to inactivate the protease inhibitors, both in the purified state and in food systems.

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Heat Capacity of Tristearin in the Presence of Food Emulsifiers

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The specific heats of tristearin in the presence of some food emulsifiers were determined by differential scanning calorimetry. Solid emulsifiers show Cp curves different from those of pure tristearin, indicating that a new mixed crystal has been obtained through the incorporation of the surfactant within the fat.

The specific heat (Cp) of simple saturated triglycerides has been determined in previous investigations by differential scanning calorimetry (1). The heat capacities of the different polymorphic forms were recorded, and it was evident that in the unstable α form the Cp values are significantly higher than in the β stable form. Simpson, Hockett and Harris (2), comparing the specific heats of tristearin and trimargarin, showed a change in the slope of the heat capacity curves at 300 K for both α and β forms.

Following previous works (3), it was postulated that this break point represents the beginning of two-dimensional melting, which is involved in an increase of heat capacity rather than latent heat. This proposed mechanism is based on the existence of different bond strengths in the three dimensions of the lattice and is applied to lipids and liquid crystals because of their structure of layers which are composed of alkyl chain molecules with lateral strong bonds, but weaker interactions between the layers themselves. According to this theory, prior to melting, a gradual increase in dislocations is possible inside the layer and leads to defects, with no latent heat involved, but rather an increase in the heat capacity. It is well known from previous work that addition of fatty acid sorbitan esters at low percentages affects polymorphic behavior of fats and triglycerides; because of this kind of action, these surfactants are called "crystal structure modifiers." It was shown that in tristearin the α-β transition was prevented (4); similar results were obtained during aging of unstable forms in fats (5) and cocoa butter (6). From these works it is apparent that solid emulsifiers are efficient in affecting the polymorphic behavior of solid fats, while liquid emulsifiers are ineffective.

The present work uses similar food emulsifiers which previously were tested for their ability to prevent the α-β transition in tristearin; their effect on the heat capacity of tristearin also was checked. The purpose of this work is to extend and deepen our comprehension of the mechanism of action of the emulsifiers as crystal structure modifiers through understanding their mode of incorporation among the fat molecules.

MATERIALS AND METHODS

Tristearin was purchased from Sigma Chemical Co., St. Louis, Missouri, and was 99% pure. The emulsifiers were commercially available from Grindsted Products, Denmark, and Atlas Europol A.p.S., Italy.

The following types of additives were tested: sorbitan monostearate (Span 60), sorbitan tristearate (Span 65), sorbitan monolaurate (Span 20), ethoxylated sorbitan monostearate (Tween 60), glycerol monostearate, citric acid ester of monoglyceride (Acidan), triglycerol ester of stearic acid (3G1S), stearyl alcohol and stearic acid. All the additives were introduced at the level of 10 wt %, and the mixtures were heated to above melting point and blended well outside the DSC prior to any experiment.

The thermal measurements were performed on a Mettler Differential Scanning Calorimeter (TA3000) calibrated with indium, lead and zinc for accuracy of caloric data and temperature readings. The samples were sealed in aluminum pans and an empty, similar pan was used as reference. All the heat capacity measurements were corrected automatically with a blank experiment measuring the heat flow of an empty pan, in order to eliminate the additional heat flow arising from difference in weight of sample pan and reference pan, and to compensate for any asymmetry in the measuring system. The Cp data of each experiment are the means of five independent runs.

The weight of sample was between 10 and 20 mg, and the heating rate was 10 C/min.

The α form was crystallized in the DSC before the measurement, by quenching the melted fat. The β form was obtained aging the sample crucibles which contained the α form, at 45-50 C, until complete transformation occurred.

RESULTS AND DISCUSSION

The specific heat data of polymorphs α and β in tristearin were recorded first for comparison with the results obtained from other authors (Table 1). The mean values received are lower than those obtained by Simpson but slightly higher than the values computed by Hampson.

The characteristic thermograms of β forms in tristearin in absence and presence of Span 60 are shown in Figure 1.

A feature characteristic to all the solid emulsifiers is a significant increase in the slope of the baseline close to the melting point which indicates a progression in the lattice breakdown close to the melting temperature; for this reason, the comparison of Cp between the different samples obviously was extended to a temperature lower than the initial melting temperature of the pure α and β forms of tristearin.

The specific heats in the presence of Span 60 and Span 65 are plotted in Figures 2 and 3.

The heat capacity of both α and β were plotted using correlation factors in order to detect the point at which the change of slope occurs (Figs. 2A and 3A). The points seem to correspond more or less to those detected by Simpson, near 20 C (293 K). The existence of other discon-

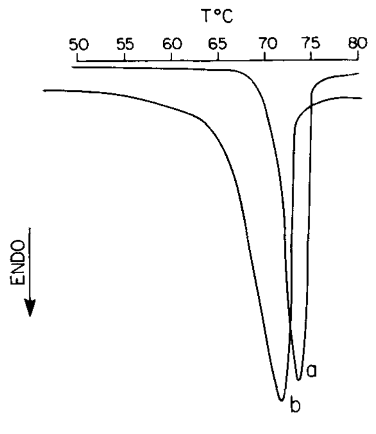

FIG. 1. Thermograms of β tristearin in the absence (a) and in the presence (b) of Span 60.

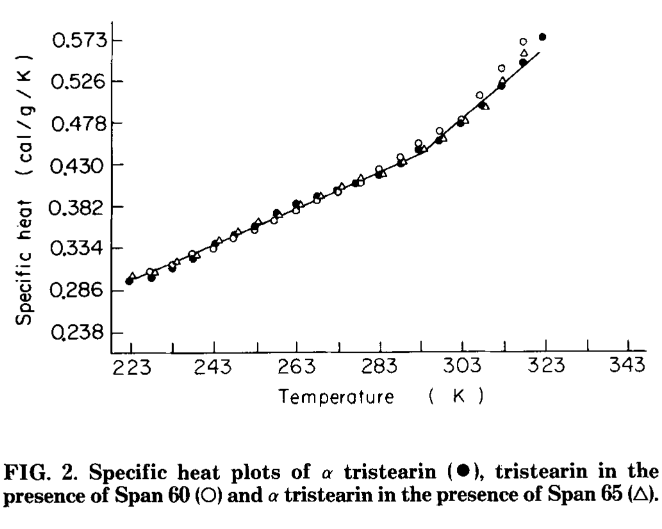

FIG. 2. Specific heat plots of α tristearin (●), tristearin in the presence of Span 60 (○) and α tristearin in the presence of Span 65 (△).

TABLE 1

Specific Heats of Tristearin α and β Forms (cal/g/K)

Temperature (K)	Garti, Schlichter, Sarig		Temperature (K)	Hampson (1)		Simpson (2)	
	Cp (α)	CP (β)		Cp (α)	Cp (β)	Cp (α)	Cp (β)
223	0.2986 ± 0.0078		220	0.279	0.252	0.313	0.278
233	0.3129 ± 0.0062	0.2795 ± 0.0087	230	0.309	0.265	0.335	0.289
243	0.3392 ± 0.0061	0.2867 ± 0.0073	240	0.330	0.268	0.354	0.301
253	0.3583 ± 0.0091	0.2986 ± 3.013	250	0.356	0.285	0.376	0.314
263	0.3822 ± 0.0070	0.3129 ± 0.013	260	0.375	0.299	0.397	0.330
273	0.3989 ± 0.0061	0.3273 ± 0.014	270	0.402	0.321	0.420	0.344
283	0.4157 ± 0.0089	0.3368 ± 0.013	280	0.419	0.327	0.443	0.358
293	0.4443 ± 0.0075	0.3559 ± 0.014	290	0.454	0.359	0.464	0.371
303	0.473 ± 0.0089	0.3727 ± 0.013	300	0.468	0.355	0.490	0.385
313	0.516 ± 0.00104	0.3942 ± 0.015	310	0.510	0.374	0.534	0.406
323	0.5757 ± 0.00822	0.4157 ± 0.012	320	0.577	0.398	0.577	0.430
333		0.4443 ± 0.014	330		0.419		0.455
343		0.778 ± 0.015	340		0.437		

tinuities in the slope, which were detected by Simpson at much lower temperatures, was not verified.

Comparing with the heat capacity of the triglyceride alone it can be seen that the presence of these emulsifiers gradually increases the Cp of the β form starting near the same point at which the slope changes; interestingly, below this temperature, the Cp values of the β form are not affected by these emulsifiers. On the other hand, the Cp values of the α form do not vary when these emulsifiers are added. Span 60 and Span 65 are known, from a separate work in the DSC, to prevent the crystallization of the β form in tristearin when the α form is heated at 5-10 C/min. The same effect has been demonstrated for other solid emulsifiers having different hydrophilic groups associated by esterification with stearic acid. The addition of these surfactants to tristearin also has been tested with regard to their effect on heat capacity. Figures 4 and 5 show the effect of glycerol monostearate, Acidan and 3G1S on the specific heat of tristearin. Unlike the Spans, these surfactants apparently increase the Cp of α and β tristearin through all temperature ranges, constant and parallel to the Cp of the pure triglyceride.

The effect on the Cp of two liquid emulsifiers, Span 20 and Tween 60, which have proven to be less efficient in preventing β crystallization of tristearin during heating of the α form, is shown in Figures 6 and 7. When Span 20 or Tween 60 were added to tristearin, the Cp curves of the fat were much less regular in their slope, with a large scatter of Cp values. In this case it is more difficult to give an interpretation of the results; it may be that this scatter of points may be caused by segregation of the liquid emulsifier, which has distinct thermal properties. Stearic acid and stearyl alcohol, which do not affect

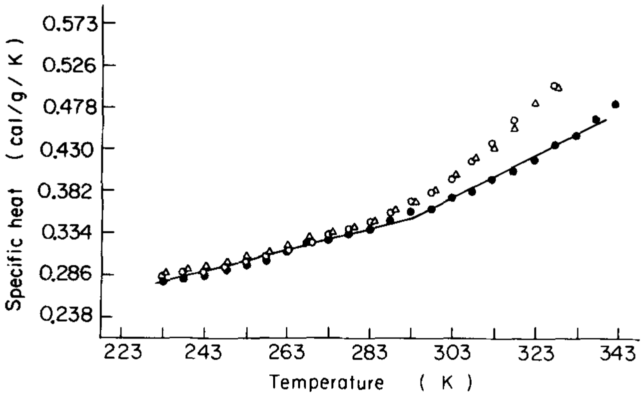

FIG. 3. Specific heat plots of β tristearin (●), β tristearin in the presence of Span 60 (△) and β tristearin in the presence of Span 65 (△).

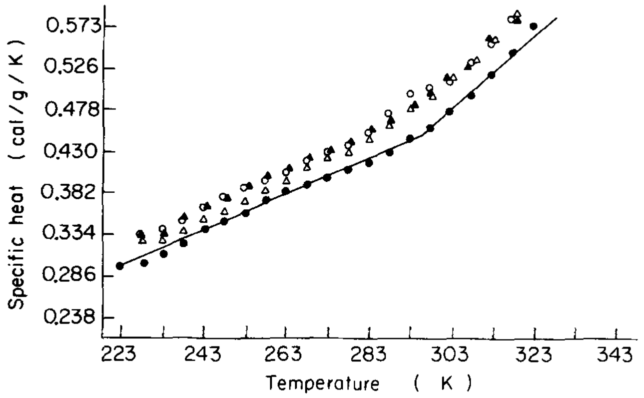

FIG. 4. Specific heat plots of α tristearin (●), α tristearin in the presence of glycerol-monostearate (▲), α tristearin in the presence of Acidan (△) and α tristearin in the presence of 3G1S (○).

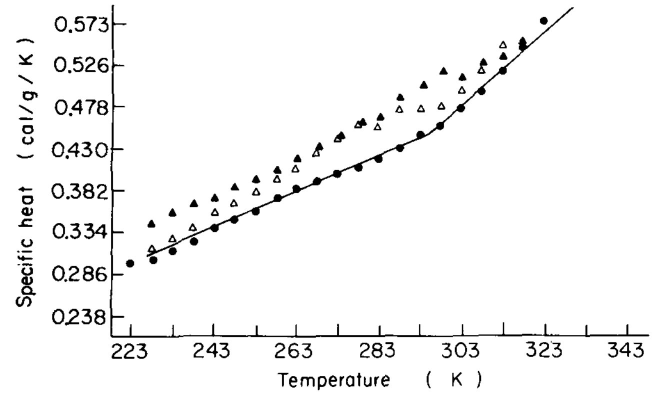

FIG. 6. Specific heat plots of α tristearin (●), α tristearin in the presence of Span 20 (△) and α tristearin in the presence of Tween 60 (▲).

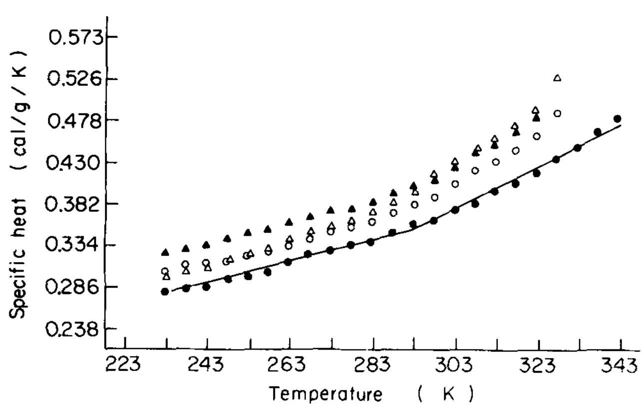

FIG. 5. Specific heat plots of β tristearin (●). β tristearin in the presence of glycerol-monostearate (▲), β tristearin in the presence of Acidan (△) and β tristearin in the presence of 3G1S (○).

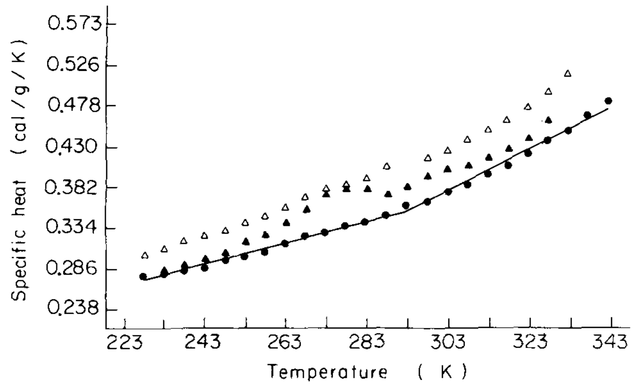

FIG. 7. Specific heat plots of β tristearin (●), β tristearin in the presence of Span 20 (△) and β tristearin in the presence of Tween 60 (▲).

the crystallization of β during heating, do not affect the specific heats of tristearin. In the thermograms of these mixtures distinct peaks clearly appear which belong to the fat and the emulsifier, indicating the two substances are immiscible and thus crystallize separately.

In order to verify that the values of the heat capacity recorded in the various experiments correspond to the new mixed crystal in which the emulsifier was incorporated during the crystallization process, an artificial mechanical mixture of 50% α and 50% β tristearin, which were obtained separately, proved that in the case of solid mixtures the Cp values computed are the arithmetic average of the individual compounds (Fig. 8). Solid mixtures of α and β tristearin with acidan in the ratio 10:1 clearly exhibit different patterns from those in Figures 4 and 5, being the average of the Cp values of the fat and the Cp values of the pure surfactant.

Although a very low concentration of emulsifier in the fat (1–2%) is enough to cause observable effects on polymorphic behavior, for the investigation of the Cp, a relatively high concentration was chosen in order to obtain drastic results which facilitate comparison.

The thermal behavior of the solid fat below its melting temperature evidently is a fundamental feature leading to understanding the polymorphic phenomena.

The concept of two dimensional solid melting defines the mode of transition from the solid to the liquid phase as a gradual breakdown of crystal lattices during a premelting stage. As illustrated by Brinkman (3), in a two dimensional solid thermal fluctuations cause dislocations of molecules and disorder in the network, permitting still a relative stability of the crystal; this differs from a three dimensional solid, which abruptly disgregates into the liquid form. This kind of transition is involved in a gradual change of the specific heat rather than latent heat. Hence, it seems that the change in the slope of specific heat is related to the initial formation of defects in the lattice previously defined as two dimensional melting.

The ability of the surfactant to crystallize within the fat in conformity with the crystallographic dimensions implies that a physical structure affinity exists between the molecules of the surfactant and those of the triglyceride, and the thermal analysis allows detection of differences in molecule fluctuations in the crystal state. It seems that the amphiphilic character of the additive is the first condition which permits the packing of the long chain emulsifier within the fat, because stearic acid and stearyl alcohol do not integrate in the lattice. However, it does not appear that every solid emulsifier incorporated in tristearin affects the heat capacity of the fat in the same manner. Span 60 and Span 65 probably best fit the space between the chains of the triglyceride, showing that they crystallize in the hexagonal structure within the α form without affecting the Cp. On the other hand, 3G1S, Acidan and glycerol monostearate increase the Cp of the α form of the fat, suggesting that they do not perfectly fit the physical structure of the fat and create imperfections in the lattice, which increase rotational freedom of the molecules. With regard to the β form, Span 60 and Span 65 increase the Cp starting from the point at which the two dimensional melting is supposed to initiate. This fact may give rise to the hypothesis that in β form the forces between the molecules of triglyceride and surfactant are weaker than those between the molecules of triglyceride. Hence the progressive mobility of the molecules due to their lateral fluctuations is enhanced as an effect of these surfactants' presence; the other solid surfactants do not seem to affect the increasing fluctuation of the molecules, but they increase their rotational mobility.

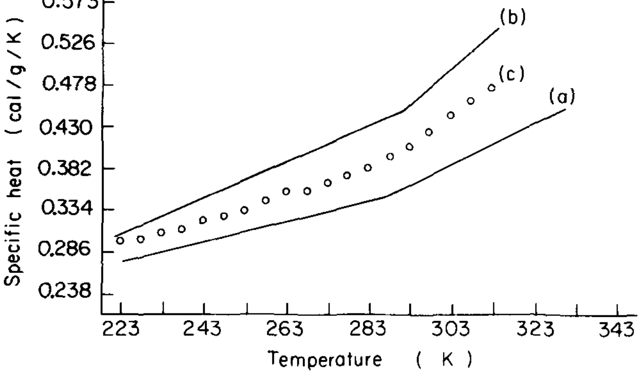

FIG. 8. Specific heat plots of α tristearin (a), β tristearin (b) and a mechanical mixture of 50% α and 50% β tristearin (c).

Because Span 20, stearic acid and stearyl alcohol have been proved to segregate from the fat, they clearly do not affect the Cp. On the other hand, Tween 60, which is a liquid emulsifier, apparently can incorporate within the fat, altering its heat content because the long ethoxylated chains clearly prevent a good compatibility with the physical structure of the fat.

The results presented show that the surfactants having hydrophobic chain equivalent to the alkyl chain of the triglyceride, through their incorporation in the fat, alter the heat content of the crystal, influencing the freedom of rotation of the molecules. Differently, Span 60 and Span 65 do not show any effect on the rotational mobility of the molecules, but they do alter thermal behavior during the two dimensional melting of β form.

Indeed, the presence of the surfactant may affect some thermal behavior patterns of the fat, while retaining the crystallographic and spectroscopic features. This means that the emulsifier interferes in the solidification and melting processes of the fat without detectable alterations of the crystal packing. This fact alludes to the ability of the surfactant to change the kinetics of phase transition, which is related to the mobility of the molecules rather than to the thermodynamic stability of the molecules' arrangement.

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