

Differential Scanning Calorimetry of Confectionery Fats. Pure Triglycerides: Effects of Cooling and Heating Rate Variation

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Differential scanning calorimetry (DSC) measurements of the crystallization and melting phenomena of pure forms of the three principal triglycerides present in cocoa butter and related confectionery fats are presented. The results are used to exhibit the usefulness of the DSC technique for potential application in quality control of these types of material, but also as a warning of the difficulties in interpretation of data. The results also serve as a reference for future use in DSC studies of similar materials.

KEY WORDS: Confectionery fats, differential scanning calorimetry, heating, melting, quality control, triglycerides.

An essential aspect of the industrial manufacture of edible oils and fats is the ability to measure the physical properties of the materials. This is true from the point of view of basic component selection and also for the assessment of the final product. As the industry has progressed and become more sophisticated, so too have the methods employed for assessing physical properties. These methods range, for example, from straightforward measurements of time-temperature cooling characteristics, with simply a thermometer and a time piece, to the measurement of behavior at a molecular level, by using sophisticated techniques such as nuclear magnetic resonance (NMR) or X-ray diffraction.

The physical properties of greatest interest are the crystallization and melting phenomena that encompass both solid fat content and polymorphic behavior. At the molecular level, NMR and X-ray diffraction probe the state of individual atoms (such as their relaxation behavior or relative spatial arrangement). From these data a solid/liquid content or structure can be calculated. They are, in a sense, direct measurements, which could be used to monitor changes that occur during cooling or heating. At the macroscopic level we can measure the consequences of molecular changes by observing some gross physical condition of the oil or fat, such as the density change on crystallization or the temperature hold-up as the heat of fusion is dissipated throughout the sample. These methods are therefore indirect.

In this paper we address ourselves to the use of a technique that measures the fundamental quantity energy change during cooling or heating, but on a macroscopic quantity of sample. In differential scanning calorimetry (DSC) those energy changes are measured that reflect specific heat capacity and changes of phase as materials are either cooled or heated (1). DSC is preferred to other similar calorimetric methods, such as differential thermal analysis (DTA), because in the former it is the fundamental quantity of energy that is measured. In the latter the temperature difference between the sample under test and some reference is recorded as they are both heated (or

cooled) at the same rate. In these circumstances an independent knowledge of the sample's specific heat capacity is required at all temperatures in order to convert temperature difference to energy. To an advanced degree the DSC technique is now commonly available in industrial as well as academic laboratories.

In the oils and fats industry a major use of physical property measurement is for quality control. Another area where physical property data are used is in the innovation and development of new products. For both services there is an ever pressing need to search for more precise, reproducible and speedy methods of characterization; recent developments in the technical aspects of DSC (namely computerization) provoke a re-appraisal of its use in estimating the characterization of confectionery fats. Computerization brings with it enhanced control of the DSC experiment and more powerful data acquisition capabilities. A wider range of instantaneous, on-line data analysis possibilities are also available.

For many years DSC has been used in the characterization of confectionery fats, cocoa butter and pure triglycerides [for recent examples see the works and cited literature of Dimmick *et al.* (2), Gibon *et al.* (3) and of Garti and Schlichter *et al.* (4-6)]. General effects on the form of the thermogram resulting from variations in cooling or heating rate of any material are already known. For example, faster rates shift peaks to lower temperatures in cooling and to higher ones in heating. Peak broadening occurs progressively as rates are increased. Such effects result from thermal lag, which occurs as temperatures are scanned at different rates. To a large extent this effect can be removed by calibration of the calorimeter at every scanning rate used and correcting each sample scan as appropriate. Effects of cooling and heating rate imposed on the characteristic polymorphic behavior properties of specific confectionery fats have not been reported, however. Previous studies (7) have, nonetheless, dealt largely with elucidation of details of polymorphism. Such studies have concentrated on the manipulation of cooling and heating to achieve as many aspects and details of polymorphism as possible and employed optimized and judicious processes of thermal cycling to do so.

The major fat components of cocoa butter and related fats are the symmetrical disaturated, monounsaturated (SUS) triglycerides. Since the crystallization and melting characteristics of a fat will depend to a large extent on the major components, pure SUS triglycerides provide ready model materials which can be used to investigate the DSC technique. Results are reported here from experiments performed on three pure triglycerides, which show effects of variation of cooling and heating rate on the data produced. It is the objective of this study to review the effects on DSC thermograms of variation in the cooling/heating protocol and to give an indication of the sensitivity obtainable with a view to quality control. It is not intended to review the detailed polymorphic behavior of each of the materials studied because this can be found elsewhere in a comprehensive review (8). In ad-

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dition, it is intended that the discussion of the data presented maintains an eye on the difficulties of interpretation of DSC experimental data.

EXPERIMENTAL PROCEDURES

Materials. Quantities of pure (better than 98%) 1,3-dipalmitoyl-2-oleoyl glycerol (POP), 1,3-di-stearoyl-2-oleoyl glycerol (StOSt) and 1-palmitoyl-2-oleoyl-3-stearoyl glycerol (POSt) were synthesized at Unilever Research Laboratory in Vlaardingen. Prior to study they were each dissolved in hexane and passed through a silica column to remove diglycerides and other polar materials. The triglycerides were then re-crystallized from acetone. Chemical analyses of carbon number gas-liquid chromatography (GLC) and silver phase high-performance liquid chromatography (HPLC) were performed, and the results appear in Table 1.

DSC apparatus. The calorimeter used for the experiments was a DSC7 module made by Perkin Elmer (Perkin-Elmer, Norwalk, CT) with a PE7500 computer to record and analyze the cooling and heating thermograms. This instrument has a maximum sensitivity of $8 \mu\text{W/s}$ and temperature precision and accuracy each of $\pm 0.1^\circ\text{C}$. Heating and cooling rates are available between $0.1\text{--}500^\circ\text{C/min}$ in increments of 0.1°C .

Materials used to calibrate temperature and energy were gallium (melting point 29.78°C) and indium (melting point 156.6°C and heat of fusion 28.45°J/g). The scanning rate used for calibration of the temperature scale was 5°C/min and the calibration was checked by measuring the melting point of deionized, distilled water.

Experimental procedure. Samples of each triglyceride (approximately 8 mg) were accurately weighed into aluminum pans. An empty pan was used as a reference. The samples were melted at 100°C for 2 min to remove all crystal nuclei resulting from previous solid states. In the first instance two cooling stages were applied to each sample in sequence. First, a rapid cooling at between 100°C and 200°C/min to a temperature of about 40°C was applied. This brought samples to a point well above that at which spontaneous crystallization would occur. Then, second, a slower cooling rate between 0.1 and 20°C/min was used for each sample down to between -10°C and -30°C , during which thermograms were then recorded for comparisons. On attaining that temperature samples were immediately heated to 50°C at a rate of between 1°C and 20°C/min . In the second instance samples were rapidly cooled from the melt straight down to -10°C . These samples were then reheated at a rate of between 1°C and 20°C/min . Therefore, three types of thermogram were recorded for each of POP, POSt and StOSt: i) Cooling at a rate between 0.1 and 20°C/min ; ii) reheating at 5°C/min of each sample cooled as in i); and iii) reheating at rates of between 0.1 and 20°C/min for samples that had been rapidly cooled from the melt. In addition, samples of each triglyceride that had been recrystallized from acetone were heated in the calorimeter at 5°C/min to produce melting point data of the most stable (β) polymorphic form.

The cooling and heating thermogram traces were analyzed and records were kept of the temperatures of the peak maxima and minima, peak onset positions (the temperatures associated with the steepest part of energy release or take-up), and overall energy associated with each

process. Complex thermogram features that were not easily quantified, such as shoulders not separable from peaks, were noted.

RESULTS AND DISCUSSION

The chemical analysis of the triglycerides is given in Table 1. The thermograms are presented in Figure 1 for POP, Figure 2 for POSt and Figures 3 and 4 for StOSt. They show some of the various cooling and heating conditions applied for each sample. From all the Figures and the data for individual samples, certain general observations can be summarized. These are in addition to the experiment-dependent features (peak shifting and broadening) that occur as thermograms were analyzed following an instrument calibration at a single scanning rate: i) The number of peaks in any single trace depends not only on the cooling and heating rate but also on the individual triglyceride; ii) rapidly cooled samples underwent progressive polymorphic transitions on subsequent reheating; iii) the degree of transformation on heating was greater at slower heating rates and greatest for POP. It was least for POSt; iv) the heats of melting for the most stable polymorphic forms encountered in this study were POP = 124°J/g , POSt = 130°J/g , StOSt = 133°J/g (these are 103.2 , 111.8 and $118.1^\circ\text{kJ/mole}$, respectively). These are to be compared with 174.4 , 176.3 and 178.2°J/g obtained from the solvent-recrystallized solids; and v) on heating, a total of four polymorphic forms were recorded for StOSt, POSt and POP.

In Figure 1(a) two cooling traces (0.1 and 20°C/min) are shown for POP. Scans at rates between these extremes (not shown) display intermediate behavior. The ordinate scales are individually set to accommodate the peak maxima and minima. Energies of fusion decrease with faster cooling rates but remain constant above 1.0°C/min . The changes in peak shape are clearly evident for the relatively simple process of cooling POP. The peaks broaden and the peak position shifts to lower temperature (from about 23°C to about 7°C) as the cooling rate is increased. In Figure 1(b) the change of behavior at a cooling rate of 0.1°C/min is also seen in the heating trace. As the cooling rate increases, a greater proportion of the solid formed is in the unstable phase, which melts at $\approx 14^\circ\text{C}$. At the very slow cooling rate (0.1°C/min), formation of this unstable material is almost entirely absent.

Figures 1(c)–(e) show the difficulty in assigning polymorphic form from a heating trace alone. After rapidly cooling the POP in the same way, subsequent heating rates appear to generate different polymorphic forms from the same original solid. Clearly the sample is undergoing different polymorphic transitions, which are dependent on the rate of temperature change in the heating process. For example, on heating at 1°C/min transitions appear at about 14°C , about 26°C and about 32°C . For more rapid heating at 5°C/min , [1(d)] melting at about 14°C is more clear, and it finally becomes the dominant process at 20°C/min .

For POSt the cooling process is somewhat more complicated than for POP. In Figure 2(a) cooling rates of 0.1°C/min and 20°C/min are shown. Relatively slow cooling rates ($<0.8^\circ\text{C/min}$) cause the crystallization of only one polymorphic form. But at faster cooling rates ($>\approx 3^\circ\text{C/min}$), a certain amount of the fat crystallizes in

DSC OF CONFECTIONERY TRIGLYCERIDES

TABLE 1

Carbon Number GLC and Silver Phase HPLC Analysis of the Pure Triglycerides Used

Triglyceride	Carbon number					HPLC		
	C48	C50	C52	C54	Other	SSS	SOS	SSO
POP	0.1	99.5	0.3	—	0.1	0.1	99.9	—
POSt	0.2	0.2	99.6	—	—	0.2	99.2	0.6
StOSt	—	0.3	0.3	99.2	0.2	—	99.2	0.8

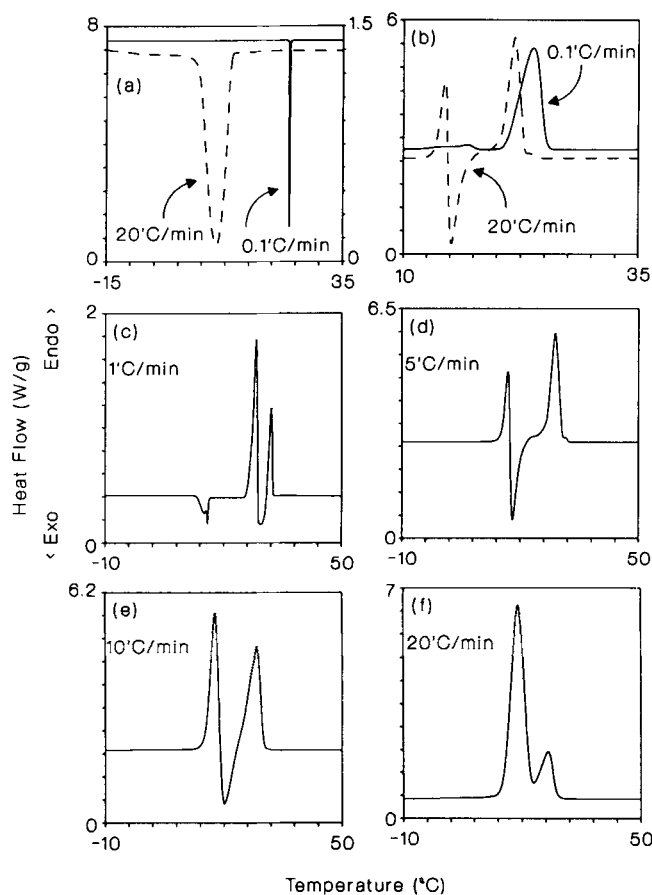


FIG. 1. Effects of cooling and heating rate variation of the thermograms for POP. All samples were first melted at 100°C for 2 min and then rapidly cooled. (a) Shows subsequent cooling from about 40°C at 0.1 and 20°C/min. For the faster cooling rate the left hand ordinate should be used and for the slower rate the right hand one is relevant. (b) Shows reheating at 5°C/min of the samples in (a), where it can be seen that the sample cooled slowly has formed only the stable polymorphic form. The sample cooled at 20°C/min shows transition of the unstable form and then melting. (c)-(f) Show the thermograms for samples immediately reheated at 1, 5, 10 and 20°C/min, respectively, after rapid cooling down to -10°C from 100°C. As the heating rate is increased, less and less of the more stable form (melting at about 30°C) is seen until, at 20°C/min, the melting at about 17°C dominates.

a less stable form at lower crystallization temperature (crystallization continues down at about -10°C and is seen for a cooling rate of 20°C/min), presumably because the temperature was lowered too quickly for complete crystallization of the stable form. Figure 2(b) also shows that even when cooling traces appear similar (*viz.*, cool-

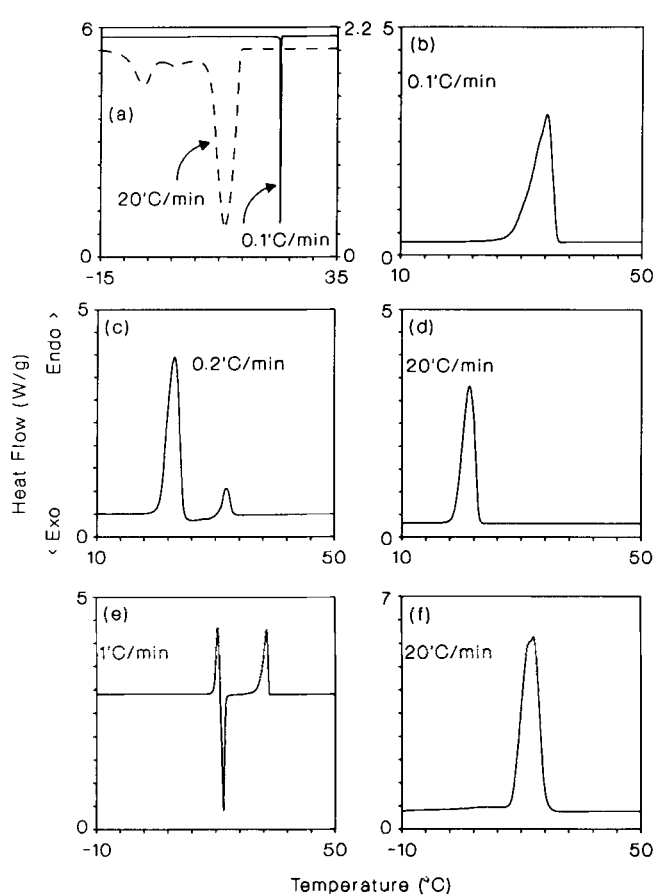


FIG. 2. Effects of cooling and heating rate variation on the thermograms for POST. All samples were first melted at 100°C for 2 min and then rapidly cooled. In (a), traces are shown of cooling from about 40°C at 0.1 and 20°C/min. As in Figure 1, ordinate scales are individually set. As for POP, the feature broadens and shifts to lower temperature for the faster rate. Additionally, crystallization at lower temperatures is sustained. In (b), (c) and (d) the subsequent heating traces at 5°C/min after cooling at 0.1, 0.2 and 20°C/min are shown. The reheating shows that only the more stable polymorph, which melts at about 20°C, was produced on rapid cooling. (e) and (f) show heating at 1 and 20°C/min after rapid cooling from 100°C. The slower heating rate allows transition from the less stable to the more stable form, but the rapid heating only permits melting of the less stable form.

ing at 0.1°C/min and 0.2°C/min), the subsequent heating at 5°C/min does not necessarily reveal the crystal formed to be of the same polymorphic type. In Figure 2(b) the first polymorphic form is seen to melt between about 30-35°C, whereas the more rapid cooling at 0.2°C/min produced a lot of unstable form, which melted at about 22°C.

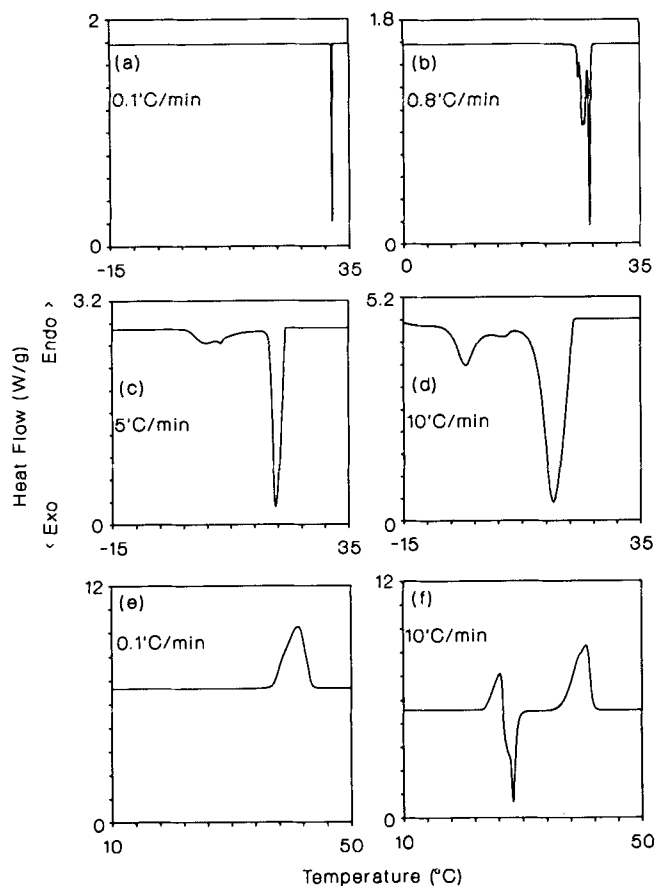


FIG. 3. Effect of cooling rate variation and heating on StOSt. All samples were first melted at 100°C for 2 min and then rapidly cooled. In (a)–(d) cooling curves from about 40°C at different rates of 0.1, 0.8, 5 and 10°C/min, are shown respectively. These reveal the development of complex crystallization behavior as the cooling rate is increased. In (e) and (f) are shown the reheating at 5°C/min of samples previously cooled at 0.1 and 10°C/min, respectively. As for both POP and POST, these show that on slow cooling (0.1°C/min) only the more stable polymorph is produced, which melts at about 40°C. At the higher cooling rate of 10°C/min a significant amount of the less stable form is produced, which transforms on heating.

Cooling at 0.2°C/min is sufficiently slow to allow some crystallization of the stable form, Figure 2(c). However, cooling at 20°C/min produced melting at a subsequent heating rate of 5°C/min of only unstable crystals [Fig. 2(d)]. This indicates that two different polymorphic forms may appear to have the same nucleation temperature, but that the kinetics of formation dominate the equilibrium thermodynamics.

Figures 2(e) and 2(f) show the melting behavior at different rates after quench cooling in POST. The results are similar to that in POP, whereby slow heating rates facilitate polymorphic transitions as the temperatures rise slowly [Fig. 2(e)]; but the faster heating does not permit the recrystallization process to the higher form. Only slow heating at 1°C/min shows a melting process at $\approx 32^\circ\text{C}$.

Figures 3(a)–(d) show that the cooling behavior of StOSt is as complex as that for POST. Rapid cooling (at 20°C/min) resulted in continued crystallization at low temperatures as for POST. For reheating at 5°C/min [in Figure 3(e), and (f), at 0.1 and 10°C/min, respectively]

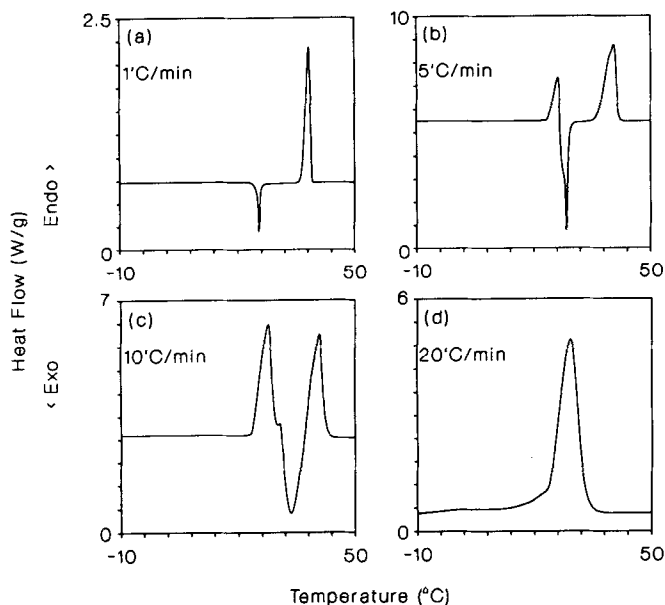


FIG. 4. Effect of heating rate variation on StOSt after rapid cooling from 100°C down to -10°C . As the heating rate is increased, less and less of the unstable form converts to the more stable form until, at 20°C/min, only melting of the unstable form occurs.

fast cooling (20°C/min) of POST produced only one polymorph; a cooling rate of only 5°C/min of StOSt produced at least two forms on heating. Figure 4 shows the effects of heating rate variation of 1, 5, 10 and 20°C/min [Figure 4(a)–(d), respectively] after rapid cooling. Clearly the heating rate enables transitions of polymorphic form to take place, e.g., Figure 4(a)–(c), but rapid heating produces only melting of the least stable form. In reviewing Figures 1(c), 2(c) and 4(a), it is clear that the propensity for polymorphic transformation is greatest for POP and least for POST.

Table 2 shows a summary of the melting ranges obtained for the distinct polymorphic forms obtained here and the energies associated with them. The assignment of polymorphic form given is based on comparisons with the summary data of Hagemann (8). In our work, despite the only simple form of the heating scan, four of the polymorphic forms of each triglyceride were revealed. For StOSt and POP these are each a sub-set of the five forms for StOSt and seven forms for POP designated by Sato *et al.* (7). The reason that the full range of polymorphic forms were not seen here is that no optimization of the heating regime [such as the cycling employed in (7)] was attempted.

The data collected at different rates of cooling and heating expose the subtle differences in kinetics that govern the transformations taking place in the three pure triglycerides.

DSC as a measuring technique for oils and fats. Clearly emerging from this series of DSC experiments on model systems for confectionery fats, is the speed and ease of data acquisition brought about by having powerful computer control. This is evident from the distinctive effects that result from changes in cooling and/or heating techniques applied. In the future, this degree of control may be

DSC OF CONFECTIONERY TRIGLYCERIDES

TABLE 2

Melting Point-Ranges and Energies Measured for the Polymorphic Forms of the Pure Triglycerides Determined by DSC^a

Triglyceride	Melting point (onset [°C])	Heat of melting (J/g)	Polymorph
POP	36.4	174.4	β
	27.3-27.5	133 -134.8	β'
	25.9-26.9	114.5-116.5	β'
	15.4-15.5	83.1- 85.1	α
POSt	34.9	176.3	β
	29.7-30.7	127.6-132.9	β'
	19.9-20.7	90.5- 93.5	α
	18.8-19.0	86.8- 88.1	α
StOSt	40.8	178.2	β
	36.3-36.8	131.2-132.5	β'
	23.2-24.5	81.6- 85.2	α
	16.8-17.4	68?	—

^aAssignment of the polymorphic form was by comparison with data tabulated in reference 8. Data for the β polymorphic forms were obtained on samples recrystallized from acetone. Due to the very rapid transformation of the unstable phase of StOSt melting at about 17°C, a confident calculation of the energy could not be achieved.

put to good use for distinguishing simple melting from recrystallization, both of which take place in a similar region in the temperature scale. By manipulating the rates progressively, the kinetically controlled process of crystallization could be separated from the thermodynamically controlled melting process.

The demonstrated accuracy with which data are obtained is very encouraging and, that, in combination with the sensitivity to subtle changes in the chemical composition

of the sample, makes it an attractive possibility for development as a quality control procedure.

DSC data of pure triglycerides. The data presented on the pure triglycerides, which constitute the major components of cocoa butter and cocoa butter equivalent fats, are the first of their kind to show how quality control procedures by DSC might be constructed for confectionery fats in general. Detailed scrutiny of the polymorphism of the three triglycerides is given by other authors (7-10), against which background our data should be viewed.

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