Detection of Animal Fats in Butter by Differential Scanning Calorimetry: A Pilot Study

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Because of its high price, butter has always been the object of adulteration by addition of less expensive vegetable or animal fats. Although a number of methods have been proposed for the detection of butterfat adulteration, none has found widespread use. For this reason, a study was conducted to assess the use of differential scanning calorimetry (DSC) for detecting the presence of added animal fat, i.e., chicken fat, in butter. The results obtained show that DSC is an efficient method for characterizing pure animal fats as well as their mixtures. Furthermore, the accuracy with which data are obtained, in combination with the sensitivity of DSC to subtle changes in chemical composition of the sample, makes DSC an attractive possibility for development as a quality control procedure.

KEY WORDS: **Animal fats, butterfat adulteration, differential scanning calorimetry, fat quality control.**

Product quality has become extremely important over the last few years, particularly in relation to foodstuffs. The certification of product quality takes on added importance as an indicator for food marketing within a competitive system, such as the European one. Quality becomes even more important since commercial guidelines have predicted a 5% trade expansion in food products by the end of the century.

All foodstuffs present a quality problem but even more so when they have a high intrinsic commercial value. Within this framework, butter is of commercial importance because it is one of the most expensive fats, and consequently, it must have elevated standards of quality. In this connection, the European Community has developed, by special regulation, strict standards of identity for butter and has established that butter must be obtained exclusively from cow's milk or cream (1). In spite of this, butter has always been subject to adulteration by the addition of less expensive vegetable or animal fats such as beef tallow, lard and, recently, chicken fat.

From the above considerations, the detection of nonmilk fats in butter is emphasized today more than ever, and a number of investigations have been carried out in recent years by several research groups to develop analytical methods for this purpose (2–5). When the added fat is of vegetable origin, it is usually easy for the analyst to recognize the addition (6,7). In contrast, if the added fat is of animal origin, few analytical methods are available, and most are either difficult to perform or time-consuming (8–13). Furthermore, the natural variability in composition of butterfat—caused for the most part by differences in the animal diet—can give rise to problems in interpretation of the data; an example is the fatty acid characterization of butterfat. Numerous investigations have been conducted on the relationship between fatty acid profile and integrity of butter, but the validity of this approach is still causing controversy (14–21). In fact, addition of foreign animal fat up to 10–15% cannot be proven for certain by fatty acid analysis because of their natural variations (22–24). Because the percentage of foreign fat used for the adulteration of butter is usually below 10%, it is impossible, in most cases, to fall back on this approach successfully.

In light of the above considerations, a study was undertaken with two distinct, yet complementary, objectives: (i) to individualize analytical parameters capable to detect fraudulent practices in butter production and (ii) to test the suitability of the new instrumental differential scanning calorimetry (DSC) method reported herein for monitoring the genuineness of butter.

This paper presents the results achieved during the first phase of the overall investigation and is restricted to the identification and quantitation of an animal fat, i.e., chicken fat in butter. In Italy, butter adulteration by chicken fat addition has drawn considerable attention in the last year, when our institute, Istituto Superiore di Sanitá (Rome, Italy), received information from some health control laboratories on a probable presence of this kind of animal fat in some butters of foreign origin. Consequently, there was an urgent need to develop a proper analytical method and to evaluate how widespread this practice is. To meet this aim, it was necessary to devise an analytical technique that is capable of accurate and precise measurements and, at the same time, is applicable to routine analyses. These requirements have been met for the most part by DSC. Therefore, an extensive use of the DSC technique was made in this study (3,24).

EXPERIMENTAL PROCEDURES

Samples analyzed. The following seven sets of samples (ten each) were examined: domestic butter, chicken fat, butter with 2, 5, 10, 15 and 20% of chicken fat.

Sample preparation. Fat characterization by DSC requires preliminary removal of water from the fat so as to avoid any interference during fat solidification and/or melting processes (25,26). In general, no standard procedure for dehydration is proposed. It should be as simple as possible, its ultimate goal being the complete removal of residual water. Various procedures, both physical and chemical, have been tried. After comparison among their respective performances, it was decided that preference should be given to a relatively simple approach to meet the requirements of reliability and reproducibility that are necessary for this kind of calorimetric analyses. The main steps of our treatment were as follows: (i) Transfer about 5 g of sample into a test-tube; (ii) melt the sample at 60°C in water bath; (iii) centrifuge the sample at 10,000 × *g* for 10 min and remove the aqueous phase; (iv) transfer the fat into another test tube containing anhydrous sodium sulfate; (v) centrifuge the sample at 10,000 × *g* for 10 min; and (vi) transfer the supernatant fat into a beaker and cool at 5°C.

Analytical determinations. Information on the instrumentation used and the operating conditions adopted are listed in Table 1. The instrument was calibrated with indium, tin and *n*-decane to ensure the accuracy of the caloric data at different range settings. Samples of *ca.* 10 mg were weighed into aluminum pans to the nearest 0.1 mg, and covers were crimped into place. An empty, covered

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TABLE 1

Instrumentation and Working Conditions for DSC[a]

Calorimeter	Perkin-Elmer[b] DSC 7 equipped with dry box
Cooling system	Perkin-Elmer Intracooler I with one-stage freon-based mechanical cooler
Thermal analysis controller	Perkin-Elmer TA 1020
Printer	Okidata Microline 320[c]
Heating program	T start: −30°C T final: 50°C Heating rate: 10°C/min Time at T start: 15 min Time at T final: 1 min
Cooling program	T start: 50°C T final: −30°C Cooling rate: 5°C/min Time at T start: 5 min Time at T final: 1 min
Nitrogen flow	Purge gas for the sample holder: 20 cc/min Pure gas for the dry box: 10 cc/min

[a]DSC, differential scanning calorimetry; T, temperature.
[b]Perkin-Elmer (Norwalk, CT).
[c]Okidata Microline 320 (Oki Electric Industry Co., Mont Laurel, NJ).

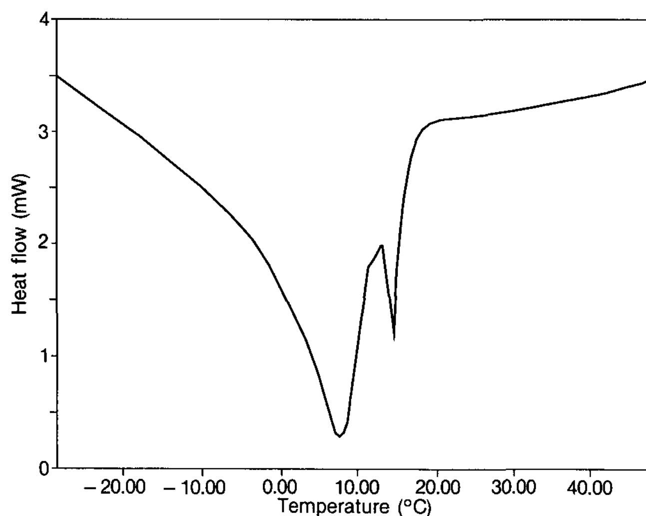

FIG. 1. Differential cooling calorimetry cooling thermogram of pure butterfat. Cooling rate of 5°C/min.

pan was used as a reference. After the sample and reference pans were placed in the calorimeter at room temperature, the cell block of the DSC was cooled to −30°C and flushed with nitrogen. The nitrogen flow rate was then adjusted to a minimum rate, sufficient to maintain a dry and inert atmosphere within the sample area without giving rise to changes in temperature. Both heating and cooling cycles were made on each sample. In the first instance, the samples were subjected to the following temperature program: −30°C isotherm for 15 min; heat from −30 to 50°C at 10°C/min; hold at 50°C for 5 min. The same sample was cooled from 50 to −30°C at 5°C/min. Heating and cooling thermogram traces were analyzed; maxima and minima peak temperatures, onset peak positions, i.e., the first deviation from the baseline established where the tangent to the slope intersects the baseline, and overall energy associated with each process was recorded.

RESULTS AND DISCUSSION

In the heating thermograms, complex features that were not easily interpretable, such as shoulders not separable from peaks, were noticed. Furthermore, the shape of the heating curve of chicken fat strongly resembled that of butterfat. Finally, the tempering or storage temperature to which butterfat was subjected significantly affected the shape of the heating curve (27,28). This is a consequence of the known phenomenon of polymorphism of animal fats that is strongly dependent on the thermal history of the sample. The detailed polymorphic behavior of each of the fats examined can be found elsewhere in a comprehensive review (29).

For all the aforementioned reasons, it was decided to focus our attention on cooling curves of the fats, which were influenced only by the chemical composition of the sample and not by the initial crystalline state because the analytical is initiated from a melted sample. Furthermore, the cooling curves produced more characteristic thermograms for the two animal fats studied. Various cooling curves are shown in Figures 1, 2 and 3. Figure 1 shows a typical solidification curve of pure Italian butterfat: The first peak occurs at about 14.5°C, with the major peak near 7.5°C. Both are exothermic peaks and are associated with crystallization phenomena of polymorphic forms. Figure 2 shows the solidification curve of chicken fat, which also displays two exothermic events. The comparison between chicken fat and butterfat thermograms shows several differences: Chicken fat exothermic peaks occur at lower temperatures (about 12.0 and 0.5°C, respectively), they are broader, and finally, after the minimum is reached on the main peak, a shoulder appears at approximately −3.5°C. The addition of chicken fat (20%) to butterfat changes the solidification curve in two respects (Fig. 3): (i) The two exotherms are shifted to lower temperatures (6.5 vs. 7.5°C and 14.0 vs. 14.5°C); and (ii) the total enthalpy change decreases (57.5 vs. 69.5).

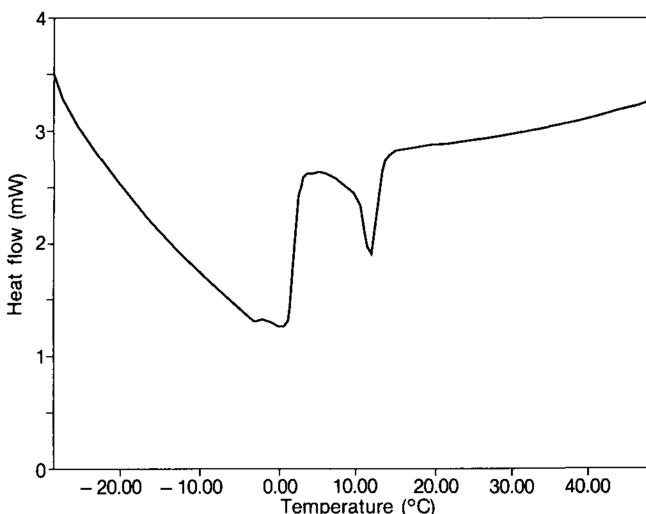

FIG. 2. Differential cooling calorimetry cooling thermogram of pure chicken fat. Cooling rate of 5°C/min.

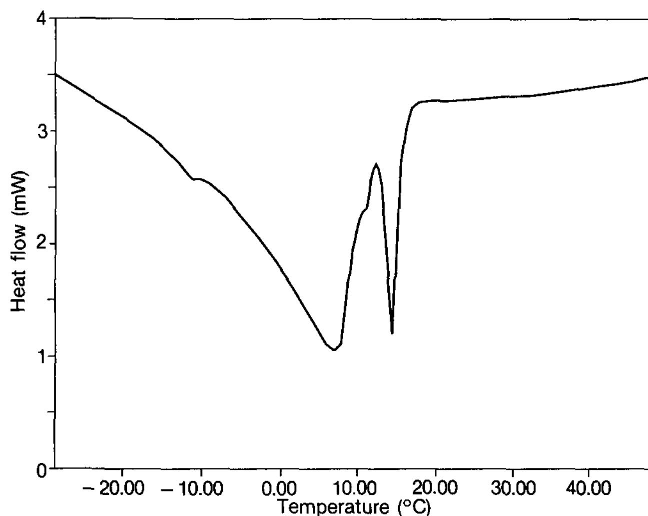

FIG. 3. Differential cooling calorimetry cooling thermogram of mixture of butterfat and 20% chicken fat. Cooling rate of 5°C/min.

TABLE 2

Mean Values and Relative Standard Deviations (RSDs) of Peak Area of the Subtracted Curves

Sample	Number of samples	Mean (J/g)	RSD (%)
Butterfat[a]	10	0.005	40
Butterfat + 2% chicken fat	10	0.031	35
Butterfat + 5% chicken fat	10	0.078	19
Butterfat + 10% chicken fat	10	0.175	7
Butterfat + 15% chicken fat	10	0.287	6
Butterfat + 20% chicken fat	10	0.362	4

[a]In this case, the peak area represents the instrumental background noise.

Statistical evaluation of these changes by the Roos criterion (30) showed that, in mixtures containing less than 10% chicken fat, the standard error was too great for quantitative analysis.

Because of the above, an alternative approach was used to reach the aims of this study. By exploiting the data handling options of the instrument software, the normalized butterfat thermograms were subtracted from the thermograms of the normalized butter fat/chicken fat mixture. The resulting curves showed a small peak at about −12°C (Fig. 4). The area of this latter exotherm was directly proportional to the amount of chicken fat added to butterfat. In Table 2, the mean values of the −12°C peak area and the respective standard deviations for butterfat and mixtures of butterfat and chicken fat are listed. Figure 5 shows that, in the range of compositions of practical interest (2–20%), there is a good linear correlation (R = 0.998) between the −12°C exothermic peak area and the chicken fat content of the mixture.

To evaluate the possibility of considering the aforementioned peak as a useful indicator of chicken fat additions, DSC analyses were performed on several butters of various origin and on their respective mixtures with chicken fat. In spite of obvious variations in breeding, feeding, environmental conditions and production technology, there were no changes in the position and shape of the peak of concern, although, in some instances, the overall thermal behavior of the butters differed substantially.

To assure the specificity of this method for the detection and quantitation of chicken fat in butterfat, further calorimetric analyses were made on mixtures of butterfat with other animal fats, i.e., beef tallow and lard. The corresponding cooling thermograms—mathematically elaborated as previously described—do not give rise to the

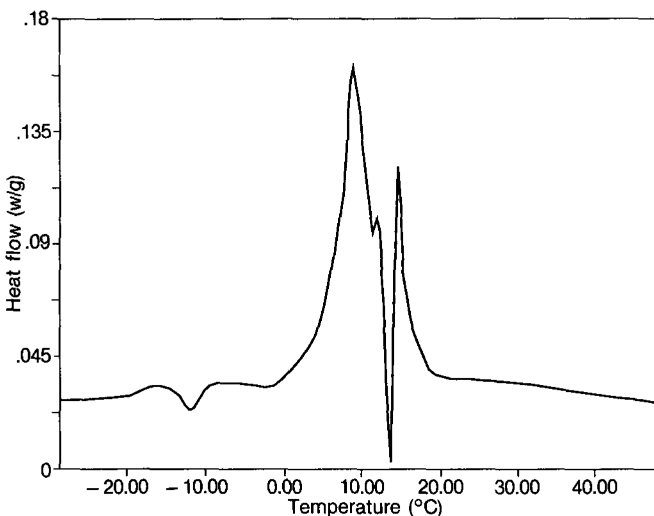

FIG. 4. Thermogram generated by subtraction of the cooling data curve of pure butterfat from the cooling data curve of mixture of butterfat and 20% chicken fat.

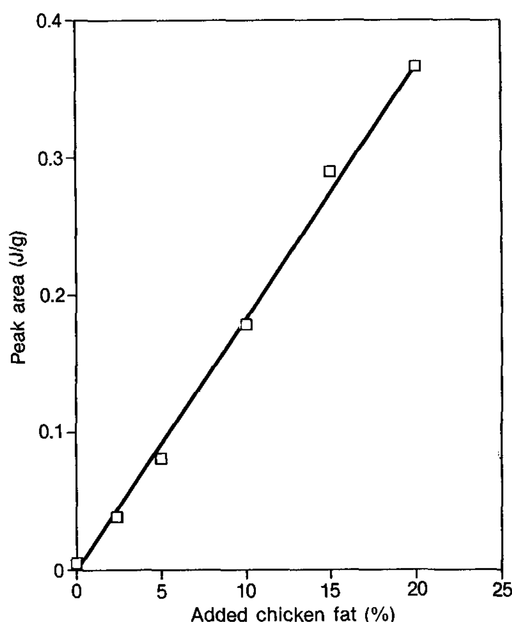

FIG. 5. Correlation between mean values of peak area and the percent amounts of chicken fat in butterfat.

TABLE 3

Fatty Acid Composition of the Animal Fat Samples[a]

Fatty acid	Butter (%)	Lard (%)	Tallow (%)	Chicken fat (%)
$C_{4:0}$ (Butyric)	2.8	0.1	0.1	—
$C_{6:0}$ (Caproic)	1.8	—	—	—
$C_{8:0}$ (Caprylic)	1.1	—	—	—
$C_{10:0}$ (Capric)	2.5	0.1	0.1	—
$C_{11:0}$ (Undecanoic)	0.2	—	—	—
$C_{12:0}$ (Lauric)	3.5	0.3	0.4	—
$C_{14:0}$ (Myristic)	11.2	1.5	2.7	1.4
$C_{14:1}$ (Myristoleic)	0.9	—	0.3	0.3
$C_{15:0}$ (Pentadecanoic)	1.4	0.1	0.7	—
$C_{16:0}$ (Palmitic)	29.9	22.9	24.9	22.5
$C_{16:1}$ (Palmitoleic)	1.2	2.0	3.0	5.1
$C_{17:0}$ (Margaric)	0.9	0.3	1.1	0.3
$C_{17:1}$ (Margaroleic)	0.6	0.3	1.2	0.3
$C_{18:0}$ (Stearic)	10.8	13.2	18.6	7.0
$C_{18:1}$ (Oleic)	22.7	41.5	37.2	40.6
$C_{18:2}$ (Linoleic)	2.2	13.2	4.7	17.3
$C_{18:3}$ (Linolenic)	0.5	0.9	0.4	1.9
$C_{20:0}$ (Arachidic)	0.2	0.2	0.1	—

[a]Fatty acid compositions were determined by gas chromatography and are expressed as mean average weight percentage on a fatty acid basis. Trace acids (less than 0.1%) are not reported.

characteristic peak at about −12°C. This supports the hypothesis that chicken fat has a peculiar crystallization exotherm at this temperature. The meaning of this finding cannot be elucidated at the moment, even though one is tempted to identify a different fatty acid composition as the cause. To extract any possible information from the data obtained in this investigation, the three animal fats—chicken fat, beef tallow and lard—were analyzed by gas chromatography to determine the relative proportions of fatty acids (Table 3). The results show that the stearic acid percentage of chicken fat, compared with the other two fats, is much lower, whereas some short-chain fatty acids, such as butyric, capric and lauric acids, are completely absent. On the contrary, linoleic and linolenic acid percentages are much higher in chicken fat. This finding represents one explanation for the different thermal behavior of chicken fat during crystallization.

The speed and ease of data manipulation, brought about by computer control, during this study represent a further benefit of DSC analysis on model systems of animal fats. It is evident that this capability allows for determining distinctive changes that arise from the cooling technique applied. In the future, this degree of control may be used to distinguish complex mixtures of animal fats.

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