevertheless, the behavior patterns are in some way associated with chemical structure.

During the cooling of the melt at the rate of 0.05 C/min, the β form has been obtained in pure tristearin.

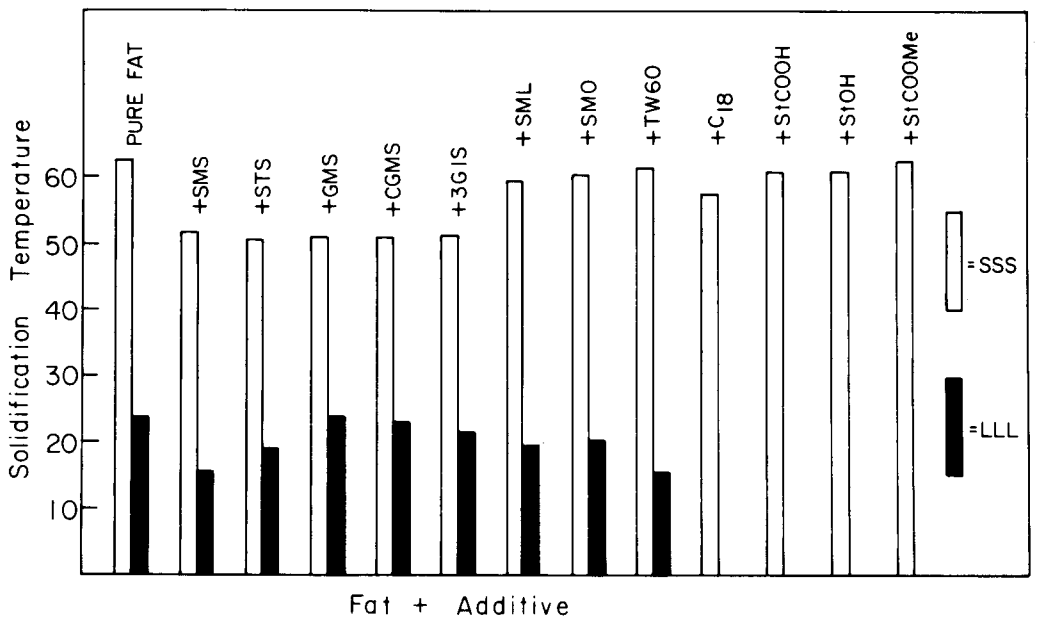

FIG. 3. Solidification temperatures of tristearin and trilaurin in the presence of different additives.

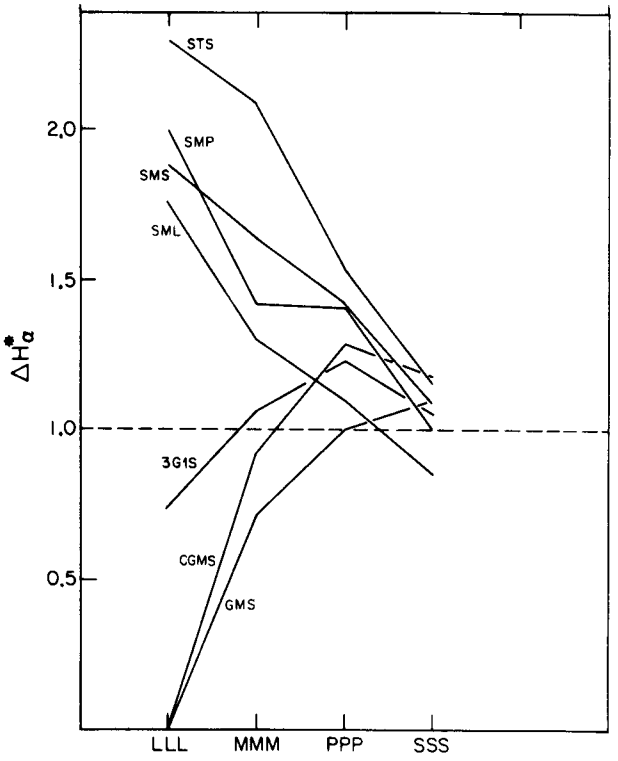

FIG. 4 . ΔH_α^* values of trilaurin (LLL), trimyristin (MMM), tripalmitin (PPP) and tristearin (SSS) in the presence of STS, SMP, SML, SMS, 3GlS, CGMS and GMS.

The addition of solid surfactants depressed the solidification point of tristearin (Fig. 3); moreover, it prevented the formation of β and induced the formation of β'. On the other hand, the addition of liquid surfactants and the additives tested below (Fig. 3) had no significant effect on the solidification of tristearin. It also can be seen that β crystallization directly from the molten state is prevented specifically by solid surfactants.

Effect of emulsifier on polymorphism of different chain length triglycerides. The effects of various surfactants on α-β transformation in trilaurin, trimyristin, tripalmitin and tristearin were compared. The values ΔH_α^* (the ratio between ΔH_α of the sample and ΔH_α of the pure triglyceride) and ΔH_β^* (the ratio between ΔH_β of the sample and ΔH_β of the pure triglyceride were measured during screening in the DSC at constant heating rates (20). The heating rates used were: LLL, 20 C/min; MMM, 10 C/min; PPP, 10 C/min; SSS, 5 C/min. In Figure 4 the effect of some solid emulsifiers on ΔH_α^* vs different triglycerides is shown. Their effect on ΔH_α of trilaurin is very significant: although solid sorbitan esters do not affect ΔH_α values of tristearin, they cause an increase in the ΔH_α value of trilaurin. Other solid surfactants decrease it to zero. The great enhancement of ΔH_α in trilaurin indicates that some emulsifiers hinder the solid-solid transformation and direct the α-form to melt. Other emulsifiers, decreasing the ΔH_α value of trilaurin to zero, facilitate the solid-solid transformation. Interestingly, all those emulsifiers that increase ΔH_α are sorbitan esters.

The polymorphic behavior, characteristic to each

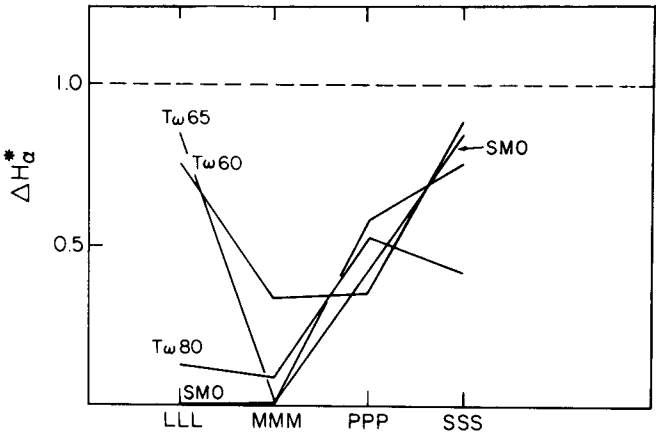

FIG. 5. ΔH_α^* values of trilaurin (LLL), trimyristin (MMM), tripalmitin (PPP) and tristearin (SSS) in the presence of ethoxylated STS (Tw65), ethoxylated SMS (Tw60), ethoxylated SMO (Tw80) and SMO (Sp80).

compound, depends on the balance between hydrophobic and hydrophilic interactions. As shown in our previous work (20), when trilaurin is heated in the DSC at a constant heating rate (20 C/min), the α-β transformation occurs mainly through the solid state, owing to the shorter hydrocarbon chain length in comparison to tristearin. The difference between the polymorphic behavior of tristearin and trilaurin is emphasized when certain solid surfactants are added to the fat (Fig. 4). In Figure 5 the effects of some liquid surfactants are shown: all of them decrease the ΔH_α value of trilaurin. It is interesting to note the effect of SML (Fig. 4): in spite of being a low melting surfactant, it behaves like the solid sorbitan esters. This confirms that in trilaurin the α-β transformation is related mainly to the glycerol moiety configurational change, because the effect of the emulsifier depends more on its hydrophilic head (see SML) and less on its hydrophobic chain length.

The ΔH_α^* value is an indication of the nature of transformation, but it has nothing to do with the extent of β that is formed. In Figure 6 it is seen that no emulsifier intereferes with the β crystallization in trilaurin. This means that the effect of the surfactants is specific to the step of "rearrangement;" sorbitan esters hinder the solid-solid transformation, while the solid surfactants promote this transformation. On the

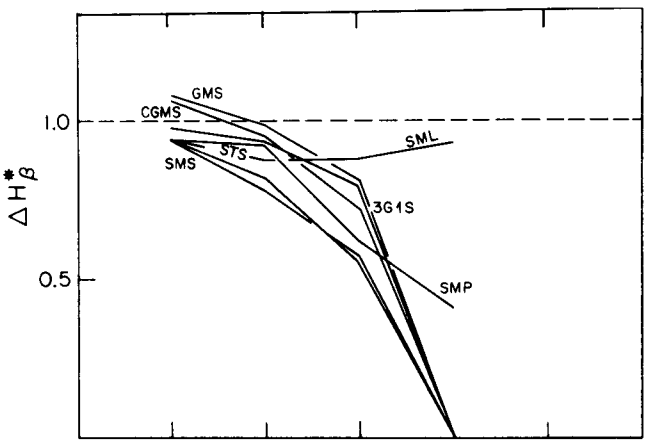

FIG. 6. ΔH_β^* values of LLL, MMM, PPP SSS in the presence of different emulsifiers.

other hand, it is evident in Figure 6 that all of the solid emulsifiers prevent, indiscriminately, the crystallization of β in tristearin ($\Delta H_\beta^* = 0$) as pointed out in the previous section, suggesting that the crystallization of tristearin is related mainly to the packing of the hydrocarbon chains.

In trilaurin, the crystallization of the β-form directly from the molten state is related to the glycerol moiety as from α-transformation. As seen in Figure 3, the solidification point of trilaurin (cooling rate = 0.5 C/min) is suppressed mainly by sorbitan esters and 3GlS, while GMS and CGMS have less effect on it.

Model of incorporation and performance. After a comprehensive investigation of the kinetic control on polymorphic transformations in triglycerides by emulsifiers, a model which describes the probable mode of the emulsifier's incorporation and performance is presented.

Heat capacity (Cp) measurements shown in a previous report (21) support the supposition of a homogeneous incorporation of the emulsifier within the fat. The modification of the heat capacity values of α and β tristearin by the emulsifier's addition suggests that new mixed crystal is formed between tristearin and surfactant, with new physical properties.

The presence of the additive does not cause any change in the crystallographic dimension of tristearin, as can be seen from the X-ray powder diffraction pattern within the range $2\Theta = 8\text{–}25°$, characteristic of short spacings. This means that the emulsifier's molecules are included in the crystal lattice without interfering with the triclinic packing of the fat. Moreover, no additional peaks outside this range that belong to the emulsifier were found.

The emulsifier's molecule penetrates among the triglyceride hydrocarbon chains in a way described schematically in Figure 7. According to Figure 7, the incorporation of the emulsifier's molecule creates vacancies among the hydrocarbon chains that cannot be detected by X-ray diffraction. As already reported in a previous work (22), the NMR spin-lattice relaxation time (T_1) is an indication that such holes are created. Briefly, this technique can distinguish between different degrees of rotational freedom of the hydrocarbon chains near the methyl end group. NMR T_1 relaxation time is the time required by protons aligned in a magnetic field to recover longitudinally (23) after being subjected to a radio frequency pulse. The mobility of the molecules favors the energy interchanges between protons and thus reduces the T_1 relaxation time. The addition of surfactant at different percentages drastically decreases the T_1 value of the β form in tristearin. This result was interpreted with the help of the model shown in Figure 7. The defect created by the introduction of the surfactant permits a higher mobility of the surrounding molecules in their methyl end groups; in such a way the motion of hydrocarbon chains diffuses to the neighbor molecules as a halo thus decreasing the T_1 value already at low percentage of additive.

The performance of the additive as a controller of polymorphic transformation has been interpreted with

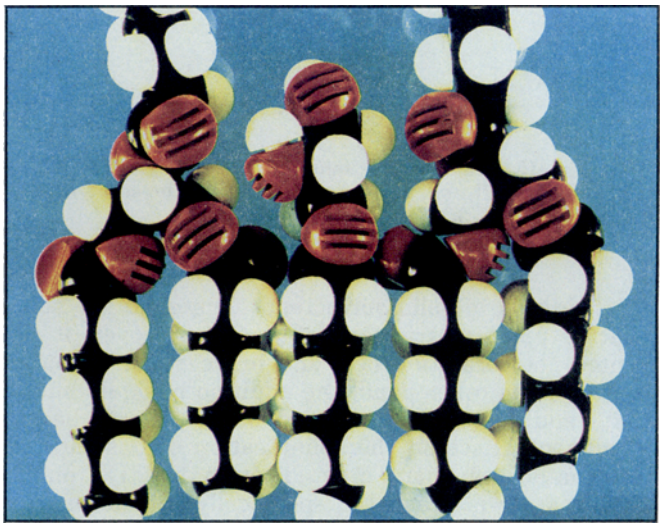

FIG. 8. Molecular model of SMS between two molecules of triglyceride in α-form.

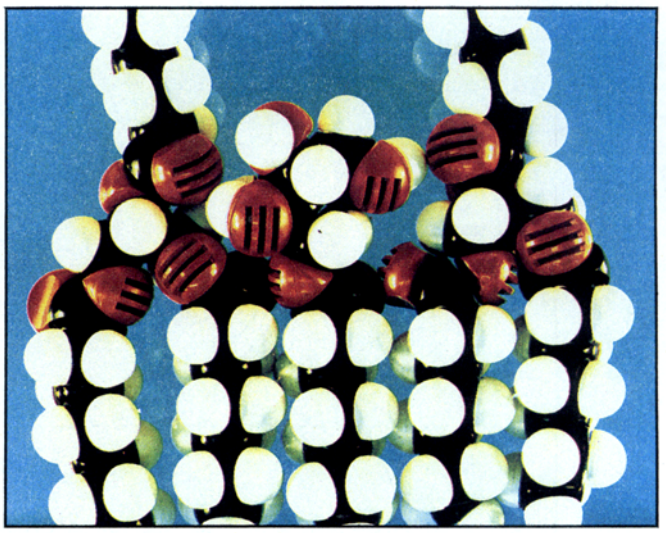

FIG. 9. Molecular model of GMS between two molecules of triglyceride in α-form.

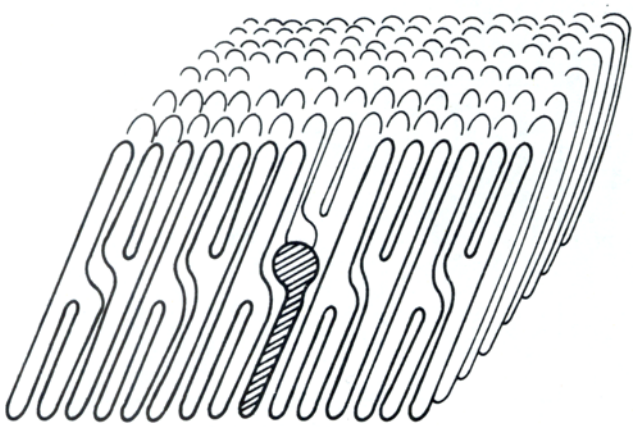

FIG. 7. Model of emulsifier incorporation within the fat.

the aid of molecular models. Figure 8 shows the model of sorbitan monostearate arranged between two molecules of the triglyceride in α-form. Figure 8 emphasizes the steric compatibility between the hydrophilic head of the emulsifier and the polar sites of the triglycerides, so that both hydrophobic and hydrophilic interactions are maximized. The two hydroxyl groups of sorbitan monostearate, seen in the molecule model, point to the carbonyl groups of the two neighboring triglyceride molecules. This arrangement permits the additive to hold the two molecules by hydrogen bonds and thus temporarily prevent the triglyceride from the configurational modification α-β.

The ability of the additive molecule to hold two chains of triglycerides arranged in the α-configuration in one point and to preclude them from switching configuration to β that can be obtained by sorbitan monostearate can be called "the button syndrome." It is not due only to the particular chemical structure but also to the particular structural fit that permits the interlocation of this additive among the fat molecules with minimization of defects and an optimal localization of the functional groups for hydrogen bond formation.

Three-glyceryl-monostearate (3GlS) that also has a retarding effect on the solid-solid α-β transformation has a bulky hydrophilic head that can arrange along the hydrocarbon chains, but still the two hydroxyl groups can approach the carbonyl groups for a hydrogen bond. On the other hand, LGMS and GMS (Fig. 9) having a different chemical structure in the hydrophilic moiety do not form hydrogen bonds from both sides.

The particular structural affinity of sorbitan monostearate to the glyceride is shown in Fig. 10. The α-β transformation in tristearin was performed at a constant rate of 5 C/min after different rates of cooling for α crystallization previous to the experiment. Figure 10 presents the ΔH_β values of tristearin with different emulsifiers added as a function of cooling rate previous to the experiment. While in neat tristearin the ΔH_β value does not change as a function of previous cooling, in the presence of solid surfactants the decrease in ΔH_β value depends on the cooling performed previously. After the slow cooling the β crystallization is less inhibited than after fast cooling. On the contrary, in the presence of SMS the prevention of crystallization is, in the same experimental conditions, maximal. This can be interpreted as higher structural affinity of sorbitan monostearate to the triglyceride than the other solid surfactants. Presumably, during slow cooling the less compatible surfactants aggregate into clusters and then decrease their effect; on the contrary, it seems that the performance of sorbitan monostearate is not affected by the previous solidification rate because the molecules do not form clusters even during slow cooling. The particular structural affinity can be further supported by the Cp measurements (21) and in phase diagrams (24).

It is interesting to note the difference between addition of emulsifier and addition of tripalmitin at the same percentage. Their effect on the polymorphic behavior of tristearin during heating is completely different. While the presence of the surfactant does not influence the polymorphism of tristearin (Fig. 11), in the presence of tripalmitin the intermediate β'-form is apparently stabilized. This is a clear demonstration that the emulsifier interferes with the kinetic process of transformation, but does not change the polymorphs obtained.

It is possible to summarize the results below as follows:

(i) The emulsifier is incorporated among the hydrocar-

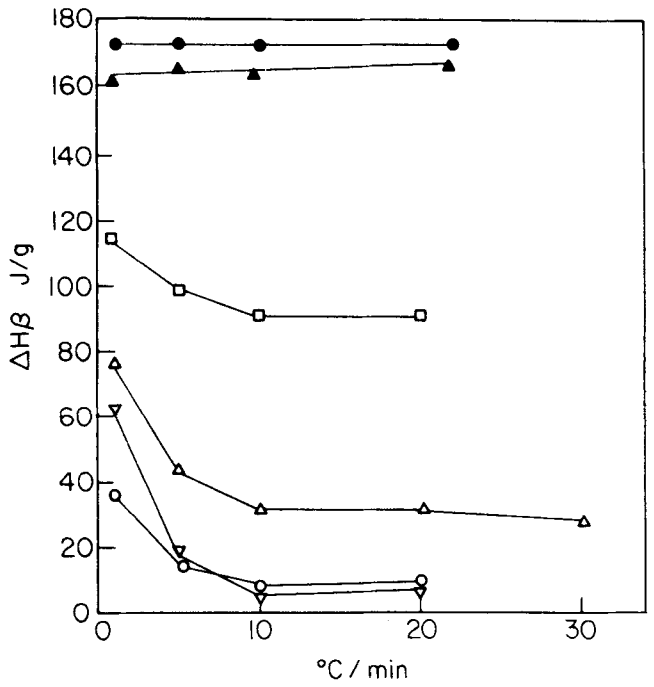

FIG. 10. ΔH values of β-form in tristearin transformed from α at 5 C/min, as a function of the cooling rate from melt prior to the experiment. ●, neat tristearin; ▲, with SML added; □, with SMP added; △, with CGMS added; ▽, with 3GlS added ○, with GMS added.

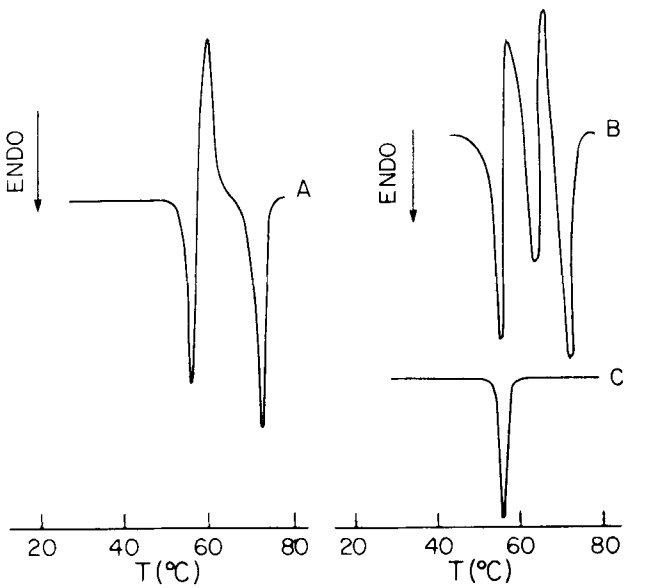

FIG. 11. Thermograms at 5 C/min heating rate of: A, neat tristearin; B, tristearin with 10 wt% tripalmitin added, and C, tristearin with 10 wt% STS added.

bon chains of the fat, which form the two dimensional layer, and causes the formation of defects which enhance the mobility freedom of the hydrocarbon chain methyl ends.

(ii) The hydrophilic moiety is situated near the polar sites of the triglycerides. If it contains two hydroxylic groups in the right position for forming hydrogen bonds with the carbonyl groups of the triglycerides, it causes a temporary prevention of β transformation.

(iii) The hydrophilic moiety of the emulsifier must be small enough to allow the insertion within the crystal lattice, but bulky enough to perform the "button syndrome."

(iv) It follows that the requirements of the emulsifier to be a controller of polymorphic transformation in tristearin are both structural and chemical.

(v) The presence of the emulsifier does not dictate the formation of any preferred polymorph but rather controls the mobility freedom of the molecules and their facility to undergo configurational changes.

The conclusions drawn in this work serve as a base for better understanding the effect of additives on polymorphism of mixtures of fats; in such cases the emulsifier may act as a seeding agent and as a dynamic controller of polymorphic transformation. It also can affect the liquid portion of the fat, which seems to be a determining factor in blooming phenomena.

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