Isolation and Thermal Characterization of High-Melting Seed Crystals Formed During Cocoa Butter Solidification

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Seed crystals which formed during early stages of cocoa butter solidification have been isolated and determined to have extremely high melting points. The melting points of the seed crystals generally exceeded 60°C, in contrast to cocoa butter, which melts between 30-35°C. In addition, the melting point of the seed crystals decreased as a function of crystal growth time. Evidence suggests that the high-melting seed crystal is not an additional polymorphic form of cocoa butter, but rather a distinct crystalline entity. Consequently, a unique compositional make-up is suspected as being responsible for the elevated melting point. A technique to separate seed crystals from the molten cocoa butter mass has been developed. The procedure has been shown not to alter the thermal and compositional properties of the isolated seed crystals.

Numerous studies (1-5) have been conducted pertaining to the solidification of cocoa butter, both in the pure form and in chocolate products. Most of the studies have been directed toward achieving a high quality end product, one that possesses certain desired physical attributes such as snap, high gloss, smooth mouth-feel, narrow melting point, proper contractibility and high fat bloom resistance. The crystalline or polymorphic forms of cocoa butter and chocolate have been thoroughly investigated since the crystalline form has a major impact on the final quality of a confectionery product. To ensure that the fat solidifies in the proper form, tempering must be performed. Tempering is the controlled formation of stable seed crystals which promote the crystallization of the remaining liquid butter mass into the proper crystalline form. These seed crystals must be present in the proper form, size, and number; and form at the appropriate time to achieve the desired characteristics. Studies relating tempering to the attributes of the final crystal and to the prevention or retardation of the fat bloom are common. However, research efforts directed toward understanding the stable seed crystal or the formation of the stable seed crystal have been limited. The objectives described in this study are a) to develop an isolation procedure to separate the solid seed crystal from liquid mass and b) to investigate the thermal properties of cocoa butter seed crystals.

MATERIALS AND METHODS

Sample preparation. Approximately 250 Kg of commercially roasted Ivory Coast cocoa beans were pressed for butter (110°C, 6000 psig, 30 min). The expressed butter, approximately 100 Kg, was filtered through an EV-16 Ertel Engineering Company liquid handling apparatus to remove shell fragments and other coarse debris. The filtration apparatus was equipped with Eimco Filter Media Division grade 534-54 paper filter pads and the liquid butter was subjected to five passes through the apparatus. The temperature of the filtration process ranged from 104°C for the initial pass, to 57°C for the final pass. The average time per pass was approximately 15 min. Further purification of the filtered butter was achieved by centrifugation in a Beckman model J2-21 centrifuge using a Beckman JA-14 rotor. Cocoa butter was heated to 80°C and held for at least one hour prior to centrifugation at 30,000 × g for 15 min at ambient temperature. The clarified cocoa butter was carefully decanted from the fine particulate matter and used in subsequent crystallization studies.

Visible light spectrophotometry. A Gilford Response model utlraviolet/visible spectrophotometer equipped with a Gilford Thermoset temperature controlled sample chamber (26.5°C isothermal) was used to monitor the process of cocoa butter solidification. Absorbance values at 500 nm were recorded as a function of time at 2 min intervals for up to 17 hr. Corn oil was employed as the reference material as it does not crystallize at the temperature of interest. Approximately 2 ml of cocoa butter were placed into quartz cuvettes and heated to 110°C and maintained at the temperature for two hours to destroy crystal memory prior to assay.

Differential scanning calorimetry. Thermal analyses were conducted on a Perkin-Elmer model 4 differential scanning calorimeter (DSC-4) equipped with an Intracooler I freon refrigeration unit. The instrument was calibrated daily with pure gallium (99.999%) to within ± 0.5 degrees of its 29.76°C melting transition temperature and to within ± 0.5 cal/g of its 19.35 cal/g heat fusion. A heating rate of 20 deg/min was used in all cases (6). Onset melting points were determined by a Perkin-Elmer model 3600 Thermal Analysis Data System (TADS). Area integrations were performed by the TADS system as well as manually with the aid of an IBM personal computer utilizing the Omnitech Easy Digit software package and a Science Assessories Corporation graf/bar digitizer. the DSC-4 served two basic functions during these studies: (a) to serve as an isothermal incubation chamber for studying static solidification of small samples of cocoa butter under strictly regulated incubation temperatures, and (b) to obtain thermal profiles of crystals formed during cocoa butter solidification.

Seed crystal isolation procedure. Approximately 10 ml of cocoa butter was placed into a conical, glass centrifuge tube and heated to 110°C for 2 hr to destroy crystal memory. The samples were incubated at 26.5°C. After a specified period of isothermal incubation, cocoa butter seed crystals were isolated from the molten mass. All steps in the isolation procedure were performed at room temperature unless otherwise noted. The conical, glass centrifuge tubes containing the seed crystals suspended in the molten mass were centrifuged at 2000 × g for 2 min. Immediately following centrifugation, the liquid cocoa butter was decanted and the tube was centrifuged

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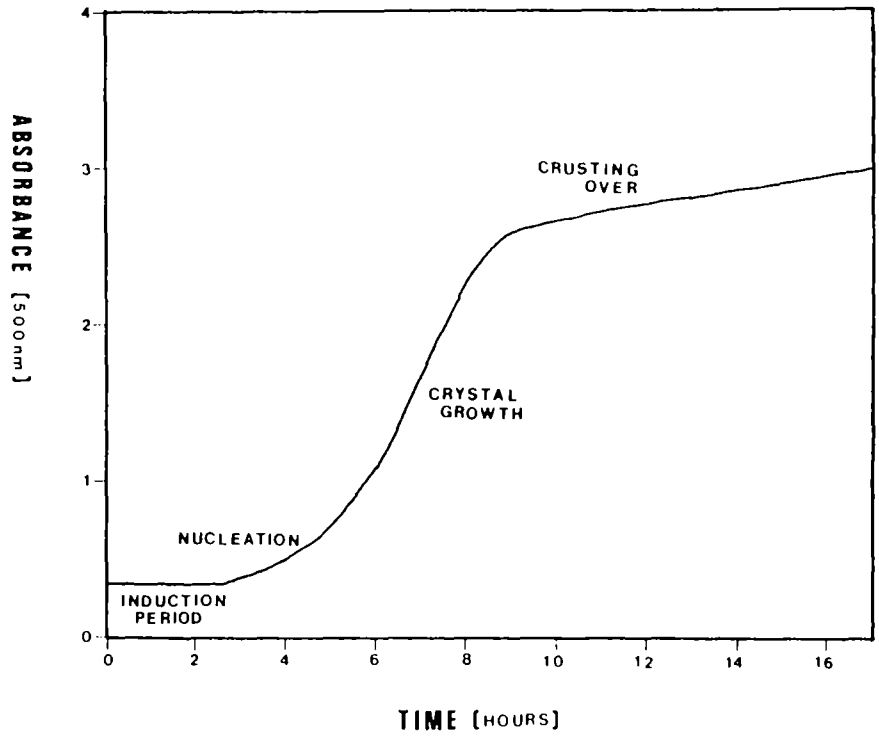

FIG. 1. Solidification curve of Ivory Coast cocoa butter solidified at 26.5°C.

inverted at 2000 × g for 1 min in a centrifuge rotor cup insert (7). The insert allowed the liquid cocoa butter to flow freely from the seed crystals while trapping crystal aggregates on the filter support screen, thus preventing significant loss of seed material. The seed crystals were then washed with a 3 ml aliquot of HPLC grade acetone (26.5°C) and resuspended in the tube by vortexing for less then one second. The suspension was centrifuged again at 2000 × g for one minute in order to concentrate the seed crystals. Again, the liquid was decanted and the tube centrifuged inverted to remove the acetone/liquid cocoa butter fraction. A second solvent washing was performed to remove any extraneous liquid butter. Concentration was again accomplished via centrifugation. Finally, the acetone fraction was decanted and the tubes centrifuged inverted, yielding cocoa butter seed crystals virtually free of liquid cocoa butter contamination.

RESULTS AND DISCUSSION

A solidification curve was produced by plotting the spectrophotometric absorbance reading of the cocoa butter sample as a function of crystallization time. A typical solidification curve for Ivory Coast cocoa butter crystallized at 26.5°C is illustrated in Figure 1. As crystallization progressed, the number and size of the crystals increased, as reflected by the continual increase in the absorbance value. The process of solidification can be divided into four stages—induction, nucleation, growth and crusting-over. The induction period is represented by the region of the curve from 0 hr to slightly less than 3 hr elapsed crystallization time. Nucleation was believed to occur when the absorbance of the sample initially increased, implying crystalline regions were scattering light as it passed through the sample. The crystal growth stage was defined by the exponential increase in absorbance corresponding to the exponential increase in the number and/or size of the crystals. Crusting-over is represented by the plateau of the solidification curve as suggested by the decrease in the crystallization rate. Seed crystals are assumed to be crystals present in the early stages of the crystal growth stage of solidification. In these studies, crystals present at 3-6 hr were considered to be seed crystals. Initially, the 0, 1, and 2 hr cocoa butter samples were clear, but became slightly turbid after 3 hr elapsed crystallization time. The clouding phenomenon gradually continued until distinct crystalline regions or structures could be visibly identified after six hours. Crystal structures increased in size as crystallization progressed while the amount of liquid butter decreased considerably. After 10 hr elapsed time, the sample appeared solidified, but the butter was not completely hardened. Only minor changes in the visual appearance of the sample occurred after nine hours. The spectrophotometric method of monitoring solidification provides a means of rapidly determining a solidification curve. This curve can be used to determine the stage of crystallization from which crystals were isolated.

Melting points of crystals (onset or transition temperatures) formed under static crystallization conditions at 26.5°C and estimates of the degree of crystallization during solidification were determined using DSC. Figure 2 illustrates the melting points of seed crystals present in Ivory Coast cocoa butter crystallized statically at 26.5°C in the DSC sample cell. The thermograms were produced by scanning from 26.5°C to 110°C at 20 deg/min after isothermal incubation for the specified time. Scans were initiated at 26.5°C so as not to cause secondary crystalli-

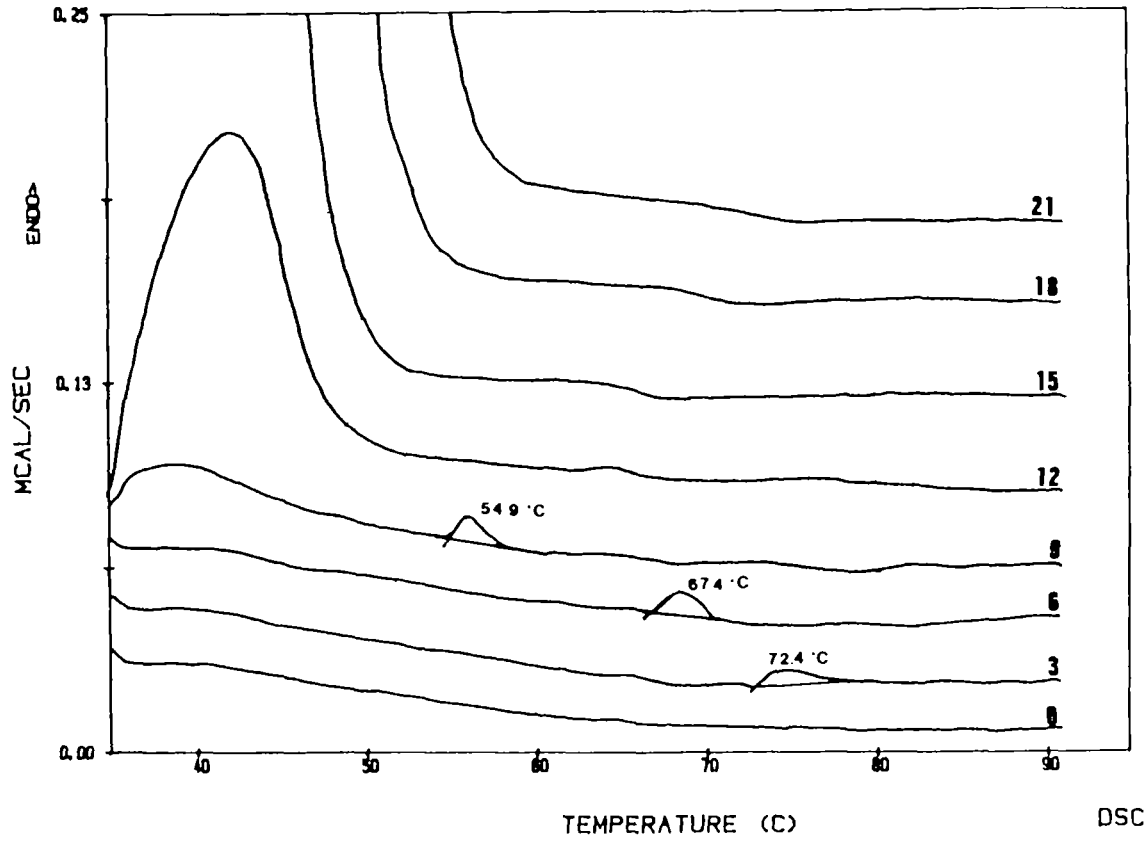

FIG. 2. DSC thermal profiles of seed crystals present after 0, 3, 6, 9, 12, 15, 18 and 21 hr static crystallization at 26.5°C.

zation upon cooling. As expected, seed crystals were not present at 0 hr since crystal memory was destroyed prior to incubation. An endotherm was first evident in the 3 hr incubation profile. The presense of an endotherm implies crystalline material has undergone melting. The crystals formed after 3 hr crystallization displayed an elevated melting point of 72.4°C. This melting point is significantly higher than the melting point previously reported (8) for the high-melting "bow-tie" seed crystal structure (39.4°C). Such an elevated melting point for cocoa butter crystals has not been reported. As crystallization progressed, the melting point of the crystalline material present decreased. An onset melting point of 67.4°C was observed for the six hour incubation profile while that for the nine hour elapsed crystallization time was 54.9°C. The exact melting transition of the low melting endotherm could not be determined since the scan was initiated at 26.5°C. The melting points were estimated to be <40°C.

The continual decrease in melting point for the crystals as crystallization progressed may be explained by the addition of lower melting glyceride species. These lower melting glyceride species are likely integrated into the existing crystal resulting in the reorganization of the crystalline structure. Such an integration/reorganization phenomenon explains the presence of a single endothermic peak. The trend of glyceride integration should continue until the final melting point of the crystal is reached—approximately 33°C for Form V (9) cocoa butter crystals. This hypothesis is supported by the presence of the lower melting endotherm in the 9, 12, 15, 18 and 21 hr thermograms. As crystallization progressed, the magitude of the low-melting endotherm increased, implying that the crystals were growing rapidly. Hence, the crystalline material present can be referred to as seed crystals.

Differential scanning calorimetry was also used to provide an estimate of the extent of crystallization. The percentage of liquid cocoa butter crystallized as a function of time was determined by comparing the area under the endotherm(s) of a partially solidified cocoa butter sample to the area under the endotherm of the same cocoa butter sample which has been completely solidified. The ratio of areas was used as an estimate of the degree of solidification. A completely solidified sample was obtained by quench cooling a cocoa butter melt in liquid nitrogen and scanning from 0°C until completely melted. The most stable polymorphic form of cocoa butter will not be formed, but the sample will be completely solidified in the lowest melting form (13°C) (10). Table 1 presents the values obtained for the degree of solidification as a function of time for the endotherms presented in Figure 2. The degree of solidification values reveal that the high-melting seed crystal is formed early in the solidification process in terms of the quantity of liquid cocoa butter solidified. In fact, less than 0.01% of the liquid phase was solidified when the seed crystals first appeared. The estimates for the degree of solidification further support the division of the solidification process into various stages (Fig. 1).

TABLE 1

Solidification for Ivory Coast Cocoa Butter Crystallized Statically at 26.5°C

Incubation time (hr)	Melting point (°C)	Degree of solidification (%)	Stage of crystallization
0	—	0.00	Induction period
3	72.4	<0.01	Nucleation
6	67.4	0.01	Crystal growth
9	54.9	0.07	Crystal growth
12	30-35	1.74	Crystal growth
15	30-35	4.16	Crystal growth
18	30-35	17.42	Crystal growth
21	30-35	31.40	Crystal growth

*Onset or transition temperature determined by DSC.

Seed crystals isolated from the liquid mass generally displayed melting points in the 60-65°C melting range. Numerous trials were performed with similar results obtained for each trial. The exact melting point of the isolated crystals, however, varied because crystallization processes, even for the duplicate samples, are never identical. Figure 3 illustrates the thermal profile of seed crystals isolated after six hour isothermal incubation at 26.5°C. The thermogram in Figure 3 reveals two interesting facts. First, the existence of a high-melting seed crystal (62.0°C) was confirmed. Second, the sharp melting transition indicates that the seed crystals are uniform with respect to thermal properties. This implies the seed crystals were similar in size and were representative of a narrowly defined region on the solidification curve. The presence of only a small low-melting endotherm indicates the isolated seed crystals have been effectively separated from the surrounding liquid mass.

In situ seed crystals (seed crystals not subjected to the isolation procedure) formed under identical conditions displayed a melting point similar to that of isolated seed crystals (Fig. 3). Comparison of the DSC scans shows similar melting points of 62.0°C and 63.1°C for isolated seed crystals (A) and *in situ* seed crystals (B), respectively, formed under the same conditions. The slight discrepancy in melting point was attributed to the inherent variance of the crystallization process. Hence, the separation technique does not appear to drastically alter the melting properties of the seed crystals.

The acetone washing step utilized in the separation procedure also could have altered the composition of the seed crystals by solubilizing both the liquid and solid phases instead of only the liquid phase as intended. Although this is difficult to prove, an experiment was designed to provide an estimate of the amount of solid cocoa butter dissolved by the acetone washings. Tempered cocoa butter was chilled to -5°C in order to induce "complete" solidification of any liquid present. The solidified cocoa butter was then subjected to acetone washings similar to those utilized in the isolation procedure. The acetone fraction was collected and the acetone evapo-

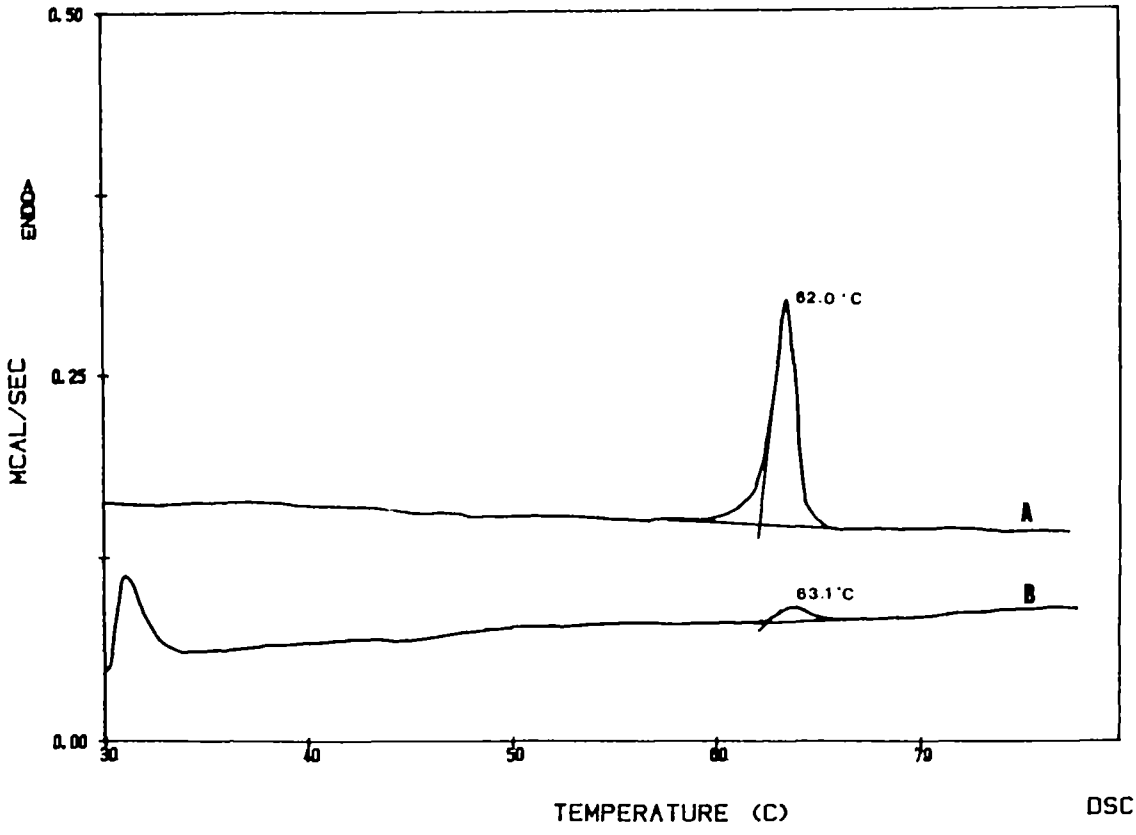

FIG. 3. DSC thermograms of high-melting seed crystals; A) isolated seed crystals; B) *in situ* seed crystals, formed after 6 hr static incubation at 26.5°C.

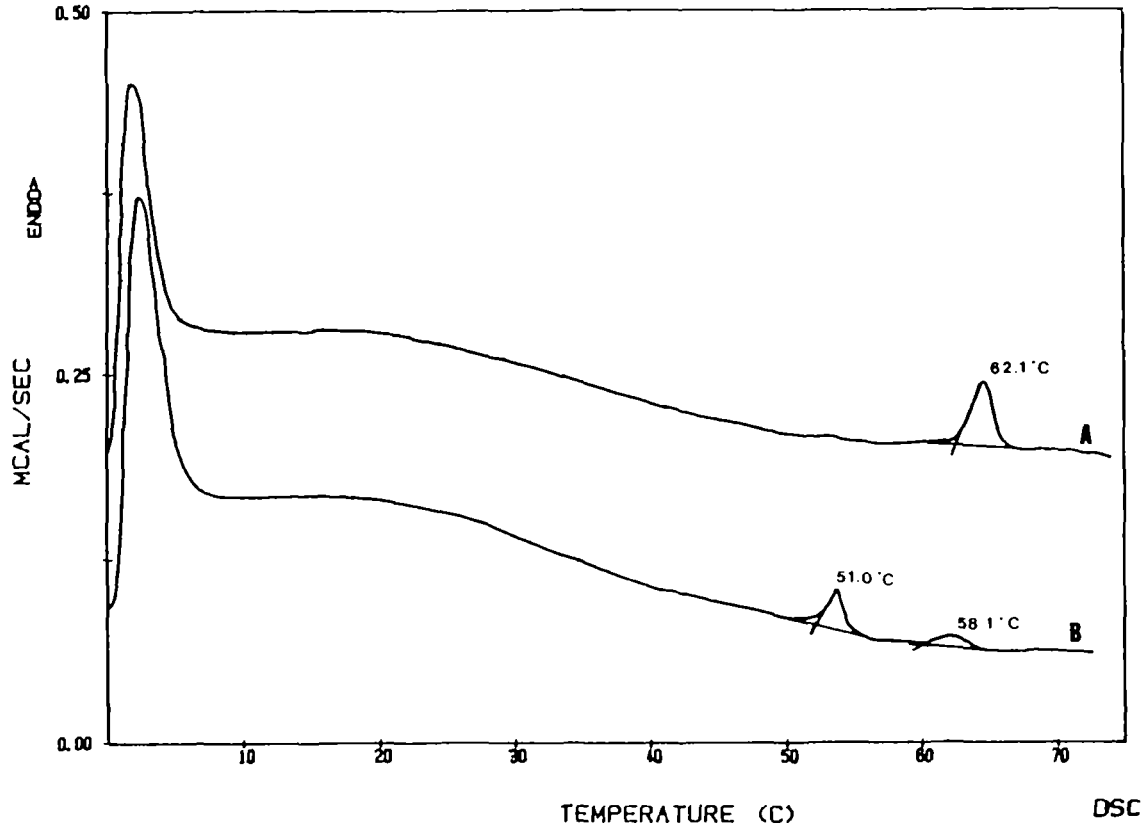

FIG. 4. DSC thermal profiles of A) isolated seed crystals, and B) lower melting polymorphic forms of isolated crystals.

rated to determine the amount of cocoa butter present.Results revealed that on average less than 5% (3.45% ± 0.93%, n = 3) of the total sample was dissolved by the acetone washings. This implies that the solvent washing step did not solubilize crystalline cocoa butter to any significant extent. Hence, the isolation procedure was considered not to drastically alter the composition of the seed crystals.

As demonstrated by the results of DSC studies, the seed crystals formed during the early stages of cocoa butter solidification possess unusually high melting points for cocoa butter crystals. Such elevated melting points can possibly be accounted for by a difference in the chemical composition in relation to the other forms of cocoa butter crystals, or a difference in the molecular packing of the molecules. Polymorphism is the ability of a substance to exist in more than one crystalline form. The high-melting seed crystal was not considered an additional polymorphic form of cocoa butter because the seed crystal was in the midst of the growth process. Therefore, the crystal was continually changing in chemical composition. In order to be considered a true polymorph, the seed crystals would have to possess a chemical composition identical to the other polymorphic forms of cocoa butter. Previous research (8) reported an elevated 1,3-distearoyl-2-oleoyl triacylglycerol content of the "bow-tie" crystal in comparison to liquid cocoa butter.

The high-melting seed crystal was not an additional polymorphic form of cocoa butter based on dissimilar composition. Isolated seed crystals that melted at 62.1°C were heated to 110°C and held for 15 min to destroy all crystal history. The melted sample was then rapidly cooled to 0°C and maintained at 0°C for 1 min before scanning from 0°C to 110°C. Rapid cooling assured that the sample solidified in its lowest polymorphic form(s). Two lower melting, less stable polymorphs formed as a result of the cooling process—a major endotherm with a 51.0°C melting transition and a minor polymorph possessing a 58.0°C melting point (Fig. 4). Cocoa butter treated in the same manner also resulted in two lower melting endotherms— polymorphic Forms I (13.1°C) and II (17.7°C) (Fig. 5). It was therefore concluded that the seed crystal was not a polymorphic form of cocoa butter.

Several phenomena observed visually during the course of these solidification studies add to the general understanding of the process of cocoa butter solidification. A high-melting seed crystal fraction was completely melted and allowed to statically crystallize at room temperature. Generally, resolidification to a hardened form occurred within a few minutes. Conversely, tempered cocoa butter crystals treated identically required several hours to resolidify to a similar hardened form. More interestingly, cocoa butter melts from which the seed crystals were removed required considerably longer periods to resolidify to a hardened form than the original cocoa butter melts. These observations imply that the seed crystal material plays a critical role in the initiation of the crystallization process and any information pertaining to seed crystal formation can be used to better understand the mechanism of solidification.

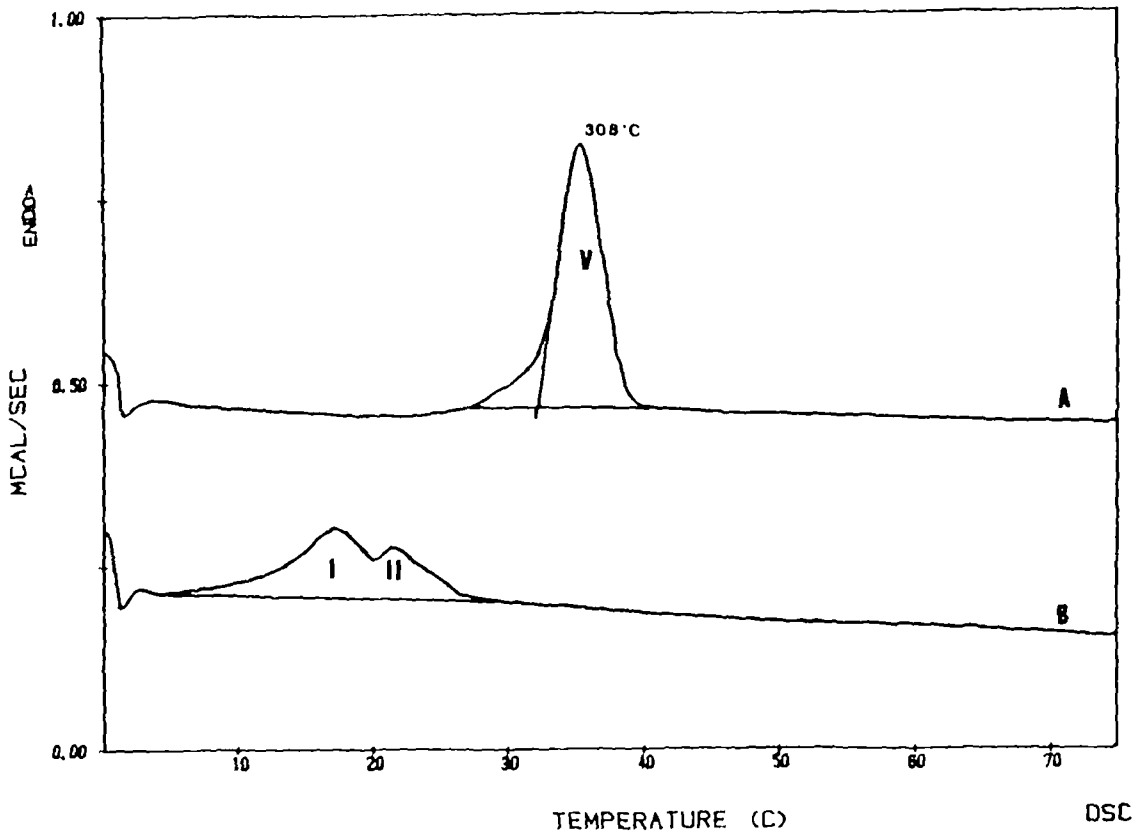

FIG. 5. DSC thermal profiles of A) tempered cocoa butter crystals (Form V), and B) lower melting polymorphic forms (Form I and II).

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