Calorimetric Studies of the Heat of Melting of Milk Fat by Differential Thermal Analysis

R. A. YONCOSKIE, Dairy Prolucts Laboratory, Eastern Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture, Washington, D.C.

Abstract

A differential thermal analysis calorimeter was used in determining the heat of melting of milk fat. The heat of melting values for milk fat, studied on samples conditioned at different temperatures, varied from 20.4 cal/g (average value) to 25.1 cal/g (average value).

Introduction

THE INTERESTING POSSIBILITY of measuring the heats of melting of fats and oils with differential thermal analysis (DTA) was expressed by Hannewijk et al. (1). That the area under a thermogram curve might be related to energy changes came as no surprise to earlier investigators, who also pondered this possibility. An early attempt to find a theoretical basis for a quantitative treatment of the area was made by Spiel in 1945 (2). Further modification and development of the theory was made by Kerr and Kulp (3). A more recent development of the mathematical equations used in quantitizing peak areas has been illustrated by Borchardt (4) in a study of organic reactions by DTA. The use of the Borchardt equations has been further expanded by David (5) for determining the heats of fusion of inorganic and organic compounds.

Though conventional DTA can, in restricted cases, be employed in calorimetric studies, a number of difficulties confront the analyst who uses this technique. It has been found that such variables as the size and shape of samples, heat conductivity, specific heat, and dilution of sample tend to make peak areas difficult to reproduce (6). A cell design, different in construction from conventional DTA, which eliminates these difficulties is illustrated by Barrall (6) and David (5). A comparison of the results obtained from conventional DTA and calorimetric DTA is demonstrated by the former (6).

The Du Pont DTA calorimeter employed in this study is similar to those just mentioned and follows the design principles suggested by Boersma (7) in 1955. The need also to establish a calibration curve by using high-purity reference samples has been emphasized by prior authors (6,8).

The method of standard calorimetry (9), though undoubtedly of the greatest accuracy for determining heats of fusion, is tedious and time-consuming to perform. By contrast the method of DTA calorimetry is comparatively simple, rapid, and fairly accurate, requiring only small amounts of material. An application of this method for determining the heats of melting of milk fat as a possible model material for other fats and oils was the main objective of the work reported in this paper.

Experimental Section

Preparation of Milk Fat

Freshly pasteurized cream (obtained during August from mixed herd milk, Beltsville, Md.) was churned in a Hamilton Beach Mixer, Model 30. The resulting butter was heated to 52C, cooled, and held at 4C for 18 hr. After removal of the butter serum, the butter was reheated to 60C and centrifuged twice at 5,000 rpm (International Centrifuge, Size 1, Type SB), followed by decantation of the clear oil. The clear oil was dried by stirring magnetically with finely powdered CaH_2 for 3 hr. The dried milk fat was stored at −18C prior to use.

Apparatus

The Du Pont Calorimeter, a plug-in accessory for the Du Pont 900 Differential Thermal Analyzer, was used to obtain all calorimetric data. Sample materials being analyzed are weighed into fabricated liners or cups and inserted into fixed silver sample holders. Chromel-Alumel sensing thermocouples are welded to the base of the sample holders. The thermocouples and sample holders are supported on ceramic insulators, which are imbedded firmly in a transite base. A silver heating block, machined to provide separate air cavities for sample and reference thermocouple assemblies, is surrounded by a nichrome resistance furnace. Programmed heating of the block, from 1 to 30C per minute, is controlled by a separate thermocouple located in the heating block.

Calibration

The calibration of the calorimeter was performed by using a programmed heating rate of 5C/min. Standards employed for this calibration were mercury ($\Delta H_f = 2.74$ cal/g), gallium ($\Delta H_f = 19.9$ cal/g), indium ($\Delta H_f = 6.79$ cal/g), tin ($\Delta H_f = 14.2$ cal/g), and zinc ($\Delta H_f = 27.0$ cal/g). The calibrating materials with their heats of fusion values were obtained with the instrument (values are cited from the American Institute of Physics handbook). The samples used for calibration, as well as other materials used in this study, were weighed to the nearest ±.01 mg with a Cahn Electrobalance, Model 1500. Peak areas were measured with a polar planimeter (Keuffel and Esser Company, K&E 4236). A study of the reproducibility of peak area measurements was made with indium by using 10 determinations. An area of 4.35 sq. in. with an average deviation of ±.014 sq. in. was obtained for 18.49 mg of indium. Calibration coefficients were calculated from the determined peak areas and known heats of fusion by substitution in the following formula:

$$\Delta H \text{ (mcal/mg)} = E \frac{A\,\Delta Ts}{MC}$$

where E = calibration coefficient, mcal/°C-min; A = peak area; ΔT_s = Y axis sensitivity, °C/in.; M = sample mass, mg; and C = chart speed, in./min.

During the calibration sequence, occasional checks were made by employing heating rates of 10C/min. Within experimental error the E calibration coefficient was the same as those calculated at 5C/min.

A minimum of three calorimetric determinations on different samples was made of each calibrating standard, and an average E value was used in plotting the calibration curve shown in Figure 1. A check on the

reproducibility of E for tin gave a value of 67.0 with an average deviation of ±.4; six determinations were used for this calculation. In the curve, E values are plotted as a function of sample peak temperature. Peak areas generated by calibrating standards and unknowns were measured in triplicate with the planimeter, and the results were averaged.

Sample Preparation and Materials

After the milk fat was weighed into a sample container, it was conditioned at 45C for one hour. This conditioning treatment was found necessary to destroy any previous temperature history. The effect of three different temperature histories was examined calorimetrically: immediate cooling of the sample from 45C to −90C at approximately 5C/min; tempering at 22C, followed by cooling to −90C at approximately 5C/min; and tempering at 0C, followed by cooling to −90C at approximately 5C/min.

Materials other than tristearin required no special pretreatment and were used as received in measuring their heats of fusion. Tristearin, as received, contained a small amount of the α form which tended to interfere with the peak area of the β form. It was found that α and β' forms could be eliminated by heating the sample in the calorimeter to 55C and holding at this temperature for 15 min. The sample was then cooled to about 20C before making the calorimetric measurement. Heats of fusion for tristearin as well as other materials were used to examine the accuracy of the calorimeter and are given in Table I. The ΔH measurements were made with an accuracy of about ±2%.

Trimyristin, tripalmitin, tristearin, and palmitic acid were obtained from The Hormel Institute, University of Minnesota, Austin, Minn. Their purity was given as greater than 99% as determined by thin-layer chromatography. The benzoic acid and naphthalene were obtained from James Hurton, 358 Chicago avenue, Valparaiso, Fla. These materials were prepared by zone melting, and their purity was given at 99.99+%. The silver nitrate was reagent grade and was obtained from the Fisher Scientific Company.

Procedure

All samples except milk fat and gallium were weighed into liners fabricated from aluminum foil; platinum liners were used for gallium. The aluminum and platinum liners were prepared by shaping foil (cut with cork borer No. 5, about 10 mm in diameter) over a machined dowel pin about 5 mm in diameter. These liners were found inadequate for milk fat because the oil tended to creep out through capillary effect along the liner seams. Specially fabricated stainless steel liners, 4 mm in diameter and 2 mm high, were employed for the milk fat. The weight of material required for a calorimetric determination was based on the magnitude of the heat of fusion and ΔT sensitivity value. One or two pretrial runs were made to determine an approximate suitable weight.

After the sample liner was introduced into the calorimeter holder, the heating block was reduced, where necessary, to a temperature at least 50C below the fusion point when a heating rate of 10C/min was used. An appropriate reduction in temperature of about 30C below the fusion point was used with a heating rate of 5C/min. A liner similar to the sample liner, but containing no reference material, was used in the reference holder. To establish a sufficiently defined baseline, the thermogram was recorded at least 20 degrees before the melting onset and 20 degrees after the completion of fusion. The ΔH calculation of an unknown was then calculated from the measured peak area and the appropriate peak temperature E constant by using the formula previously defined.

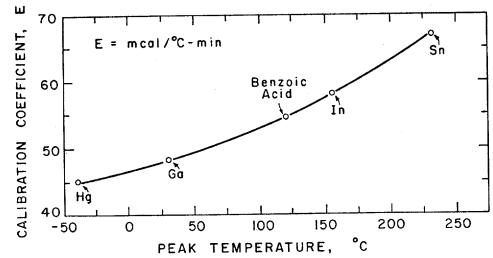

FIG. 1. Calibration curve for heats of fusion.

Though benzoic acid and naphthalene might be expected to sublime during a calorimetric determination, no such behavior was apparent, as noted by their heats of fusion in Table 1. Anthracene however did sublime at about 180C, below its fusion temperature of 217C, as was evidenced by crystal formation on the inner calorimeter cover. A heat of fusion of about 20 cal/g was calculated, which is far below the literature value of 38.7 cal/g.

Results and Discussion

In calculating the heat of fusion of pure organic materials where fusion occurs at a specific temperature, the calories involved in the transformation are merely calculated from the following simplified formula (4): $\Delta H = EA$, where E = the calibration coefficient and A = the peak area.

An examination of the calibration curve in Figure 1 however shows that the calibration coefficient E is a variable which changes with the peak temperature. Since milk fat is a complex mixture of triglycerides possessing no single fusion temperature, the formula as given above is not directly applicable for calculating its heat of melting.

Because this mathematical expression is not directly applicable for determining the heat of melting of milk fat, other approaches for calculating this calorific value had to be considered. A first approach consisted of dividing the total peak area into small segments, as shown in Figure 2. The heat of melting was calculated for each area designated as 1,2,3. . . .9 by employing the E values corresponding to tem-

TABLE I

Results of ΔH_f Determinations

Sample	Sample size, mg	Heating rate °C/min	Cal. 15°/gram determined ΔH_f	Cal. 15°/gram known ΔH_f
Indium	19.02	10	6.89	6.79
Tin	12.06	10	14.3	14.2
Trimyristin (β)	1.68	10	49.5	50.3[a]
Trimyristin (β)	2.97	5	49.7	50.3
Trimyristin (β)	2.81	10	49.3	50.3
Tripalmitin (β)	3.17	10	52.8	53.1[a]
Tripalmitin (β)	2.45	5	53.4	53.1
Tristearin (β)	2.16	10	54.9	54.6[a]
Tristearin (β)	3.02	5	55.0	54.6
Benzoic acid	2.01	10	34.1	33.9[b]
Benzoic acid	4.03	5	33.6	33.9
Benzoic acid	2.97	5	33.9	33.9
Naphthalene	3.57	5	36.2	35.6[b]
Naphthalene	3.34	10	35.8	35.6
Naphthalene	3.23	10	35.1	35.6
Palmitic acid	3.05	10	51.0	50.6[a]
Silver nitrate	8.46	10	16.4	16.2[c]

[a] Reference 12.
[b] Reference 13.
[c] Reference 14.

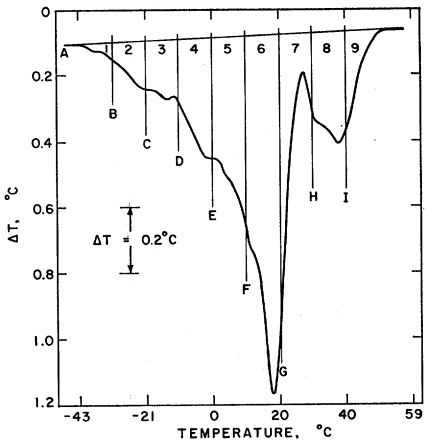

FIG. 2. Heating thermogram of milk fat conditioned at 22C for 47 hr.

peratures designated as A,B,C. . . .I. A summation of the calorific values for each area increment gave a value corresponding to the total heat of melting for milk fat. The heat of melting calculated by this method for the milk fat sample illustrated in Figure 2 was 21.36 cal/g.

A second method was to use the areas previously calculated for Figure 2 and to plot the percentage area swept out to t_1 t_2. . . .t_n (where t = temperature). A plot of area versus temperature was constructed, and the E calibration value corresponding to the temperature at 50% of the area was used for determining the heat of melting. The value obtained from this method was 21.4 cal/g.

Prior work on the heat of melting of milk fat between −10C and 60C gave a value of 19.5 cal/g (10). A more recent paper (11) gave values of 24.52 and 19.43 cal/g, based on two different thermal treatments of cream from which the calculations were made.

In all subsequent determinations the second method was employed for calculating the heat of melting of milk fat. Once a plot had been constructed for a sample tempered under a certain set of conditions, the same calibration coefficient was used for all samples in that series. The heats of melting of milk fat, as determined by the DTA calorimeter, are given in Table II.

The time periods used for tempering the milk fat were far in excess for complete crystallization, as indicated by the work of de Man (15).

The technique of DTA calorimetry, demonstrated with milk fat, should be applicable in determining the heats of the melting of other fats and oils. As contrasted to standard calorimetry, the method requires less time to perform, gives fairly accurate data, and needs only milligram amounts of sample. This last feature makes it extremely useful in analyzing minor components isolated from fats and oils. Though reproducibility was not studied statistically in this work, values within a tolerance of ±2% should be obtainable. A calibration curve, established with materials of high purity, appears to remain constant over long intervals of time (no correction was necessary over at least a six-month period).

TABLE II

Heats of Melting of Milk Fat[a]

Butterfat tempering conditions	Sample wt. mg	Heating rate °C/min	Heat of melting cal/g
No tempering	15.21	5	20.4
No tempering	15.10	5	20.4
No tempering	11.70	10	20.6
No tempering	13.00	5	20.5
No tempering	12.74	5	20.0
Tempered at 22C for 30 hr	15.14	5	21.4
Tempered at 22C for 43 hr	11.51	5	21.7
Tempered at 22C for 44 hr	11.28	5	21.6
Tempered at 0C for 22 hr	8.72	5	24.8
Tempered at 0C for 18 hr	14.20	5	24.9
Tempered at 0C for 25 hr	8.37	5	25.6
Tempered at 0C for 26 hr	8.57	5	25.2

[a] All samples were cooled from either the conditioning or tempering temperature to −90C at about 5C/min.

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