Evaluation of the Differential Scanning Calorimetric Method for Fat Solids[1]

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Abstract

Denison et al. (1) recently reported a method for measuring the per cent solids in fats using the Differential Scanning Calorimeter (DSC). The present work evaluates that method using the Perkin Elmer DSC-1, compares it with nuclear magnetic resonance (NMR) and dilatation methods, and extends it to hard butters. Although the method gave excellent interlaboratory agreement with soft fats, extension to hard fats led to greater experimental variance than SFI. The DSC method provides greater speed (one hour elapsed time) and additional information (thermal "fingerprint" of the fat). Thus, the DSC determination of fat solids overall compares favorably with the NMR method, as well as the SFI dilatation method. The DSC method is readily adaptable to quality control use.

Introduction

It is well established that the melting point and heat of fusion of a pure triglyceride depends upon the polymorphic form of the fat. Thus, the use of DSC melting endotherms for determining fat solids requires a pretempering of the sample to insure reproducibility of curve shapes as well as areas. Figure 1 illustrates a typical example of this effect by the difference between the initial melting curve of tristearin and the melting of pure β-form after conditioning a half hour at a temperature just below the melting point. The original melting curve shows the endotherm corresponding to the melting of the α-form present, followed by the exotherm due to rearrangement to the β-form. This in turn is followed by the melting of the β-form. Conditioning of the sample above the melting point of the α-form, but below the β-form converts it to pure β-form. This is evidenced by the single melting endotherm upon remelting of the sample.

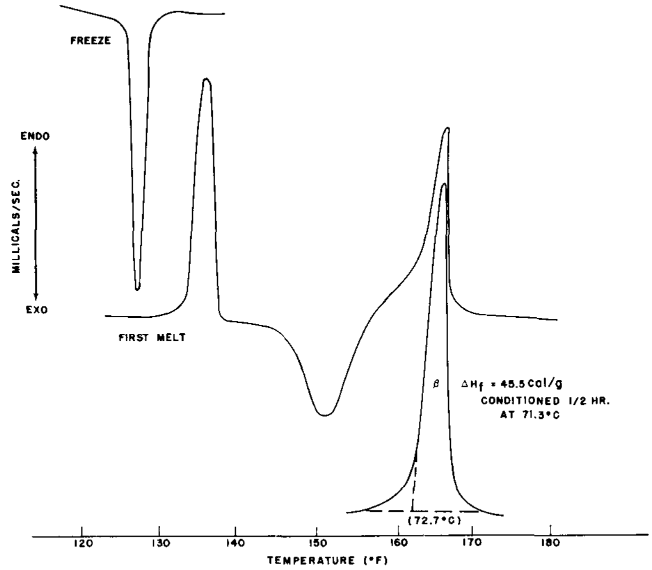


FIG. 1. Melting and freezing curves of tristearin (99+% from Applied Science Labs.).

Materials and Methods

The first approach used in this laboratory was the most direct one, namely, the complete melting of a fat sample to erase its thermal history, followed by cooling at a predetermined rate to build in a new state of temper. The results by this method proved to be less reproducible than with the tempering procedure proposed by Denison and Justin (1). The latter involves the use of the same temperature sequence as in the Standard AOCS dilatation procedure (2).

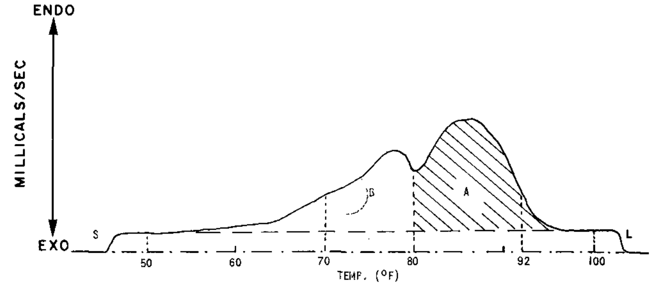
The Perkin-Elmer DSC-1 was used for these studies for two reasons. First, it is a true calorimeter in that actual energy input is measured. Thus an integration of a curve [millical/sec vs. time (temp)] yields calories directly. Second, the instrument was the only commercially available instrument which provided a convenient sample holder for fats at the time this work was initiated. Samples were weighed into aluminum pans, under ambient atmosphere.

This work evaluates the method by application to fats of varying hardness as well as by direct comparison with the NMR and SFI methods of solids determination.

Experimental Procedures

Principle of the Method

Figure 2 illustrates a typical Differential Scanning Calorimeter (DSC) curve obtained by melting a pretempered sample. One calculates the per cent solids at 80 F as shown in Figure 2. To calibrate the calories/unit area, one melts a sample of pure indium which has a known heat of fusion. Indium provides a relatively easily available, stable, reproducible



$$\%\ \text{SOLIDS} = \frac{\text{cal/unit area}^{(1)} \times \text{area (A)}}{\text{wt. of sample (g)} \times \Delta H_f^{(2)}\ \text{(cal/g)}} \cdot 100$$

(1) Based on ΔH_f of indium.

(2) Use average value of 35 cal/g.

FIG. 2. Principle of DSC method for per cent solids.

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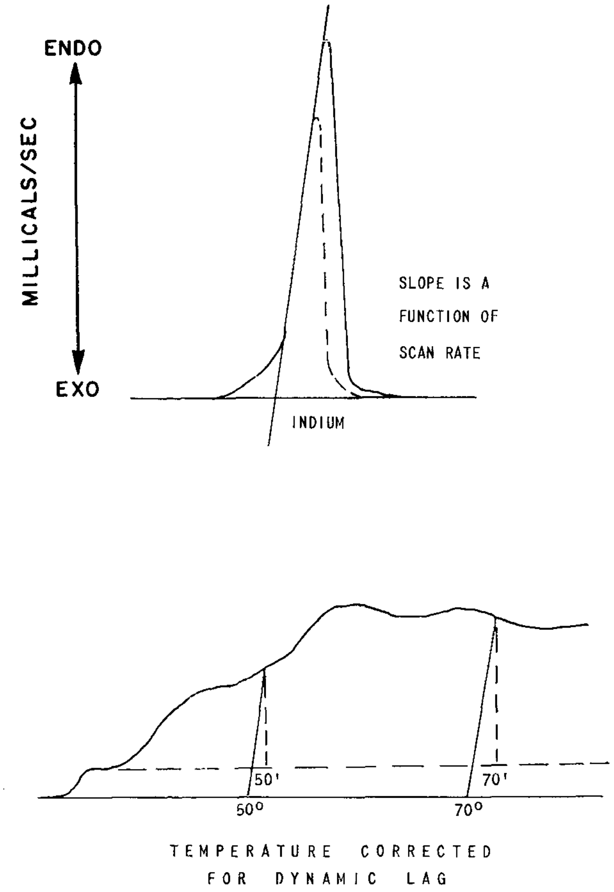

FIG. 3. Temperature corrections for dynamic lag.

standard. Water or pure lauric acid can be used for low temperature calibrations in the temperature range of interest (i.e. adjustment of T average control).

Procedure

The detailed procedure is as follows: (a) Weigh a 5 mg sample of fat; (a) heat above the melting point (77 C); (c) pretemper by holding 5 min each at 0, 26.7 and 0 C. These temperatures correspond to 32, 80, and 32 F of the Standard AOCS Method (2). Centigrade temperatures are specified since the instrument is calibrated in centigrade degrees. (d) Melt at a programmed rate of 5 C/min; (e) draw the base line on the chart (dashed line, Fig. 2) assuming Cp(s)/Cp(l) = 0.95; (f) draw in the corrected temperature lines, i.e., compensate for dynamic lag as described below; (g) integrate the areas of interest, e.g., to 50, 70, 80, 92 and 100 F; (h) calculate the per cent solids assuming an average heat of fusion of 35 cal/g. Calibrate the instrument daily by melting pure lauric acid or pure water to insure the accuracy of the temperature readings.

Steps c, e and h are variable. To suit the particular fat of interest, change the tempering cycle as necessary. The relative heat capacities of solid and liquids, as well as the heat of fusion, may differ from the values used. The values presented appear to represent good averages which give reasonable results. If, however, one has a fat known to be high in one component, for example, a high trilaurin content, one would use a higher heat of fusion than for a high trimyristin fat (see discussion below on NMR standard mixtures of safflower oil/tristearin and Table I).

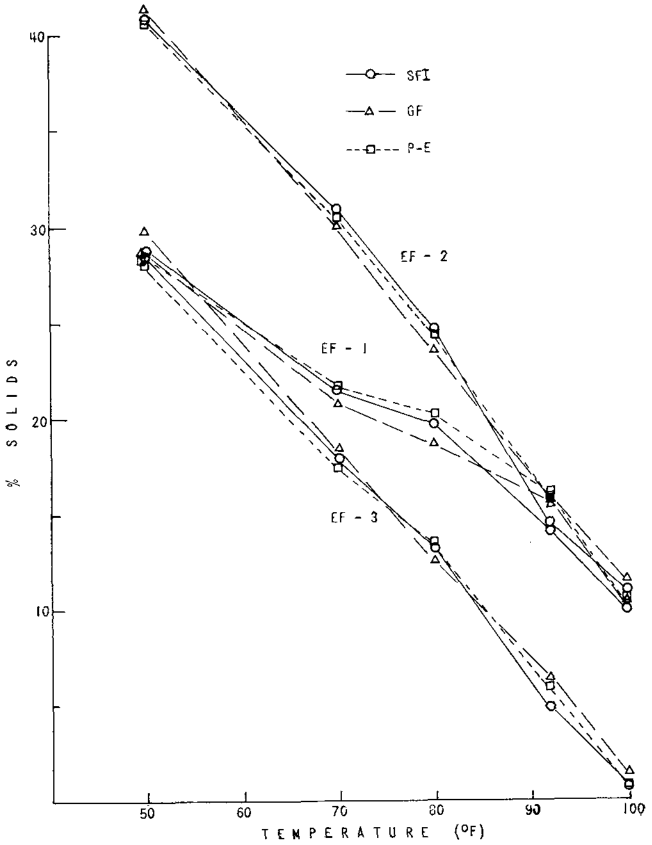

FIG. 4. Comparison of SFI with DSC method at two different laboratories using Smalley Committee Fats.

Correction for Dynamic Lag

A small dynamic lag occurs during a transition due to the finite time required for heat transfer. Figure 3 shows how to correct the abscissa (temperature) of the melting curve for this lag.

The indium curve illustrates that the leading edge has a finite slope (here exaggerated) despite the fact that the indium melt is an isothermal transition. The indium melt curve and the smaller dotted one show clearly that the amount of displacement of a peak maximum depends on sample size. Thus, the thermal lag is a function of the sample size or the height of the curve from the base line. To correct the temperature on the programmed base line (50′ and 70′, slope from the isothermal base line at the temperatures of interest (e.g., 50 F and 70 F, Fig. 3) until it intersects the melting curve. From this intersection, one drops a perpendicular to give the corrected temperature on the programmed base line (50′ and 70′, Fig. 3). The drawing exaggerates the correction which usually represents less than 0.2 C.

NMR Analyses

The NMR technique used for solids determination was that of Chapman et al. (4,5) and Pohle, et al. (6,7) using a Schlumberger NMR Model 106 (Varian Model PA-7).

Results and Discussion

Perkin-Elmer (P-E) reported (2) a linear correlation between dilatometry and DSC crystallinity values for Smalley Committee Samples EF-1, 2 and 3. The first trials of the P-E Method at General Foods (GF) were, therefore, on Smalley samples in order to test interlaboratory agreement. Interlaboratory comparisons gave exceptionally good agreement

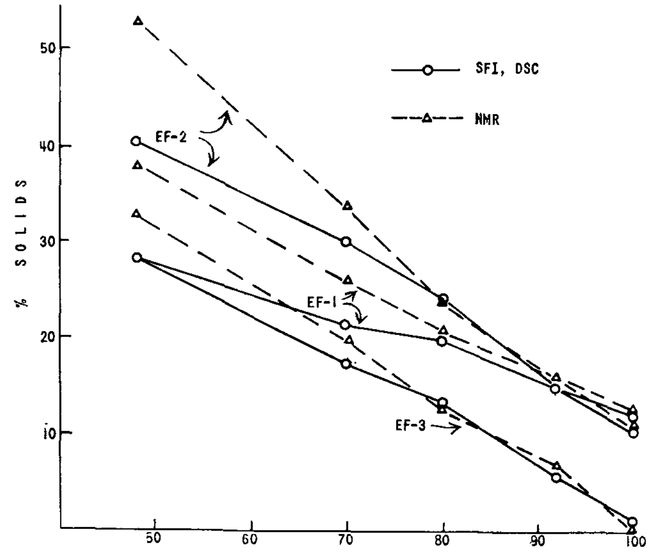

FIG. 5. NMR solids vs. DSC and SFI.

(Fig. 4), especially since the GF values were the results of single determinations.

Nuclear magnetic resonance of the same samples gave considerably higher values at lower temperatures (50–80 F) and became equivalent at the higher temperatures (Fig. 5). The calibration standards for the NMR work were known mixtures of safflower oil and tristearin. The curves (Fig. 6) were essentially linear and varied only slightly with temperature. Examination of these calibration samples by dilatation (SFI) and by DSC shows them to exhibit high solids values at the higher tristearin levels. DSC values calculated with a 45 cal/g heat of fusion (closer to tristearin than the usual 35 cal/g) achieve a better fit of the data as shown in Table I. Cottonseed oil, hydrogenated to varying degrees, gives samples of different hardness for comparison studies of DSC vs. NMR. The curves obtained (Fig. 7) are very similar qualitatively to those obtained by Pohle (7) when he compared NMR vs. SFI on hydrogenated safflower oil. In both cases, the NMR method agreed better at lower per cent solids than at higher ones.

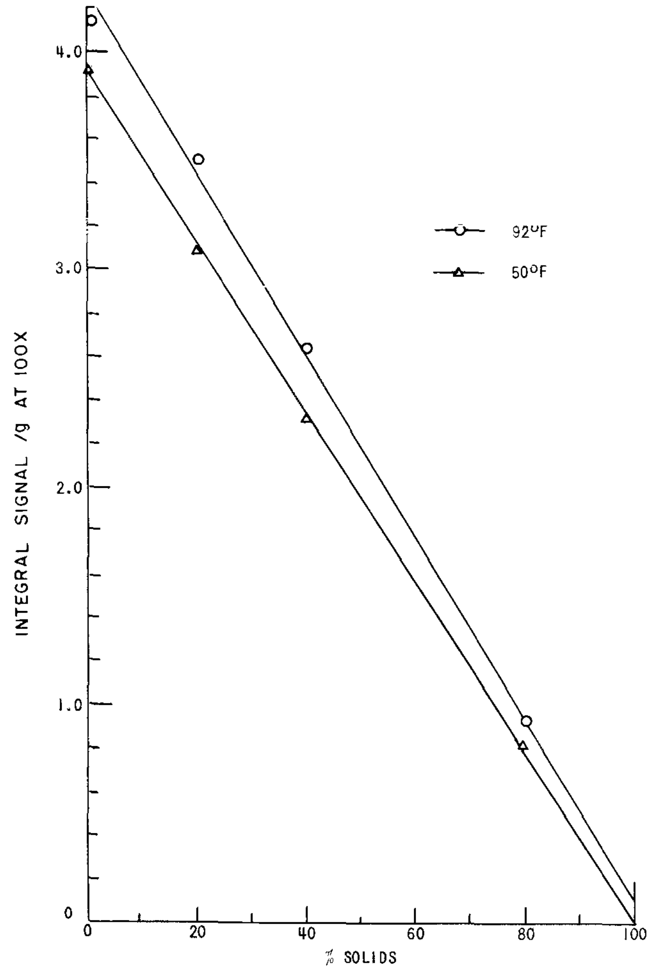

FIG. 6. NMR calibration curves (7g sample).

TABLE I

NMR Per Cent Solids vs. Dilatation and DSC at 80 F of Safflower Oil/Tristearin Mixtures

NMR	SFI	DSC[a]	DSC[b]
0	0	0	....
20	19.7	25.6	19.9
40	48.4	49.3, 52.3	38.4, 40.7
60	72.6	74.8, 74.9	58.2, 58.3
80	119.0	119.4	93
100	......	....	....

[a] $\Delta H_f = 35$ cal/g.
[b] $\Delta H_f = 45$ cal/g.

The original P-E method called for 20 mg samples. When this method was applied to harder fats than the Smalley samples, the reproducibility fell off. The reproducibility improved with 20 mg samples when the heating rate was slowed to 1.25°/min. However, a statistical study, involving 40 experiments, showed a sample weight dependence. Sample size reduction to 5 mg improved thermal equilibration, permitted the use of the 5°/min scan rate, and circumvented the weight dependency problem with hard fats.

Table II shows the NMR results for the two fats which differed in SFI only at 80 and 92 F. Below, it shows the corresponding DSC values for 20 mg samples at two scan rates. The latter numbers were calculated using 42 cal/g for the heat of fusion for this lauric-type fat.

A statistical comparison was made of the dilation, NMR and DSC methods. The statistician made the approximating assumption that all three methods gave equal precision, i.e., a difference of ±10% between two fats at 50 F is equally meaningful for any of the three methods. This is not strictly true. The

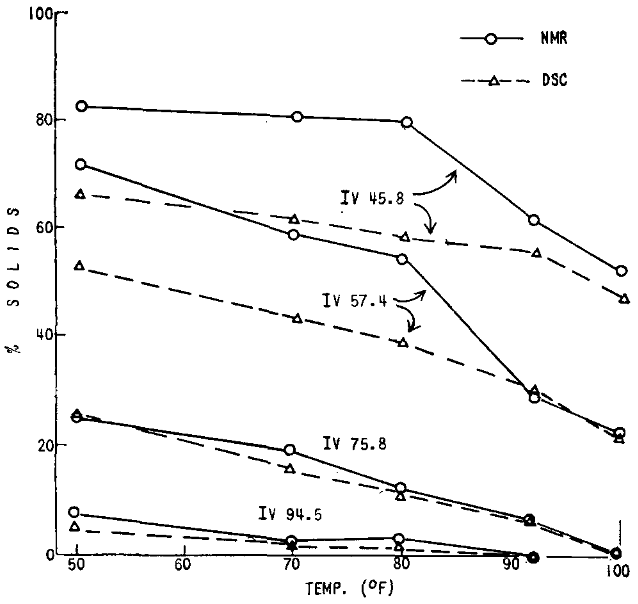

FIG. 7. NMR vs. DSC on hydrogenated cottonseed oil.

TABLE II

DSC vs. NMR vs. SFI for Hard Fats

	Temp.	NMR	SFI
Fat 1	80	74	56.6
	92	13	11.0
Fat 2	80	82	64.4
	92	13	11.4
DSC (20 mg sample)			
		5°/min.	1.25°/min.
Fat 1	80	55.5	57.5
	92	17.7	9.1
Fat 2	80	63.4	60.3
	92	19.5	10.7

dilatation method provides less variance between duplicates examined on the same day. Table III shows the comparative results of six fats using Wilcoxon's two-way classification test (9). NMR gives a favored result in only one instance. In 14 of the remaining 15 pairs, the DSC method is equal to (nine NSD) or better than (five) either or both of the other methods.

Perkin-Elmer cooperated with General Foods in a second interlaboratory study. Each laboratory used the same two fats in duplicate determinations. Also, each laboratory retempered each sample in the instrument and reexamined them. This retempering established instrumental reproducibility and the reproducibility of the tempering process while eliminating weighing and sampling errors since the same samples were used. A correlation coefficient of 0.998 resulted between duplicate temperings. Interlaboratory agreement showed no significant difference, except at 92 F where a technique factor may be responsible. Both laboratories readily distinguished the two fats despite the fact that they differed only at the higher temperature.

The pooled variance from duplicate determinations used to estimate the 95% confidence interval for a single determination are shown in Table IV. With only two determinations, it is impossible to determine whether repeated measurements would distribute normally about the mean. Therefore, the confidence intervals may not be meaningful. A larger study, or some quality control results, would give more validity to these results and perhaps narrow the ranges.

Evaluation

The above results demonstrate the advantages and disadvantages of the DSC fat solids methods described below:

TABLE III

Statistical Study Comparison Results[a] of SFI vs. DSC vs. NMR on Hard Fat

	1	2	3	4	5
1	....	....	....	....	
2	NSD[b]	....	....	....	....
3	NSD	DSC > Others[c]	....	....	....
4	NSD	DSC > NMR	NSD	....	....
5	NMR > SFI	NSD	DSC > Others	DSC > SFI	.
6	NSD	NSD	DSC > SFI	NSD	NSD

[a] Non-parametric test (Wilcoxon's two-way classification). Assumed all three methods gave equal precision.

[b] NSD = no significant difference.

[c] > = greater discriminatory power determined at a significance level of 0.10.

TABLE IV

95% Confidence Intervals

	Temperature (F)			
	50	70	80	92
Confidence Interval	±7.8	±6.5	±2.7	±2.0

Advantages. (a) The thermal history of a fat can be determined. Fats have a "memory," to a degree, and during the initial melt, the DSC can often give valuable clues as to the tempering or storage temperature to which the fat has been subjected. (b) In the fat melt curve, one obtained a "fingerprint" of the fat. This is something of importance in trying to distinguish two fats having identical SFI values. (c) There are no temperature limits. For example, the fat solids range could readily be extended to −20 F for soft fats or to 150 F for hard fats, if necessary. (d) There are no limits on the hardness of the fats accommodated by the method (tristearin can be assayed). (e) A sample can be examined in about 1 hr with 1 hr elapsed time (2 hr for duplication). This time might well be shortened in a control laboratory with a more routine operation and also with external pretempering of the fat. (f) The precision is quite good (±3% of value at 80 F) and interlaboratory agreement is excellent (see Fig. 4).

Disadvantages. (a) The small sample size (5 mg) poses the problem of representative sampling. Sampling from a melt obviates this to a high degree. (b) The variance in fat solids is not constant. It is greater at low temperatures. This would be a hindrance only for fats, differing, say at only 50 to 70 F. (c) The variance in fat solids by DSC is greater at low temperatures than is the variance by dilatation for duplicates run simultaneously. This difference will undoubtedly diminish as experience is gained in this field.

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