Differential Scanning Calorimetry of Confectionery Fats: Part II—Effects of Blends and Minor Components

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Differential scanning calorimetry (DSC) measurements of the crystallization and melting phenomena of typical confectionery fats are presented. The results show the sensitivity and reproducibility with which DSC data can be used to classify the types of confectionery fat. Calculations of the reproducibility of crystallization and melting parameters obtained from DSC are presented. In addition, the effects are shown on the thermograms of progressive changes in formulation to a typical cocoa butter equivalent. Further, the effects brought on by the presence of minor components, such as trisaturated triglycerides and other polar materials, which are common in confectionery fats, are described and quantified.

KEY WORDS: Confectionery fats, differential scanning calorimetry, heating, melting, minor components, triglycerides.

As reviewed in our earlier article, hence referred to as Part I (1), an essential aspect of the industrial manufacture of edible oils and fats is the ability to measure the physical properties of the materials. It is important to do this in a sensitive and reproducible fashion. The physical properties of greatest interest are the crystallization and melting phenomena that encompass both solid fat content and polymorphic behavior. Much of this information is accessible to us by means of a technique that measures the fundamental quantity of energy change during cooling or heating but on a macroscopic quantity of sample. This technique is differential scanning calorimetry (DSC).

In the oils and fats industry a major use of physical property measurement is for quality control of samples of one particular material. Specifications are set down for individual products in terms of cooling characteristics or solid fat content at particular temperatures. Another area where physical property data are used is in the development of new products. For both services there is an ever-pressing need to search for more precise, reproducible and speedy methods of characterization. DSC, it would seem, is ideally suited to the task of characterization of confectionery fats.

DSC has been used in the characterization of fats and oils for many years [for recent examples, see the works and cited literature of Manning and Dimick (2), Gibon *et al.* (3), Schlicter *et al.* (4–6), Merken *et al.* (7), Ziegleder and Kegel (8) and Kaisersberger (9)]. In Part I (1), a reappraisal of the technique was given with the aid of experimental data produced on model confectionery fat components. These were indeed the major fat components of cocoa butter and related confectionery fats, namely the symmetrical disaturated, monounsaturated (SUS) triglycerides. The experimental results confirmed the typical effects of cooling/heating rate variation and gave positive indication of the sensitivity obtainable. In addition, the difficulties of interpreting DSC experiments were also discussed.

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To extend the study presented in Part I, we now concentrate on the DSC measurement of confectionery fats themselves and, in particular, cocoa butter equivalents (CBE). The purpose of this is to confirm the applicability of the technique and to give us confidence in its use on complex blends of vegetable fats that constitute modern confectionery fats. This is achieved by investigating, in some depth, typical CBE and assessing DSC's sensitivity to certain composition changes apparent for different types of CBE and 'minor' components therein. Calculations of the reproducibility of the DSC technique for confectionery fat characterization are also presented.

EXPERIMENTAL PROCEDURES

A Perkin-Elmer Series 7 Thermal Analysis System with a differential scanning calorimeter (DSC7) was used to obtain cooling and heating thermograms of selected fats. The apparatus is described fully in Part I (1).

Sample weight can influence the shape of the DSC trace. High (>15 mg for the DSC7) weights give broader peaks and can lead to poor resolutions although the peak onsets remain the same. Low (<5 mg for the DSC7) weights give rise to smaller signals in the detector, and baseline noise may distort the trace. Very low sample weights may also give rise to anomalous crystallization traces because the numbers of heterogeneous nuclei in the sample are small and heterogeneous nucleation becomes less probable. A weight of 10 ± 1 mg was chosen for this study to maximize the signal without excessive distortion of the thermogram. The samples were pipetted into aluminum pans and accurately weighed. The pans were then hermetically sealed with a "cold welder." This reduced the possibility of depositing fat in the instrument's sample holder. An empty pan was used as the reference.

Except in the experiments on reproducibility, all samples were cooled and heated only once. During reproducibility experiments, some samples were scanned up to twelve times. However, repeated measurement caused deterioration of the samples. Deterioration was evidenced by a progressive change in the peak shapes and positions on cooling/crystallization. All thermograms were analyzed with the software available, and those parameters characterizing the traces, which were easily calculated, were recorded. These parameters were the peak position (maximum) on the temperature scale, peak height on the energy scale, peak area integrated as energy, and peak onset temperature as were used in the previous work described in Part I. Thermograms were normalized for sample weight prior to each peak area and height calculation. The following three sets of experiments were carried out:

Reproducibility measurements. A sample of a typical high-quality CBE (Coberine as supplied by Loders-Croklaan, Wormerveer, Netherlands) was measured repeatedly to assess the degree of reproducibility that could be achieved in the DSC experiment. A heating regime was chosen to melt out all crystals, followed by rapid cooling (80°C/min) to the starting temperature, followed by

cooling at 1.5°C/min. This latter part of the cooling process is typical of the rates used in DSC for the study of fats and oils. Thus, the sample was held at 80°C (or 100°C) for 2 min, cooled at 80°C/min to 30°C (≈1 min) and cooled to −10°C at 1.5°C/min (≈27 min). A single sample of the Coberine was scanned six times during one day and then three times the following day. The following week five further samples were scanned on one day. The Coberine was thus scanned a total of fourteen times.

Effect of triglyceride formulation. A series of blends, having different POP/POSt/StOSt compositions (St = stearic, P = palmitic and O = oleic), were prepared from three component vegetable fats (illipe, fractionated shea butter and fractionated palm oil, all supplied by Loders-Croklaan) to investigate the effects on thermograms of progressively changing POP/StOSt ratio (at constant POSt). The triglyceride compositions of these blends are given in the ternary diagram of Figure 1 and samples are designated A–E.

In Part I it was shown that by manipulating cooling and heating rates in DSC, different time-scale kinetic processes could be resolved, and the different melting points of the many polymorphic forms encountered could be measured. For comparing fats with progressively changing triglyceride composition, DSC scans with moderate rates were chosen as a compromise between lengthy scans at slow rates (which allow slow processes to be manifest but require long experiment times) and rapid scans (which act to blur information but require only short duration). The following regime was chosen: samples were held at 100°C for 2 min, cooled at 80°C/min to 40°C (≈1 min), cooled to −20°C at 2°C/min (≈30 min) and heated to 50°C at 5.0°C/min (≈35 min).

Effect of minor components on DSC traces on a high-quality CBE. A sample of Coberine was used as the starting material. It was specially prepared by selective fractionation processes to contain very low levels of trisaturated triglyceride materials. It was refined further by removing partial glycerides and other polar materials. This was achieved in an adsorption process conducted in a packed column of silica with hexane as an eluting solvent. The adsorbate (rich in diglyceride) was then recovered from the silica for later use. At this point, the stock CBE contained 0.6% diglyceride and only 1.1% trisaturated triglyceride.

A matrix of thirty samples for DSC examination was then produced from the stock by adding controlled amounts of Revel-A (supplied by Loders-Croklaan), which is 90% rich in palmitic acid in the form of triglyceride, and quantities of the diglyceride desorbed from the silica. The concentration range of trisaturated triglyceride obtained was 1.1 to 5.0% and that of the diglyceride was 0.6 to 5.0%. Cooling and heating regimes were as for those in "Effect of triglyceride formulation" section above.

RESULTS

Data are derived from the thermograms to characterize the shape of the curves. Typical parameters include the temperature positions of maxima and minima in the thermogram (peaks), the onset temperatures (calculated by drawing the maximum gradient tangent of an energy

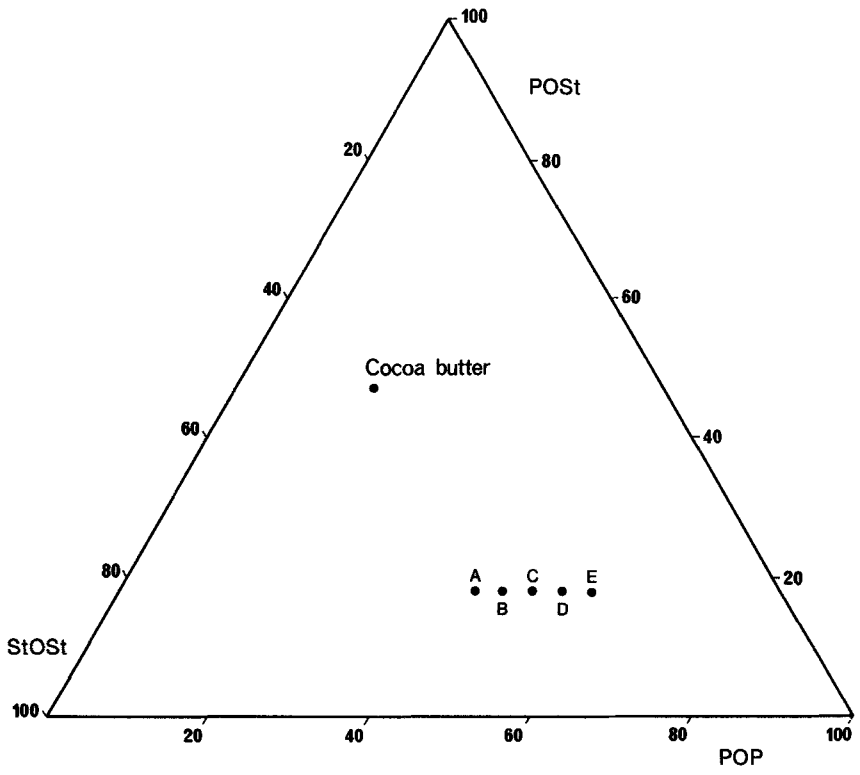

FIG. 1. Triglyceride composition (POP/POSt/StOSt) of confectionery fats A–E blended from three different vegetable fats.

process back to the baseline), and the area between the thermogram and the baseline. Some of these constructions and designations are shown in Figure 2.

Reproducibility measurements. For the repeated measurement of DSC cooling, the following parameters were extracted from each cooling thermogram: peak temperature

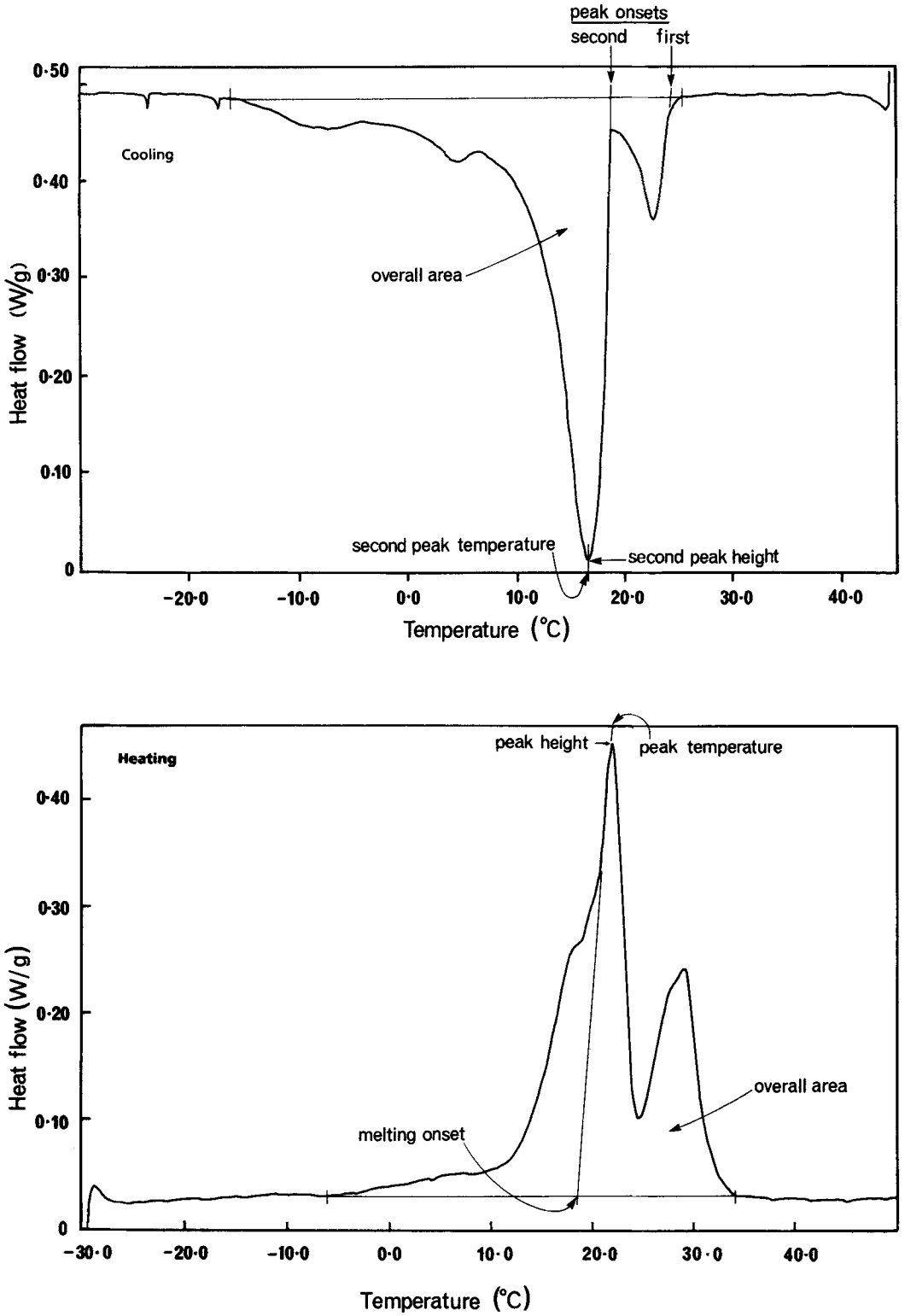

FIG. 2. Characteristic cooling and heating thermogram for confectionery fats. The parameters serving to describe the features are shown by construction lines. The ordinate scale denotes energy flow in watts per gram to or from the sample. Occasionally the temperature scanning rate used is incorporated, in which case the ordinate scale is labelled in Joules per gram.

TABLE 1

Statistical Calculations of Reproducibility Measurements for Cooling of Coberine Cocoa Butter Equivalents

	Peak temperature (°C)	Peak height (W/g)	Peak onset (°C)	Latent heat (J/g)
Mean	14.39	0.25	16.40	70.49
σ	0.06	0.01	0.13	0.54

(°C), peak height in watts per gram (W/g), peak onset (°C) and peak area (or latent heat of fusion) in Joules per gram (J/g). Statistical calculations on fourteen replicates of the same cooling thermogram for the Coberine yielded the reproducibility given in Table 1. The parameters indicate a high degree of reproducibility. For example, the main peak onset has a standard deviation of 0.13°C (which is equivalent to ±0.26°C for 95% confidence limits).

Effect of triglyceride composition. The cooling trace for all formulations consisted of two peaks (Fig. 3a) for the Coberine studied. Both peaks shifted to lower temperatures by about 0.5°C per 5.0% as the POP level increased. Similarly, many of the other features shifted in linear fashion (Fig. 4). This progressive translation, as the POP/StOSt content is changed, could be due either to a deeper under-cooling required for nucleation or to a genuine lowering of the melting point of the mixture. There appeared to be no quantifiable trend in the heat of crystallization for this series of formulations.

The heating thermograms consisted of three merging peaks and a fourth for the samples formulated with most POP (Fig. 3b). Trends were not as marked as for the cooling trace, but some shift with POP concentration to lower temperatures was noted. The first two peaks were the major features, and these changed in relative proportion as the POP content increased. This might be taken as support for the idea expressed above that crystallization occurs only at a deeper under-cooling in samples concentrated in POP. As before, no trend was discernable in heat of melting.

Effect of minor components. A summary of the traces is shown in Figure 5. All the samples from the matrix showed two distinct peaks, the lower temperature feature (later crystallizing) being the major one. The main differences in the thermograms' shape resulting from minor component content were manifest in the smaller peak.

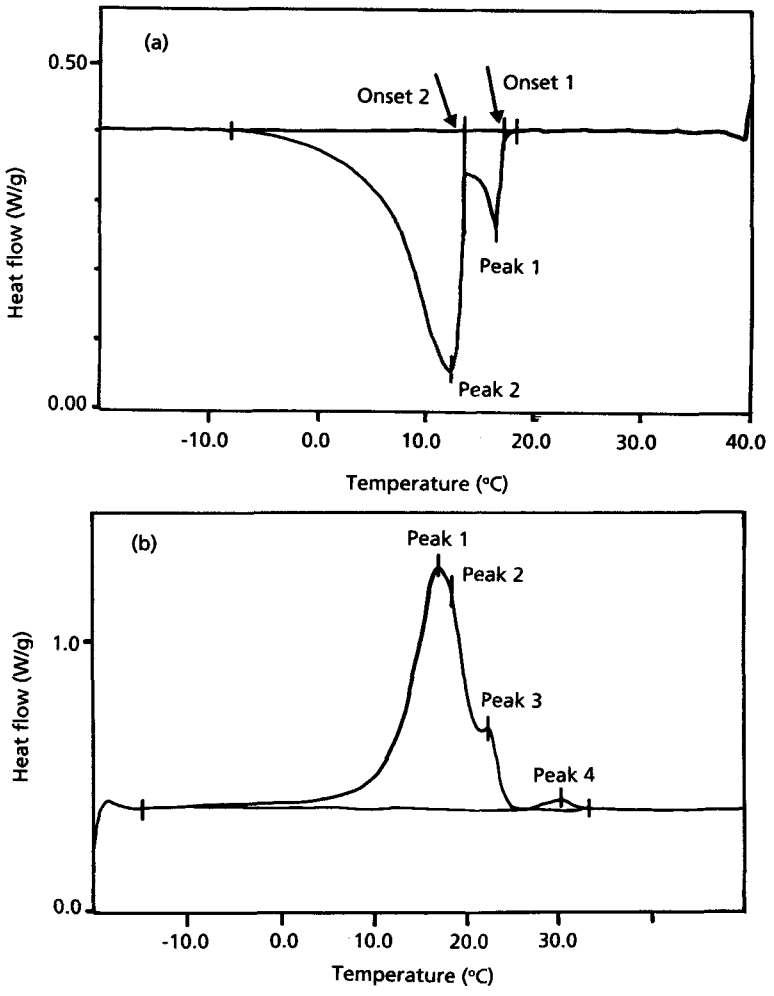

FIG. 3. (a) DSC cooling thermogram of sample E. Cooling rate = 2°C/min. (b) DSC heating thermogram of sample E measured directly after cooling. Heating rate 5°C/min.

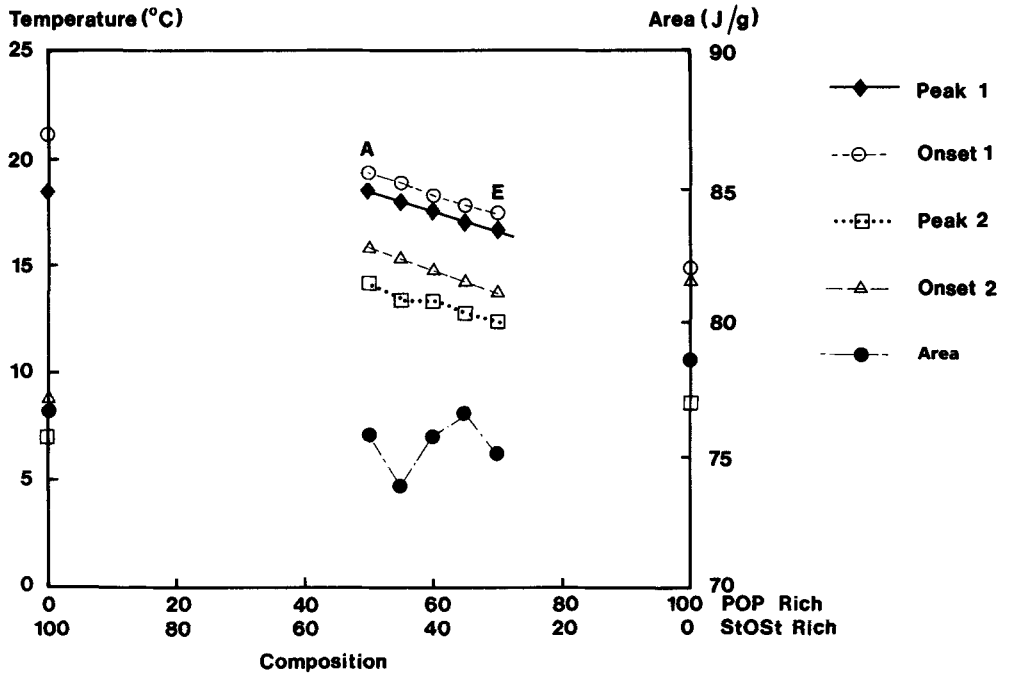

FIG. 4. Shifts of the cooling parameters as a function of POP/StOSt ratio for fat blends A–E, which all have a POSt content of about 20%.

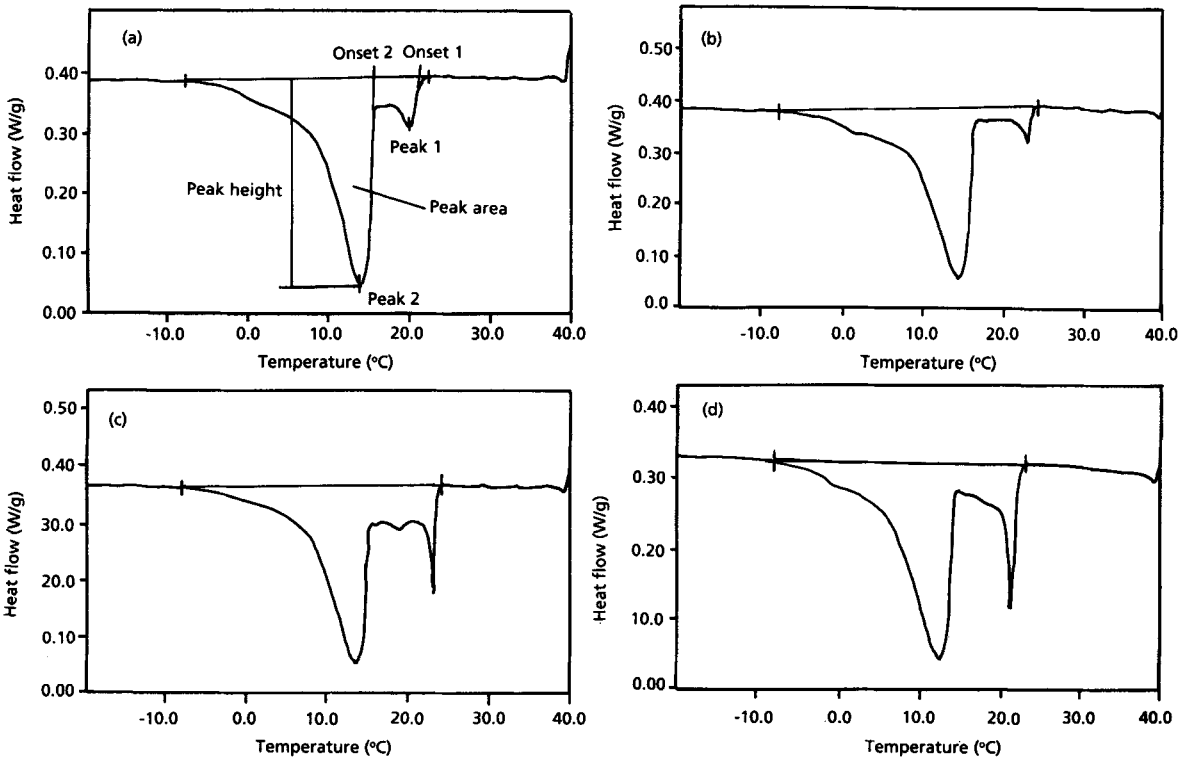

FIG. 5. Differential scanning calorimetry cooling thermograms of (a) Coberine, (b) Coberine + 4.4% diglyceride (DG), (c) Coberine + 3.9% S_3 and (d) Coberine + 4.4% DG + 3.9% S_3 (where DG = polar component desorbed from silica column after treatment of Coberine and S_3 = Loders-Croklaan Revel A). Cooling rate = 2°C/min.

Generally speaking, this peak increased in sharpness (height/width ratio), broadness (width) and area as the trisaturated triglyceride level increased, and in broadness as the diglyceride level increased.

All heating thermograms consisted of one main peak with a large leading shoulder and a smaller trailing shoulder (Fig. 6). This trailing shoulder increased with the level of trisaturated triglyceride and decreased with diglyceride. At the higher trisaturated triglyceride levels some polymorphic transformation on heating was apparent at the end of the trace. The amount of transformation decreased with diglyceride content.

Analysis of thermograms. The data from both cooling and heating thermograms were analyzed by using proprietary software statistical packages. Linear regressions of each DSC feature measured (peak position, onsets, *etc.*) in the thermograms were made against the composition of each of the minor components, both separately and as mixtures. The model used was of the type $y = m_1[S_3] + m_2[DG] + c$, where y is the DSC parameter, m_1 and m_2 are the S_3 and diglycerides (DG) coefficients, and c is a constant. The correlations for each thermogram parameter with $[S_3]$ *and* [DG] are shown in Table 2, in which the coefficients for the trisaturated triglyceride concentrations $[S_3]$ and diglyceride [DG] also appear. Peaks are numbered in the order in which they appear in cooling or heating (Figs. 5 and 6).

From the figures and the tabulated correlations, it is apparent that the DSC traces (particularly the cooling curves) are affected in a systematic way by the addition of trisaturated triglyceride and/or diglyceride. For example, there is a clear correlation between the area of the small peak (energy under peak 1 as a proportion to the total energy) in cooling and the level of trisaturated material; the correlation coefficient is close to unity and the S_3 coefficient is much greater than that for the DG. This is somewhat mirrored in the later stages of the heating process. The actual start of crystallization (defined as the temperature at which the thermogram departs from the baseline) correlates well, with both trisaturated triglyceride and diglyceride concentration making equal contributions; the crystallization begins sooner if either is present. On the other hand, certain other parameters show little or no linear correlation with change in the concentration of either minor component. This might indicate that the parameters chosen for the correlation are just not sufficiently sensitive within the simplicity of the approach adopted.

DISCUSSION

The data collected for confectionery fats with progressively changing triglyceride composition are useful because they show just how widely different characteristic traces for the fats can be. And that is essential for quality control classification of CBE of different types that have different crystallization properties used in different applications. This bodes well for the wider application of DSC in quality control. This being the case, it remains to establish the sensitivity of the technique for assessing variations in physical behavior likely for individual confectionery fat types.

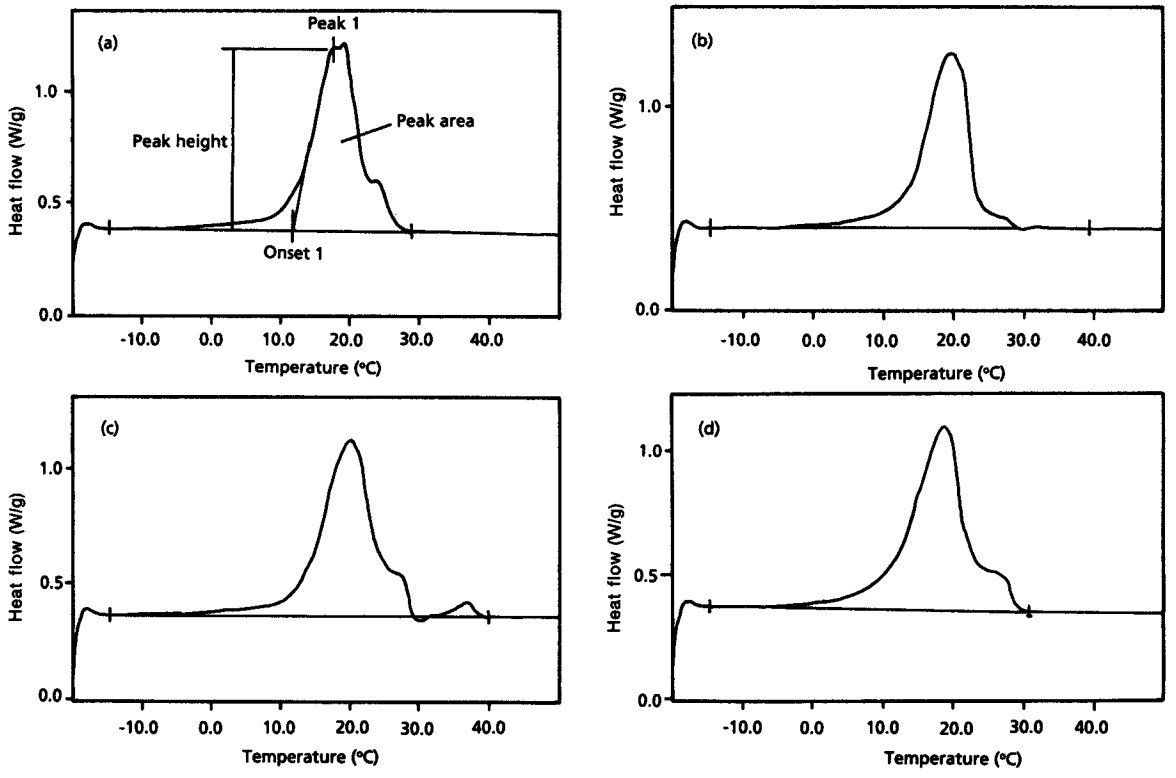

FIG. 6. Differential scanning calorimetry heating thermograms of (a) Coberine, (b) Coberine + 4.4% diglyceride (DG), (c) Coberine + 3.9% S_3 and (d) Coberine + 4.4% DG + 3.9% S_3. Samples heated directly after cooling. Heating rate = 5°C/min.

TABLE 2

Linear Regression Coefficients for Differential Scanning Calorimetry Parameters Based on Minor Component Concentration

	Diglyceride coefficient	S_3 coefficient	Constant	Correlation coefficient[a]
Cooling				
position (°C):				
peak 1	0.38	0.46	18.53	0.81
peak 2	0.02	−0.14	14.46	0.57
onset (°C):				
peak 1	0.39	0.39	19.41	0.78
peak 2	0.11	−0.17	15.94	0.82
area:				
total (W/g)	−1.05	0.13	79.37	0.53
peak 1/total (%)	−0.28	3.68	6.38	0.98
start of crystallization	0.48	0.51	19.66	0.87
Heating				
position (°C):				
peak 1	−0.06	−0.24	19.35	0.57
peak 2	−0.04	−0.07	20.47	0.14
peak 3	0.24	0.31	24.10	0.64
onset (°C):				
peak 1	−0.08	−0.28	14.08	0.75
area:				
total (W/g)	−0.63	0.30	85.54	0.38
peak 3/total (%)	−0.67	2.11	8.01	0.91

[a]Correlation is significant for this experiment when the coefficient exceeds the 0.75 level.

A great commendation for the technique for assessing the crystallization and melting phenomena associated with fat is the high degree of experimental reproducibility that can be achieved. It is worthy at this point to comment on the lack of correlation achieved with the energy measurement. Whereas for a single sample measured repeatedly the reproducibility is high (about 0.5 J/g in 70.0 J/g), the variation from sample to sample across a series can be significantly more (±1.5 J/g in the POP/StOSt series). This is because the energy calculation (and peak height) is much more sensitive to and dependent on the choice of baseline location than are any of the temperature parameters, and indeed no allowance has been made for heat capacity changes during melting or crystallization.

The second requirement is to establish the ability of DSC to characterize rather subtle changes in triglyceride composition (as opposed merely to distinguish the rather gross changes described above). The formulation changes produced in replacing StOSt with POP were readily detected and, what is more, easily quantitated. This quantitation was certainly assisted by the smoothness of change in the temperature parameters.

For the case of the confectionery fat that was carefully adulterated with typical minor components (but of constant POP/POSt/StOSt composition), the results demonstrate clearly the sensitivity that DSC has in response to modifications. Not only can the minor component changes be detected but they can actually be correlated and quantitated to a precise degree.

In summary, increased levels of trisaturated triglyceride increase the degree of crystallization in the early stages and also raise the temperature of nucleation. Diglyceride also causes crystallization to occur sooner (which might be the diglyceride itself precipitating and then nucleating further triglyceride material) but slows down the subsequent velocity of growth. Diglycerides also retard polymorphic transitions in the triglyceride on heating.

The results presented here clearly demonstrate the DSC technique as sensitive in the characterization of confectionery fats. In its modern computerized form, its main attributes include fast, accurate control with temperatures known at all times. This allows comparison of fats crystallizing or melting under identical thermal conditions.

The wide variations in confectionery fat triglyceride composition are reflected in differing calorimetric behavior. As seen from studies on low levels of minor components and studies on variation of triglyceride composition, the sensitivity of the DSC experiment copes well with the quantitation of those effects.

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