Technical

Thermal and Compositional Properties of Cocoa Butter During Static Crystallization

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Studies were conducted using differential scanning calorimetry (DSC) and high performance liquid chromatography (HPLC) to determine the thermal properties and glyceride composition of cocoa butter crystals formed under static conditions. In addition to these studies, visual characterization of the crystallites was obtained with polarized light microscopy (PLM). Crystals were formed under controlled static or motionless conditions at formation temperatures of 26.0, 28.0, 30.0, 32.0 and 33.0 C. Preparatory techniques were developed using laminated polyethylene with plastic hoops in order to grow the crystals for isolation and visual identification by PLM prior to DSC assay. Cocoa butter was also crystallized from liquid oil directly in the DSC pans prior to thermal assay. At each crystal formation temperature (26-33 C), various crystallite types grew, each with varying triglyceride composition (PLiP, POO, PLiS, POP, SOO, SLiS, POS, SOS, SOA). As an example, the 'feather' and 'individual' crystals formed at 26.0 C exhibited significant increases in SOS and significant decreases in POP compared to the original butter. It was determined that the original amount of SOS significantly increased in the cocoa butter crystallite as the incubation temperature increased from 26-32 C.

The confectionery industry has long been interested in defining the number and types of crystals present in cocoa butter. The reason for this is the impact cocoa butter crystals can exert on the overall characteristics of a confectionery product. In the confectionery industry, tempering is a process used to assure the proper crystalline form in the resulting product. The mechanisms involved in this process are not completely understood. If tempering is not properly conducted, undesirable crystal forms may be produced and will result in a surface appearance problem called 'fat bloom.' The problem of bloom manifests itself by a spotted white swirling appearance on the surface and in the interior of the molded chocolate bar. Additional symptoms of improper crystal formation include poor texture, lack of contraction, and poor surface gloss.

Characteristics and melting points of crystals are affected by the composition of the fat. Cocoa butter is a relatively simple fat when compared with other fats of multiple fatty acid composition (1). Cocoa butter is made up primarily of three fatty acids, oleic, stearic and palmitic, with minor amounts of linoleic and arachidic.

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TABLE 1.

Classification and Temperature (°C) of Cocoa Butter Crystalline Forms

Vaeck (1960)		Duck (1964)		Willie & Lutton (1966)	Lovegren et. al. (1976)	Davis & Dimick (1986)
γ	17	γ	18.0	I 17.3	VI 13.0	I 13.1[a]/17.6[b]
α	21-24	α	23.5	II 23.3	V 20.0	II 17.7/19.9
				III 25.5	IV 23.0	III 22.4/24.5
β'	28	β''	28.0	IV 27.3	III 25.0	IV 26.4/27.9
		β'	33.0	V 33.8	II 30.0	V 30.7/34.4
β	34-35	β	34.4	VI 36.3	I 33.5	VI 33.8/34.1

[a]Onset or transition temperature determined by DSC.
[b]Maximum peak temperature determined by DSC.

One of the most controversial areas in confection science is the discrepancy of data found in the literature used to classify cocoa butter crystal polymorphs. Since the discovery of polymorphism, numerous scientists have reported different numbers of polymorphs and conflicting melting points for the various crystalline forms found in cocoa butter. Presently, investigators report (Table 1) between four and six polymorphic states of cocoa butter (2-6). The various nomenclatures assigned to each classification compound the problem due to a lack of consistency. The precise melting characteristics of cocoa butter have received considerable research attention in recent years. The techniques previously used in crystallography studies include dilatometry (7), X-ray diffraction (8) and differential scanning calorimetry (DSC) (8-10). Even with these sophisticated instruments the ability to clearly define the desired stable crystal has not been resolved. In processing, the cocoa butter in a chocolate formula will seed during the dynamic tempering process. After seed formation occurs the chocolate is molded and permitted to crystallize in a motionless or static state. This research deals with those crystals formed under static conditions at temperatures near practical tempering conditions. The major objective of this work is to shed new understanding on the morphological, thermal and compositional characteristics of pure Ivory Coast cocoa butter crystals.

EXPERIMENTAL

Bristoline Bristolscope polarized light microscope (PLM) and an International Scientific Instruments (ISI) Model-60 scanning electron microscope (SEM) were used for microscopic techniques. In conjunction with the polarized light microscope, a Leitz temperature variation stage was used to control the sample temperature during examination.

For PLM and SEM studies, pure Ivory Coast cocoa butter was heated to 60 C for at least six hr. Aliquots for polarized light microscopy were removed from the

liquid cocoa butter and placed on glass slides with cover slips that were tempered to the preset incubation temperature. SEM stubs were tempered to the desired incubation temperature, and ca. 50 μl of cocoa butter was placed on the stub surface. After incubation, the stubs were placed in the ISI PS-2 coating unit, cooled for 5 min with wet ice water bath at 0–5 C and immediately coated with 280 A of gold. SEM micrographs were taken with Polaroid Type 55 film. PLM micrographs were taken with a 35mm Leica camera which fit directly into the eye piece barrel of the Bristoline microscope. Kodak 125 plus pan film was used to record crystalline structure.

At 3-day intervals for 30 days, liquid cocoa butter was placed in aluminum DSC pans and the sample weight recorded. Photography was not possible with these samples due to their enclosure within DSC pans. After prescribed incubation periods, the melting behavior was determined with the DSC according to the procedures described previously (9).

A second sampling technique for the static crystallization study involved placing small aliquots of cocoa butter between two polyethylene layers. This sandwich structure containing the cocoa butter was then stretched over a plastic hoop and incubated at various temperatures. Sample preparation was conducted at 5-day intervals for 30 days.

Crystal formation temperatures were set at 26.0, 28.0, 30.0, 32.0 and 33.0 C. After 30 days, the incubator contained 50 to 60 samples which differed in time of incubation. Prior to thermal analysis, the polyethylene hoops with crystalline butter were examined by PLM. The different crystals were photographed, isolated with a plug cutter and placed into an aluminum DSC pan after one layer of polyethylene was removed. This procedure permitted comparison of morphological and thermal characteristics of the same sample.

Thermal analysis was conducted on a Perkin Elmer DSC-2 with an attached Thermal Analysis Data Station (TADS). Pure gallium (99.9999%) was weighed in duplicate and used as a melting point standard (29.78 C; 19.35 cal/g). The melting point of the gallium standard was determined by the intersection of the onset slope with the baseline. Cocoa butter melting points were determined by superimposing the sample endotherm over the gallium standard endotherm (9). All melting points are expressed as onset temperatures. A heating rate of 20 C/min was used through a range from 15 to 45 C.

Samples for the triglyceride analysis were prepared using the static polyethylene hoop technique. After the prescribed incubation, crystals were identified with PLM and removed from the hoop with a plug cutter. The solid fat crystals were then solubilized in chloroform and filtered with a 0.45 millipore type HA filter. Triglyceride determinations were conducted using a Waters HPLC pump and differential refractometer. An acetonitrile-chloroform 6:4, v/v mobile phase was pumped at 0.7 ml/min through an Alltech Associates C-18, 5-μ reversed phase column.

The weight percent of POP, POS and SOS was determined in each crystal sample, and statistical analyses were conducted. The triglycerides PLiP, POO, PLiS, SOO, SLiS and SOA were not analyzed statistically because they comprised less than 10% of the total triglyceride composition.

Standard triglycerides (POO, SOO, POP, POS, SOS) were used to determine the identity and weight percentages of the various triglycerides present in cocoa butter and the isolated crystal samples. The identity of POO, SOO, POP, POS and SOS was determined by spiking a pure cocoa butter sample with a standard. The remaining triglycerides PLiP, PLiS, SLiS and SOA were tentatively identified by interpreting the fatty acid data of the pure Ivory Coast cocoa butter and also the triglyceride chromatographic data obtained by Shukla (12) on cocoa butter.

RESULTS

Thermal and morphological differences observed indicated numerous crystalline forms present at 26.0, 28.0, 30.0, 32.0 and 33.0 C. Due to the obvious morphological differences, the nomenclature used to designate a particular crystal was based on gross visual differences as observed by microscopy (11).

The three crystals formed at 26.0 C were designated 'temper,' 'individual' and 'feather' crystal (13). As incubation time elapsed the 'temper' crystal melting point increased from 31.5 to 33.4 C (Fig. 1). After two days at 26.0 C, the 'individual' crystal formed and exhibited a 29.7 C melting point (Fig. 2). The thermal and morphological characteristics of the 'individual' crystal remained constant through a 20-day incubation period. However, after approximately 30 days the 'individual' crystal underwent a slow transition to another less distinct crystal type which was labelled a 'blade' crystal structure. In addition, a 'feather' crystal structure formed after two days at 26.0 C on the periphery of the 'temper' crystal. The 'feather' crystal possesses a 32.4 melting point (Fig. 3).

As the incubation temperature increased to 28.0 C, small 'spiney' crystals and large 'feather' crystals formed. The 'spiney' crystals were very small and fragile; thus, isolation and identification prior to thermal analysis were difficult. The melting point of these crystals was 36.8 C. The 'feather' crystal formed at 28.0 C had a melting point of 34.4 C, which is two degrees higher than the melting point of the 'feather' crystal formed at 26.0 C.

Incubations of cocoa butter at 30.0 C produced the 'spiney' and 'feather' crystals. The initial crystal formation at 30.0 C occurred in the form of a 'bowtie' crystal previously described (11). As time elapsed the 'bowtie' form enlarged in the distal area while the central area of the crystal remained in a constricted form. Eventually, more crystals add to the structure, resulting in the distinct morphology characteristics of 'feather' crystals. The 'feather' crystal formed during the 30.0 C incubation exhibited a 35.1 C melting point (Fig. 4). As before, the 'spiney' crystals could not be analyzed thermally using the hoop technique due to their extremely small and fragile structure.

However, a 36.8 C melting point endotherm was observed on numerous occasions using the aluminum DSC pan preparations technique.

At 32.0 C incubation, a mixture of 'feather' and 'irregular' crystals predominate. The 'spiney' crystal

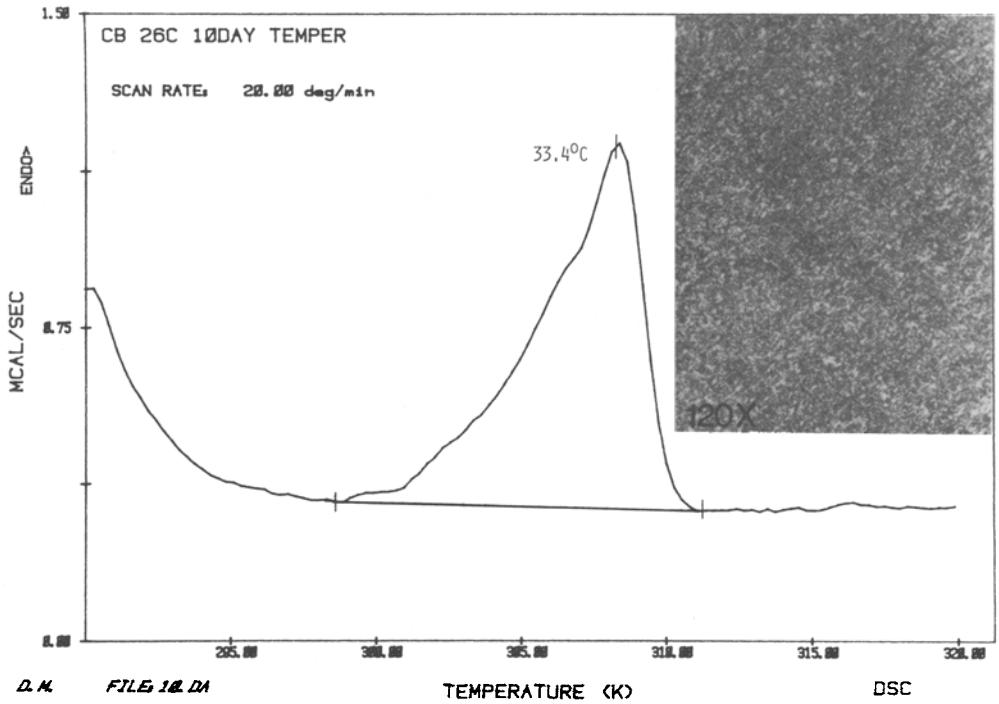

FIG. 1. DSC melting curve of the 'temper' crystal after 10 days at 26.0 C. The PLM micrograph illustrates the crystals that produced the endotherm.

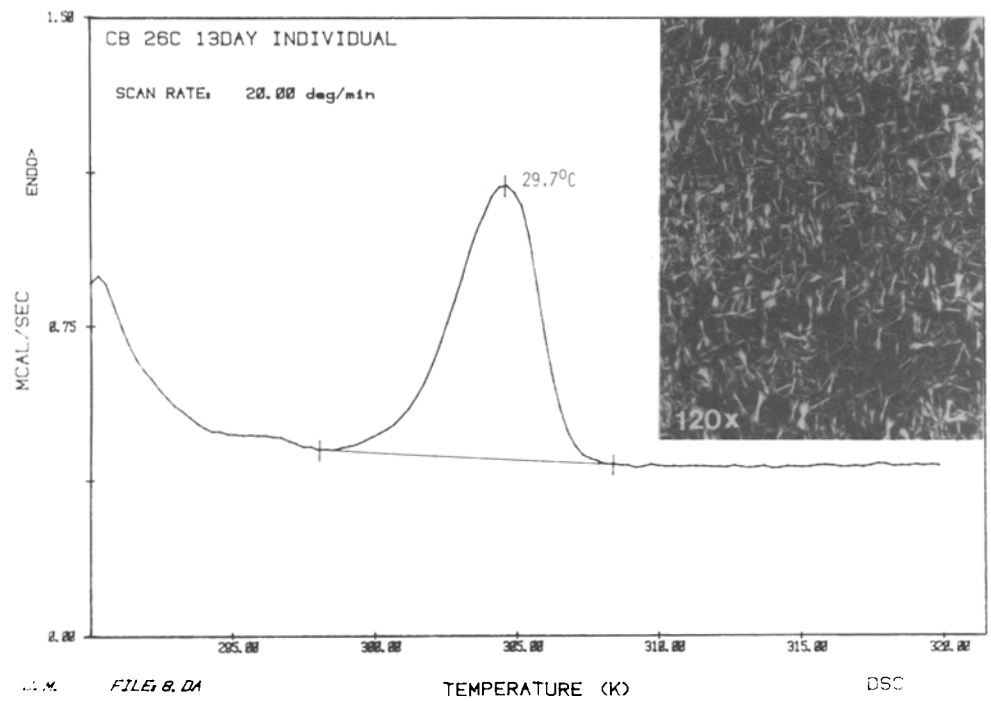

FIG. 2. DSC endotherm of the 'individual' crystal formed at 26.0 C. The 'individual' crystals which melted at 29.7 C are illustrated in the PLM micrograph.

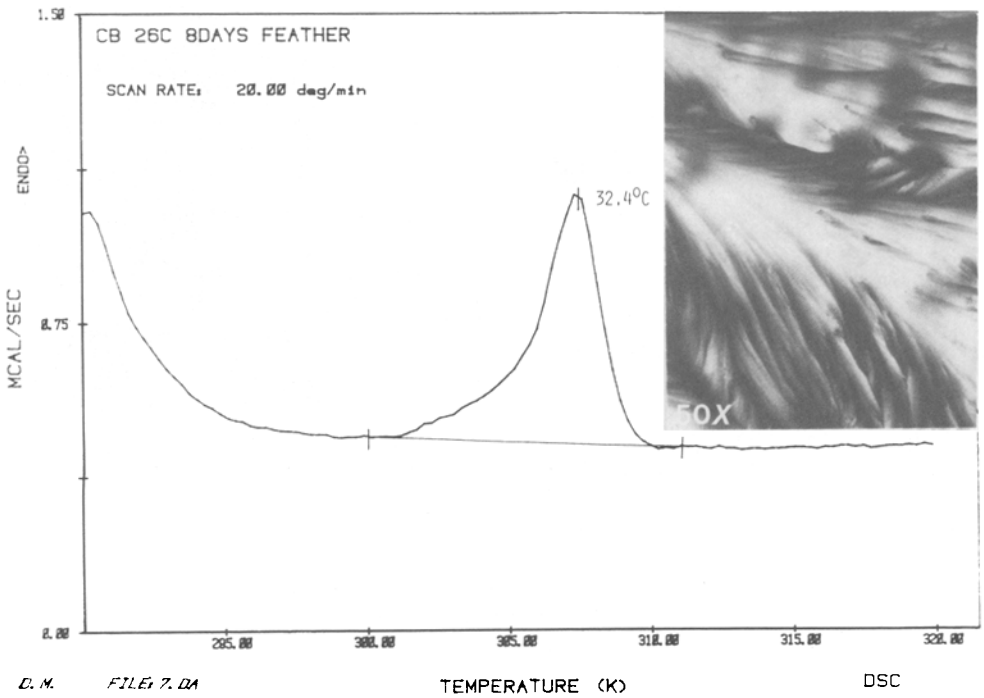

FIG. 3. DSC thermogram of the 'feather' crystal formed at 26.0 C. The 'feather' crystals that melted at 32.4 C are shown in the PLM micrograph.

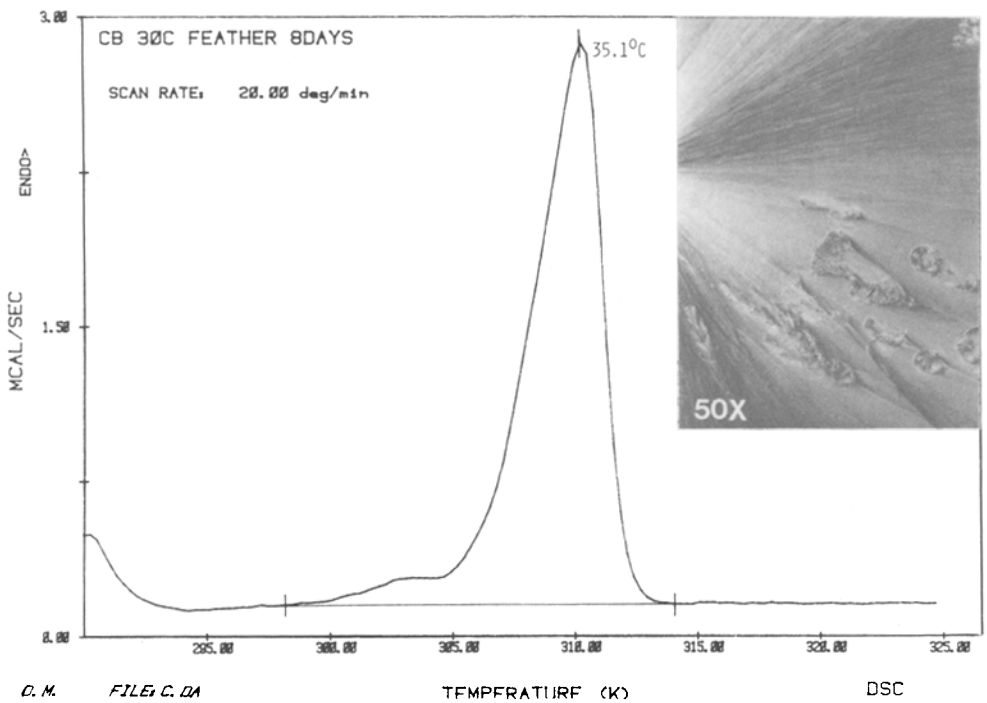

FIG. 4. A DSC thermogram illustrating a 35.1 C melting point for the 'feather' crystal with corresponding PLM micrograph.

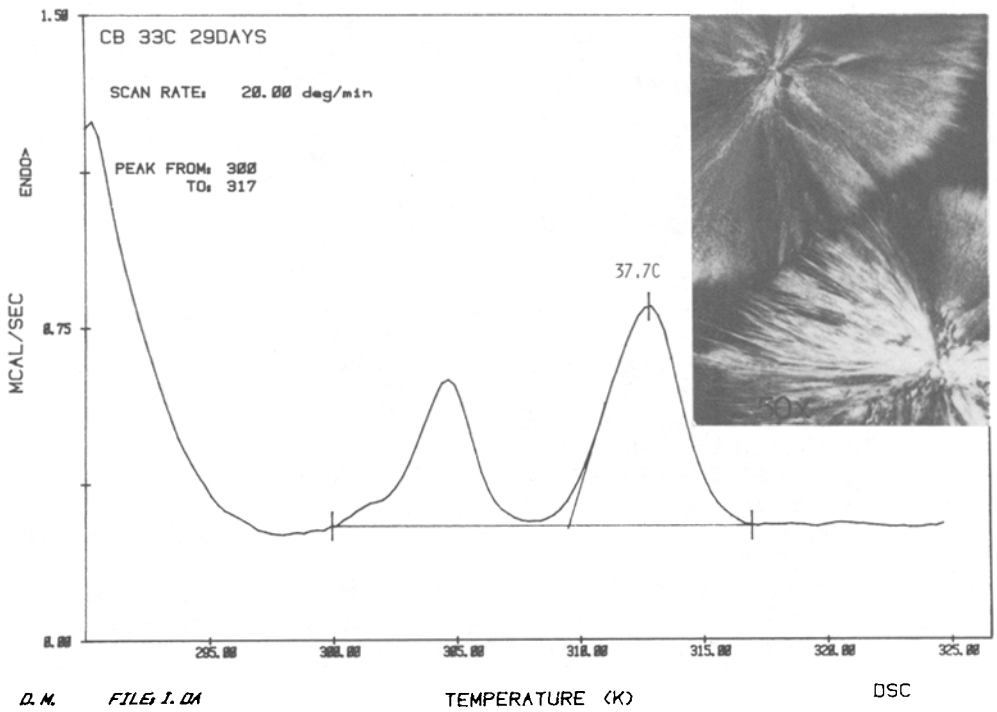

FIG. 5. A DSC thermogram illustrating a 37.7 C melting point 'feather-irregular' crystal mixture with corresponding micrograph.

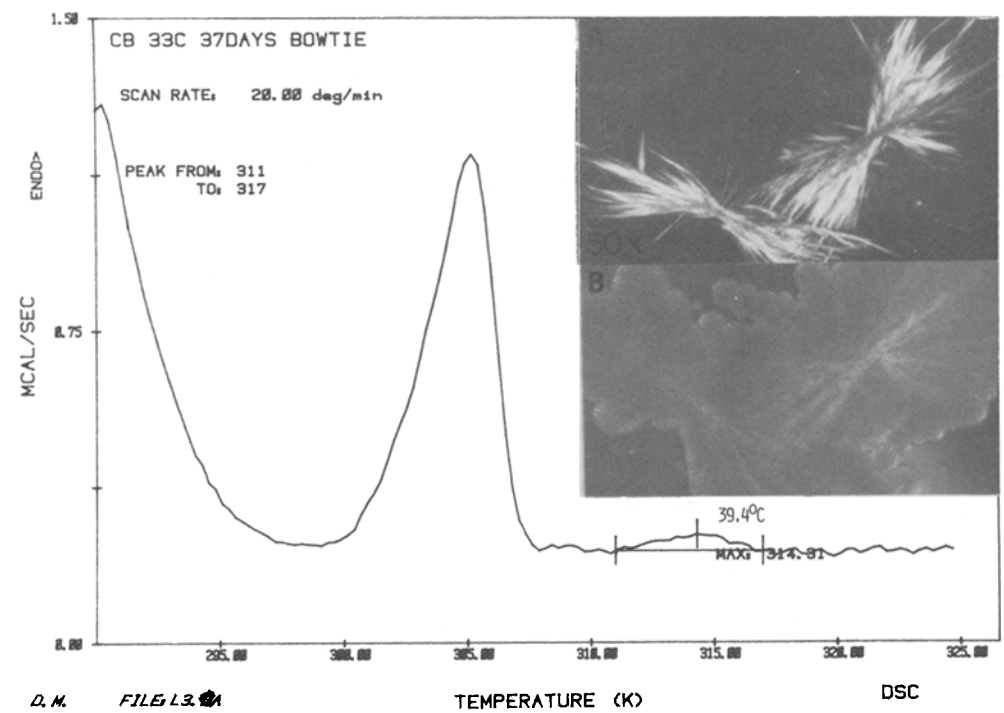

FIG. 6. A DSC melting curve illustrating low melting crystals and two large isolated 'bowtie' crystals. Micrograph illustrating the isolated 'bowtie' crystals (A); micrograph of the 'bowtie' crystals and unstable crystals which produced the two endotherms (B).

was also observed at 32.0 C, but occurred so sparsely that no analysis could be performed. After 10 days at 32.0 C, the 'irregular-feather' crystal mixture had a melting point of 36.2 C. As the crystals matured at 32.0 C, an increase in melting point to 37.2 C was observed.

At an incubation temperature of 33.0 C, 'irregular' and 'feather' crystals solidified from the cocoa butter. The melting point of these crystals was ca. 37.7 C (Fig. 5). A second characteristic of the higher melting point crystals was the observation of 'bowtie' crystal formations within the morphological matrix of the 'feather' crystal. Isolation of the 'bowtie' crystal was difficult due to its fragile structure. To isolate the 'bowtie' shown in Figure 6, Photo A, the polyethylene hoop containing the crystals was removed from the incubator and cooled to room temperature (18 C). Unstable crystals then formed by seeding off the two 'bowtie' crystals (Fig. 6, Photo B). Isolation of the 'bowtie' was accomplished by removing the unstable mass with the 'bowtie' crystals. The endotherm produced by this mass illustrated the melting of the unstable crystals at approximately 29 C and the melting of the 'bowtie' crystals at 39.4 C (Fig. 6). Based on the pure triglyceride data it appeared that the 'bowtie' crystals are made up of an SOS-rich fraction which exhibits a high melting point. The pure triglycerides POP and POS both possess Form I melting points below 39.4 C. These Form I melting points are 37.4 and 38.3 C, respectively (Table 2).

During static crystallization a trend evolved which showed an increase in melting point as the incubation temperature was increased from 26.0 to 33.0 C. In all cases, with the exception of the 33.0 C incubation, the large 'feather' crystal formations were accompanied by much smaller yet distinct solitary 'spiney' crystals. Furthermore, as the incubation temperature was increased, the gross morphology of the crystals varied as illustrated by polarized light and scanning electron microscopy.

The composition of each crystal was defined to determine the role each triglyceride assumes in the crystal formation process. Triglyceride composition of the 'temper' crystal formed at 26.0 C exhibited no significant difference in percent of POP, POS and SOS when compared to the pure Ivory Coast cocoa butter from which it was crystallized (Table 3). The 'feather' and 'individual' crystals formed at 26.0 C exhibited significant increases in SOS and concurrent significant decreases in POP when compared to pure Ivory Coast cocoa butter. The SOS rich 'individual' crystal formed at 26.0 C has a low melting point of 29.6, possibly due to the SOS solidifying in the less stable Form III polymorph (Table 2) which has a melting point of 30.0 C (16). There was no significant difference in POS in any of the three isolated crystals when compared to pury Ivory Coast cocoa butter. At 28.0 C incubation, the SOS was thought to solidify into the Form II polymorph (37.7 C) which compares well with the 37.0 C melting point of the 'spiney' crystal. The increase in SOS was offset, to a degree, by a decrease in POP, PLiP, POO, PLiS, SOO, SLiS, and SOA.

A second set of analyses was conducted on the 'feather' crystals solidified at 26.0, 28.0, 30.0 and 32.0 C. The weight percent SOS in the 'feather' crystals increased significantly with each two-degree increase in incubation temperature. A large increase in the SOS concentration was observed with a general decrease in most of the remaining triglycerides. The weight percent of SOS in the 'feather' crystal increased from 32.6% to 46.4% after the incubation temperature was increased from 26.0 to 32.0 C. In each case a large increase in percent SOS occurred with a concurrent decrease in percent POP, PLiP,

TABLE 2.

Polymorphic Forms and Melting Points of Three Pure Triglycerides Found in Cocoa Butter

Polymorph	Triglyceride Melting Points (°C)		
	POP[a]	POS[b]	SOS[b]
IV[b] (α-2)[a]	18.1	18.2	22.8
III (Sub β'-2)	26.5	24.5	30.0
II (β'-2)	33.5	33.0	37.7
I (β'-3)	38.3	37.4	42.8

[a]Lutton and Jackson, 1950 (15).
[b]Landman et al., 1960 (16).

TABLE 3.

Percent Concentration of the Triglycerides POP, POS and SOS in Pure Ivory Coast Cocoa Butter and Three Visually Different Crystals Formed at 26.0 C[a]

Triglyceride type	Ivory Coast cocoa butter[c]	Crystal types		
		Temper[b]	Feather[c]	Individual[c]
POP	14.8±0.3A	15.1±0.1A	12.9±0.8B	11.9±0.5B
POS	45.4±0.5AB	48.0±1.7A	45.8±2.0A	42.9±1.2B
SOS	28.8±0.2A	27.5±0.2A	32.6±1.9B	36.3±1.4C

[a]Means in a row with the same capital letter are not significantly different at the .05 alpha level.
[b]Mean of two determinations ± standard deviation.
[c]Mean of four determinations ± standard deviation.

TABLE 4.

Percent Concentration of the Triglycerides POP, POS and SOS in Pure Ivory Coast Cocoa Butter and 'Feather Crystals' formed at 26.0, 28.0, 30.0 and 32.0 C[a]

Sample	Triglyceride types		
	POP	POS	SOS
pure butter	14.8 ± 0.3A	45.4 ± 0.5A	28.8 ± 0.2A
26.0 C	12.9 ± 0.8B	45.8 ± 2.0A	32.6 ± 1.9B
28.0 C	10.2 ± 0.3C	45.4 ± 1.6A	39.0 ± 0.2C
30.0 C	8.4 ± 0.6D	43.3 ± 0.4A	43.2 ± 1.3D
32.0 C	8.3 ± 0.5D	37.8 ± 1.1B	46.4 ± 0.6E

[a]Means in a column with the same capital letter are not significantly different at the .05 alpha level. Means are of four determinations ± standard deviation.

POO, PLiS and SOO when compared to pure Ivory Coast cocoa butter. For the cocoa butter crystals grown at the various incubation temperatures, the weight percent POS was not significantly different for the 'feather' crystals; however, at 32.0 C a significant decrease was observed. The weight percent of POP in the 'feather' crystal significantly decreased from 12.9% to 8.3% as the formation temperature was increased from 26.0 C to 32.0 C.

Thermal analyses in these studies illustrated that as the formation temperature increased the melting points of crystals increased. Compositional data exhibited significant increases of SOS in the higher melting crystals. In general, it is safe to speculate and state that the various cocoa butter triglycerides act in a manner characteristic of fractional crystallization. In addition, it must be emphasized that structurally symmetrical triglycerides like SOS will be more suited to solidify with those triglycerides that possess similar chain lengths and symmetry. This has been illustrated with the compatibility of SOS and POS, while POP, containing four less carbons, was not as compatible. The remaining triglycerides of lesser concentration were not as compatible due to larger differences in chain length symmetry and degree of unsaturation.

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