Reconsideration of Polymorphic Transformations in Cocoa Butter Using the DSC

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Cocoa butter was crystallized in the differential scanning calorimeter (DSC) in the presence and the absence of sorbitan monostearate at different cooling rates. The solidification and fusion curves were recorded. It was found that cooling rate affects enthalpies and temperatures of phase transitions. The very slow cooling rate causes a significant decrease in crystallization enthalpy, suggesting that fractionation of glycerides occurs under these conditions. In the presence of sorbitan monostearate 5%, cooling and heating curves of cocoa butter are sharper, the solidification point is higher and the fusion point is slightly lower. Further, in the presence of sorbitan monostearate both crystallization and fusion enthalpies are lower than in pure cocoa butter. The presence of the emulsifier seems mainly to promote the fractional crystallization.

Fusion curves after different periods of isothermal crystallization suggest that higher polymorphic forms differ in chemical composition from lower ones, and that the presence of sorbitan monostearate affects the fractionation in the fat.

As well known by confectioners, cocoa butter (CB) is an outstanding example of polymorphic fat; according to proper crystallization and aging conditions, different crystal forms are obtained, which exhibit characteristic melting points and x-ray diffraction patterns (1,2).

CB actually is a blend of mixed triglycerides, the near-constant composition of which has been determined (Table 1) (3); this mixture is nevertheless assumed to crystallize homogeneously as a single compound (1-3). The polymorphic behavior of CB has been investigated and the effect of the emulsifiers on polymorphic transformations has been observed (4). Hence, the crystallization behavior of CB is generally considered from the polymorphic point of view assuming that under proper crystallization conditions a limited number of defined polymorphic forms are obtained with discrete melting points. Although pure compounds may form definite polymorphs, it may not be the case in such mixtures as CB or other natural fats. In CB it has been shown (5) that the stability of crystals, as measured by the melting point temperature, increases as the crystallization temperature rises from 26 to 30 C; concurrently the composition of the crystals changes from a lower percentage in SOS (stearo-oleo-stearin) to a higher percentage in SOS. Similarly, from crystallization of molten butter fat at different temperatures, different compositions of the crystals have been obtained (6).

TABLE 1

Triglyceride Composition of Cocoa Butter

Oleo-palmito-stearin	52%
Oleo-di-stearin	19%
Oleo-di-palmitin	6%
Palmito-di-olein	9%
Stearo-di-olein	12%
Palmito-stearin	2%

Although six polymorphs are generally recognized in CB, Merken and Vaeck (7) stated that form VI is not a distinct polymorphic form, but rather a phase differing in composition; this statement finds support in the observations of Riiner, Larsson and Paulika (private communication), according to which the V-VI transformation is a solid solution disruption of the (SUS + SSS)V form lattice into a (SUS)V and (SSS)III: in Lutton's nomenclature (β triple chain length) → (β double chain length) + (β double chain length). This can accomplish the anomaly of the SCI (Solid Content Index) curve obtained for form VI at lower temperature (2), which can imply the existence of two solid phases in the "VI" form. Merken and Vaek further state that form III itself actually does not exist and is a mixture of forms II and IV. All these results suggest that the stability of the crystal in cocoa butter does not depend only on the internal crystal structure but also on the triglyceride composition of the crystals.

The aim of the present work is to examine, using the DSC, the possibility that the so called polymorph III is a mixture or combination of two or more phases rather than being a pure polymorph, and that the crystal stability may be associated with chemical composition.

MATERIALS AND METHODS

Cocoa butter was purchased from Hamester (West Germany). The emulsifier used was sorbitan monostearate and was commercially available from Grindsted (Denmark).

The thermal data were obtained by a Mettler Differential Scanning Calorimeter (DSC TA3000). The instrument was calibrated with gallium, indium, lead and zinc to ensure accuracy of caloric data and temperature readings. The weight of cocoa butter samples tested ranged from 6 to 20 mg; the samples were closed hermetically in an aluminum pan. A similarly closed empty pan was used as reference. The crystallization of the samples was performed within the DSC, after an isothermal stage at 50 C to ensure melting. The dynamic crystallization was performed at the rate of 30 C/min from 50 to 26 C and at rates ranging between 0.02 C/min and 0.3 C/min, from 26 C to low temperatures. The isothermal crystallization was performed cooling the sample at 0.5 C/min and keeping it isothermally at 22 C for times ranging from 100 min to 20 hr. The heating was performed in all cases at a constant rate of 5 C/min.

RESULTS AND DISCUSSION

The exothermic curves of CB solidification at different cooling rates are shown in Figure 1. The solid lines

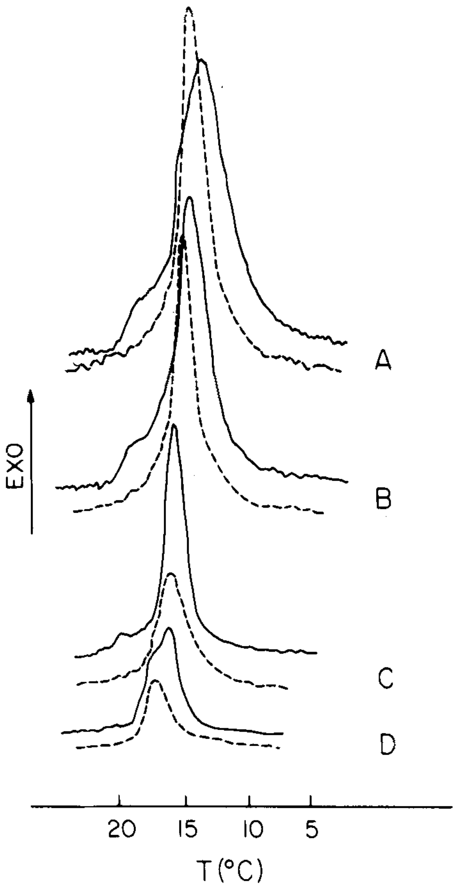

FIG. 1. Crystallization curves of pure CB (—) and CB + SMS (- - -) at different cooling rates. A, 0.3 C/min; B, 0.2 C/min; C, 0.1 C/min; D, 0.05 C/min.

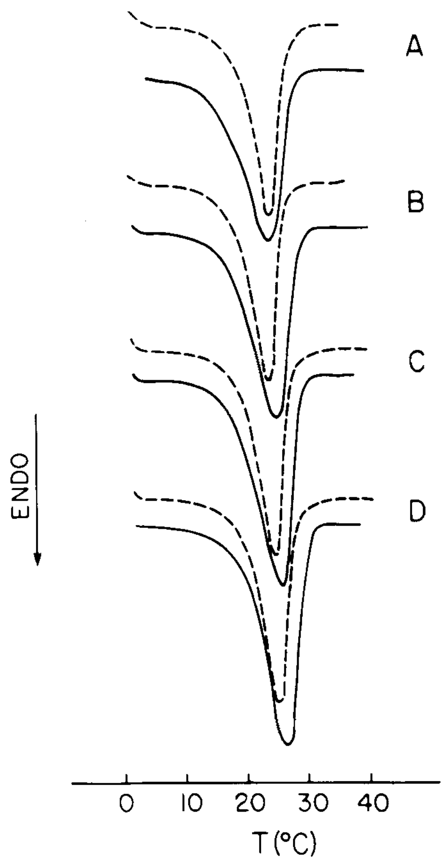

FIG. 2. Heating curves of CB (—) and CB + SMS (- - -) at 5 C/min, crystallized at different cooling rates. A, 0.3 C/min; B, 0.2 C/min; C, 0.1 C/min; D, 0.05 C/min.

represent the solidification curves of neat cocoa butter, and the dashed lines belong to the crystallization curves of cocoa butter with the addition of sorbitan monostearate.

As the cooling rate becomes lower, the fat crystallizes at a higher temperature (Fig. 1) and the exothermic curves are sharper.

After the crystallization, the same samples were heated up and heating curves were recorded. Figure 2 shows the heating curves of pure cocoa butter (solid lines) and those of cocoa butter with the additive (dashed lines). Although the heating rate was identical in all four thermograms, the fat melting point (MP) changed in correlation with the previous treatment.

The crystallization and fusion temperatures as a function of cooling rates are listed in Table 2. The mp's increase as the previous crystallization was slower, and they range between those of forms II and IV recognized in the literature (1-3); further, they do not correspond to any specific polymorph previously reported. Rather, they change gradually according to the previous cooling.

The cooling rate affects the crystallization and fusion enthalpies (Table 2). For a given substance a lin-

TABLE 2

Crystallization Enthalpies (ΔH_c) and Temperatures (T_c), Fusion Enthalpies (ΔH_f) Temperatures (T_f) of CB and CB + SMS

	No additive				With SMS (5 wt%)			
Cooling rate (°C/min)	ΔH_c (J/g)	T_c (C)	ΔH_f (J/g)	T_f (C)	ΔH_C (J/g)	T_c (C)	ΔH_f (J/g)	T_f (C)
0.3	88.7	13.2	94.5	22.8	69.1	14.2	82.2	22.4
0.2	93.9	14.2	98.7	23.7	70.5	14.8	86.3	22.7
0.1	100.7	15.5	103.7	24.6	67.1	15.9	85.9	23.7
0.05	91.8	16.8	107.1	25.3	62.1	17.2	87.5	24.3
0.02	83.1	19.2	106.5	26.3	—	—	—	—

ear relationship usually exists between fusion temperatures and the corresponding enthalpies. However, as can be seen in Figure 3, below a certain cooling rate the observed enthalpies are lower than expected. In other words, as the cooling rate becomes lower, the ΔH_f values do not increase as a function of increase in mp's. The same anomaly also is seen in crystallization enthalpy, during the cooling rate of 0.02 C/min.

The conclusions that can be drawn from these results are:

- In the experimental conditions of this study, no definite polymorph is obtained; clearly the mp's of the crystallized fat change gradually according to the cooling rate.
- Some segregation of triglycerides may occur in fat crystallized at the rate of 0.02 C/min, because the crystallization and fusion enthalpies observed are lower than those obtained at higher cooling rates.

The crystals obtained at different cooling rates may differ in polymorphic modification; the other possibility is that they may differ in chemical composition. The decrease of crystallization and fusion enthalpies in the very low cooling rate could be a suggestion that the higher mp derives not only from a more stable polymorph, but also from partial segregation of lower mp triglycerides. Presumably, the 0.02 C/min cooling enables the fat to solidify in a more compact arrangement not feasible in the presence of the more liquid triglycerides. It can be seen from Figure 2 that the higher cooling rates lead in the subsequent heating process to slightly wider and spread peaks. This may suggest that higher cooling rates produce a phase mixture instead of distinct polymorphs. The gradual change of mp with different cooling rates can support the statement of Merken and Vaeck that the III polymorph is actually a mixture of forms II and IV. The connection between the sharpening of fusion peak and the increase in homogeneity of the phase mixture is confirmed by the fusion peaks of forms IV, V and VI in cocoa butter (4,8), which clearly become sharper as the crystal stability increases; moreover, the assumed segregation of liquid triglycerides concomitant with the increased stability of the fat has been proved by Manning (5) by means of DSC and HPLC.

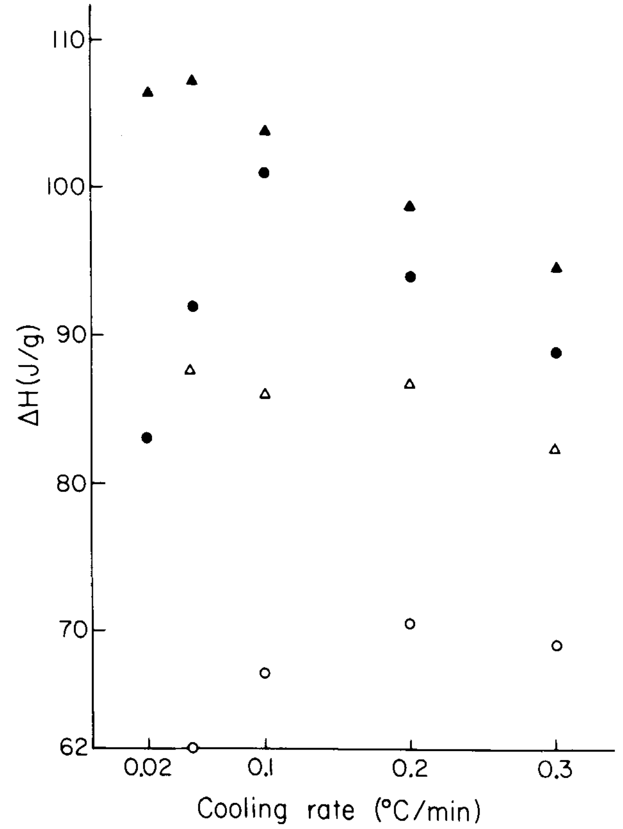

FIG. 3. Fusion and crystallization enthalpies vs cooling rates. ▲, ΔH_f of pure CB; •, ΔH_c of pure CB; Δ, ΔH_f of CB + SMS; ○, ΔH_c of CB + SMS.

The effect of cooling rate on the solification and fusion properties of CB was tested after the addition of sorbitan monostearate (SMS) at the level of 5 wt%. The blend of cocoa butter with SMS did not appear as a completely clear liquid above 50 C, but was rather turbid. The cooling curves of CB with the surfactant are sharper than those of pure CB (Fig. 1). The shoulder which appears in the cooling curves of pure CB around 20 C is not present in those of CB + SMS. The solidification temperatures are slightly higher in the presence of SMS than in pure CB (Table 2), and the enthalpies values are considerably lower. The fusion curves (Fig. 2) are clearly sharper in CB + SMS than in pure CB. The mp's of CB with the additive decrease a little and the fusion enthalpies are significantly lower compared to pure CB.

In the presence of the emulsifier it seems that crystallization takes place only partially as can be seen from the low solidification enthalpies and high solidification point values, compared to pure CB. The emulsifier, owing to its low solubility into the fat, may arrange in an ordered structure which acts as crystal seed and hence promotes crystallization of the higher mp triglycerides; the fraction of CB constituted by the lower mp triglycerides may solidify in a form without detectable enthalpy (it may be perceived as amorphous). This can be confirmed by the low values of fusion enthalpies compared to pure CB.

The seeding effect of the emulsifier may further induce the formation of smaller crystals, expressed in the slightly lower mp in comparison with pure CB after the same cooling regime. As found in solution-grown crystals of polymers (9) whose structure is characterized by successive layers of folded chains and is similar to the crystal structure of the fat, the size of the particles and thickness of the lamellae are related to the mp. Moreover, the sharpness of the melting curves of the fat with the additive suggest that in this case melting occurs in a narrow temperature range, probably due to a narrower range of crystal dimensions. This supposition also can be confirmed by the sharp and high crystallization point of the cooling curves.

The crystallization of CB also was performed during isothermal treatment at 22 C for different periods of time. The sample, after being held at this temperature, was cooled rapidly to O C in order to eventually crystallize the fat fraction that did not solidify during the isothermal period, and afterward it was scanned (Fig. 4). The gap between the two melting peaks around 22 C separates between the melting of the fraction

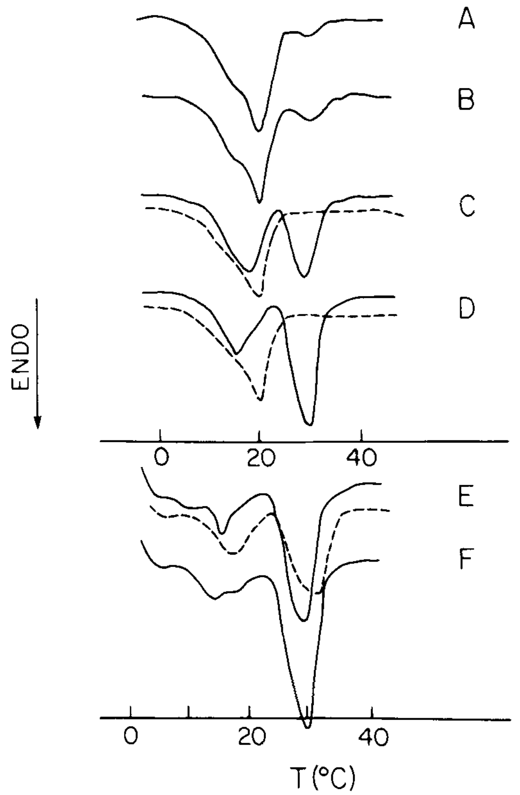

FIG. 4. Heating curves of CB (—) and CB + SMS (- - -) after isothermal crystallization 22 C at different times. A, 100 min; B, 200 min; C, 400 min; D, 600 min; E, 800 min; F, 1200 min.

solidified isothermally (above 22 C) and the fraction solidified during the subsequent fast cooling (below 22 C). As the isothermal period is longer, more fat is allowed to crystallize at 22 C (mp = 28 C). Further, the longer the isothermal period is, the lower is the melting range of the low melting fraction; this indicates that with the increase in the stable fat portion, the lower melting fraction changes composition and becomes richer in the lower melting triglycerides. The addition of the emulsifier (dashed curves) seems to produce an inhibitory effect on crystallization up to a certain time duration (600 min); above 600 min the crystallization of an even higher melting fraction, compared to pure CB, is promoted. Hence, after the fat is allowed to solidify long enough, a more pronounced fractionation occurs.

The crystallization and fractionation properties of CB in the absence and in the presence of additives have direct implications in polymorphic behavior. The possibility that more stable crystals are richer in high m.p. fractions than less stable crystals has been pointed out (5). Nevertheless, the segregation seems to occur only above a certain m.p. polymorph, where incorporation of the liquid triglycerides is limited by the increasing density of the crystal lattice; hence, the transformation from a lower m.p. form to a higher m.p. form seems to be accompanied by partial segregation. The presence of the emulsifier has been shown to promote segregation and crystallization of the higher m.p. fractions of the fat. This is in good agreement with our previous results concerning the enhancement of I → V polymorphic transformations in CB, caused by the presence of solid sorbitan esters (4).

The formation of a homogeneous solid in CB can be achieved by rapid solidification; after extensive tempering higher and lower m.p. triglycerides segregate, allowing transformation to higher polymorphs; on the basis of this assumption, the polymorphic transitions in CB are not merely associated with polymorphism, because it is considered such only when different crystalline forms exist which have the same chemical composition. Nevertheless, these transformations are related to changes in crystal structure; the presence of the emulsifier seems to be relevant with regard to fractionation properties of CB as well as to the internal arrangement of fat molecules.

It seems there are enough indications in the recent literature and our recent results that CB is not behaving differently from any pure triglyceride and consists mainly of three possible crystallographic packings; the fact that six different polymorphs have been detected is related mostly to the fact that complex mixtures of saturated and unsaturated triglycerides are present in CB. Modification III therefore can be interpreted as being a mixture of two solid phases of polymorph II and IV.

By treating the fat at different cooling rates or different aging regimes it seems possible to distinguish between phases. The presence of an impurity such as an emulsifier, which changes the rate of seeding and growth, emphasizes the distinction between those phases. A further work is required using more sophisticated methods such as imaging NMR in order to better distinguish between the phenomena of polymorphism and phase separation in complex mixtures of triglycerides.

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