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# DETECTION OF BEEF BODY FAT AND MARGARINE IN BUTTERFAT BY DIFFERENTIAL SCANNING CALORIMETRY

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## Abstract

In this study differential scanning calorimetry (DSC) melting and crystallization curves of butterfat, beef body fat (BBF) and margarine were formed by cooling gradually from 70 to –40°C. Then margarine and BBF were added to butterfat at the rates of 5, 10 and 20% in order to investigate their curves. When BBF or margarine was added to butterfat, 1. and 2. peak areas increased in crystallization curves of butterfat with 2. peak being more discernible. Results obtained show that DSC technique could be used in order to determine adulteration of butterfat.

**Keywords:** adulteration, beef body fat, butterfat, differential scanning calorimetry, margarine

## Introduction

Milk is processed to butterfat in every family farms and milk processing plants of Turkey. It is noted that the annual butterfat production is 116.000 tons according to statistical data [1]. It is frequently adulterated to meet the demand which exceeds the supply and to increase profit margins. Adulterants fall into two main categories: vegetable oils and fats, and animal body fats. Adulteration with vegetable fats and oils can be detected by several thin layer chromatographic techniques. Animal body fat adulteration is more difficult to detect [2–3]. For this purpose, chromatographic techniques cannot be applied, except in the case of tallow adulterations [4]. On the other hand, every oil or fat has characteristic fatty acids and triacylglycerol (TAG) profiles, which are unique to the type of oil and can be used in detecting adulteration [5]. In general, all oils and fats are composed of a complex mixture of 96 to 99% of TAG, which are the esters of glycerol and fatty acids. Therefore, oils and fats from plant origin can be further classified according to their fatty acids and TAG compositions. Analysis of the TAG composition of an oil or fat requires methods of separating their complex mixtures into individual components or at least into simpler mixtures that contain only a few TAG each [6]. The complex mixtures of TAG from oils and fats have usually been analyzed by reversed-phase high-performance liquid chromatogra-

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phy (HPLC). However, complete determination of TAG profile can be achieved only by several successive procedures that are tedious and time-consuming. Therefore, this approach is less practical for the oil industry, for quality-control programs, and for many research and development programs.

Thermal analysis has long been available to the oils and fats researcher [7–11]. Since applications of this technique started, and abundance of data has become available on the reproducibility of some basic quantities measured or derived from thermoanalytical curves [12]. DSC is the most widely used thermoanalytical technique for oils and fats [9]. This technique is used for studying various heat-related phenomena in materials by monitoring associated changes in enthalpy. Nowadays, DSC is preferred to other similar calorimetric techniques, such as DTA, because the former has the advantage of providing a more direct measurement of the energy accompanying the physical and chemical changes studied [13]. For many years, DSC data of oils and fats have given valuable information on melting and crystallizing temperatures as well as heats of fusion and crystallization [14]. In DSC melting curves of oils and fats, complex features were not easily interpretable. This is a consequence of the known phenomenon of polymorphism of oils and fats that is strongly dependent on the thermal history of the sample. Conversely, the DSC crystallization curve, which is influenced only by the chemical composition of the sample, and not by the initial crystalline state, is more reproducible and simpler than the melting curve [15–16]. Many studies have been conducted to investigate the thermal profile of various oils and fats products [17–19].

The purpose of this study was to detect and determine adulterated butter with BBF and margarine using DSC technique.

## Materials and methods

BBF used in this study was obtained from a major slaughter-house in Erzurum, Turkey. Commercial margarine (50% palm oil, 50% cottonseeds oil) was obtained from a local oil factory (Doyasan Oil Factory, Erzurum, Turkey). Butterfat samples were purchased from a farm in Kars, Turkey.

BBF was prepared by melting the adipose tissue at 50°C, then liquid oil phase was filtered through filter paper. All margarines, BBF and butterfat were evaporated at 50°C in an evaporator for the purpose of evaporation of all the moisture content of samples. After evaporation, remaining residues were used in the experiment.

BBF, margarine and butterfat samples were individually melted to 50°C. The resulting liquids were then mixed with butterfat by using micropipette so that the BBF and margarine fat contents would be 5, 10 and 20%. Prior to DSC analysis, all samples were stored at 4°C.

The endothermic and exothermic transitions of samples were measured as described by Lambelet and Ganguli [20] using a Shimadzu DSC-50 (Kyoto, Japan). DSC was calibrated with indium (*m.p.*: 156.4°C, $\Delta H_f$: 28.47 J $g^{-1}$). Previously melted samples of fat 10 mg were weighed into aluminium hermetic cell and sealed with a crimper. An empty, covered cell was used as a reference. The cells were initially

heated to 70°C to destroy any previous crystalline structure, and then cooled at 1°C $min^{-1}$ to –40°C to crystallize the material and subsequently reheated to 70°C at 2°C $min^{-1}$. The data treatment was determined using a Shimadzu TA-50I data processor. All experiments were conducted with three replicates.

Analysis of variance of all data was conducted using general linear models (GLM) procedure [21].

## Results and discussion

Melting and crystallization, two commonly used physical events to characterize thermal behavior of oil and fat samples, require the intake or release of thermal enthalpy. DSC is eminently suitable to determine these physical properties of oil and fat samples. Generally, in melting curves of fat samples, complex features that were not easily interpretable, such as shoulders not separable from peaks, were noticed. These results illustrate the complex nature of TAG in fat samples. Due to complexity of the recorded thermal events, all melting and crystallization points are read at the maximum/minimum of either endo-or exotherm peaks. Overall, the designation of transition temperatures for crystallization curves are clearly indicated in Table 1.

**Table 1** Influence of the beef body fat and margarine concentration on the DSC crystallization curves of butter with results of Duncan's multiple comparisons test

| Treatment | Adulterant fat/% | Peak temperature/°C | | 1. and 2. peak area (total)/J $g^{-1}$ | Percent increase in 1. and 2 peak areas |
|---|---|---|---|---|---|
| | | 1. peak | 2. peak | | |
| Butter (control) | – | 11.45±0.56$^{a}$ | 7.37±0.19$^{e}$ | 5.18±0.71$^{a}$ | – |
| Margarine /% | 5 | 12.74±0.09$^{bc}$ | 7.05±0.04$^{cd}$ | 8.10±0.05$^{b}$ | 56.25 |
| | 10 | 13.07±0.15$^{c}$ | 6.82±0.03$^{b}$ | 8.62±0.15$^{b}$ | 66.17 |
| | 20 | 12.51±0.07$^{b}$ | 6.05±0.19$^{a}$ | 8.79±0.41$^{b}$ | 69.35 |
| Beef body fat/% | 5 | 14.14±0.08$^{d}$ | 7.19±0.08$^{de}$ | 8.74±0.32$^{b}$ | 68.50 |
| | 10 | 14.13±0.15$^{d}$ | 7.12±0.06$^{d}$ | 9.73±0.65$^{c}$ | 87.56 |
| | 20 | 15.92±0.06$^{e}$ | 6.86±0.06$^{bc}$ | 11.17±0.22$^{d}$ | 115.28 |

±: standard deviation for three samples
$^{a–e}$values in a column with the same superscript are not significantly different by Duncan's multiple range test ($p<0.05$)

It is clear that the DSC curve of the BBF is different from the butterfat and margarine (Fig. 1). Melting curve of the BBF shows a big endothermic peak between 10 and 50°C, whereas the butterfat and margarine are completely molten at 40°C. There is also some difference in the melting curve in butterfat and margarine which could result from the difference in its triglyceride composition. Crystallization of BBF begins at 27°C, whereas the butterfat and margarine begin to crystallize at 15°C (Fig. 2). These differences provide a basis for the determination of adulteration in butterfat us-

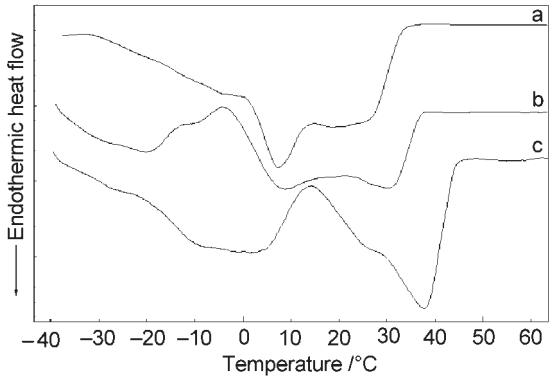


**Fig. 1** DSC melting curves of butterfat, margarine and BBF. a – butterfat; b – margarine; c – BBF

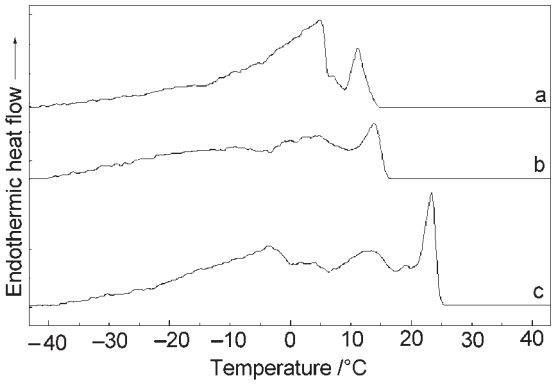


**Fig. 2** DSC crystallization curves of butterfat, margarine and BBF. a – butterfat; b – margarine; c – BBF

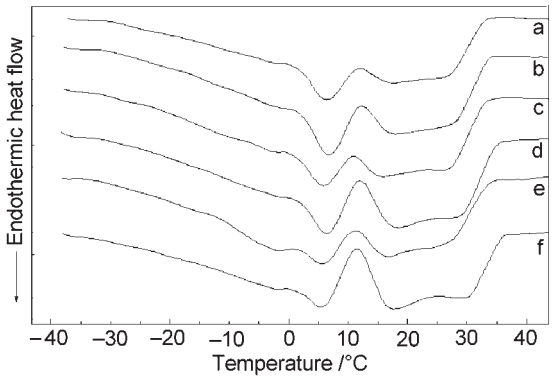


**Fig. 3** DSC melting curves of butterfat–margarine and butterfat–BBF mixtures. a – butterfat+5% margarine; b – butterfat+10% margarine; c – butterfat+20% margarine; d – butterfat+5% BBF; e – butterfat+10% BBF; f – butterfat+20% BBF

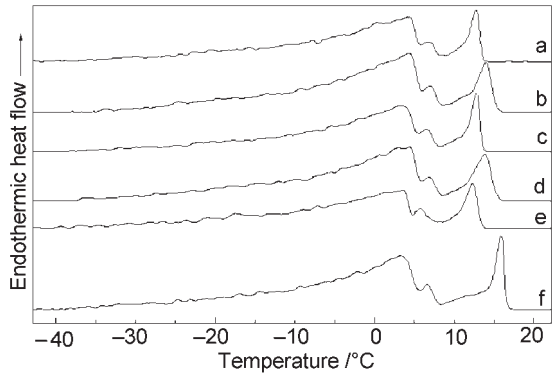


**Fig. 4** DSC crystallization curves of butterfat–margarine and butterfat–BBF mixtures. a – butterfat+5% margarine; b – butterfat+10% margarine; c – butterfat+20% margarine; d – butterfat+5% BBF; e – butterfat+10% BBF; f – butterfat+20% BBF

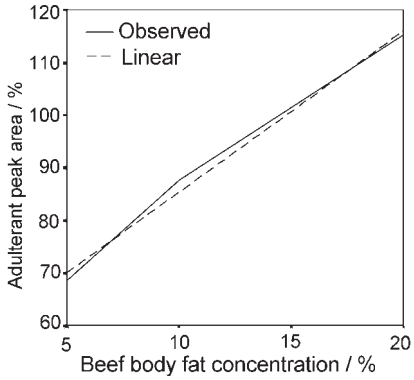


**Fig. 5** Linear relationship of butterfat–BBF mixtures

ing DSC. As can be seen in Fig. 1, the melting curve of butterfat showed one major endotherm region. The endotherm region at higher temperature consisted of a plateau with a pair of shoulder peaks, while the endotherm region at lower temperature contained a small peak. In margarine, a major endothermic peak with a shoulder peak and a small distinct endothermic peak were observed. Adding 5, 10 and 20% BBF or margarine to butterfat had a significant difference in the DSC melting curve of butterfat (Fig. 3). These differences were very clear at the level of 10 and 20%.

Polymorphism of natural oils and fats that has interested researchers for many years [15–16]. Measurement based on crystallization diagrams have the further advantage of avoiding problems linked to polymorphism of fats, so the results are independent of the thermal treatment of the samples prior to analysis. Therefore, all the results discussed below refer only to DSC crystallization curves.

In butterfat, the crystallization curve is characterized by three peaks. Similar results were also reported by Foudad *et al.* [19]. Adding 5% of BBF or margarine

caused a slight difference in the DSC curve of butterfat. But adding 10 or 20% BBF to butterfat was involved significant modification of corresponding curve of butterfat. These modifications were statistically significant for both peak temperatures and peak areas ($p<0.05$). The multiple comparison test were shown in Table 1. The first two peaks were changed in relative proportion as the BBF or margarine content increased. The main differences in the curve shape resulting from BBF or margarine content were manifested in the smaller peak. Generally speaking, this peak increased in sharpness, broadness and area as the adulterant fat level increased. This effect is greater for addition of BBF than for margarine. Lambelet *et al.* [13], and Lambelet and Ganguli [20] reported similar changes in peak areas in adulteration of cow and buffalo ghees. Also, the position of these peaks changes with the amount of BBF or margarine in a mixture (Table 1). While the first peak corresponding to the butterfat was shifted to the higher temperature region, the second peak to the lower temperature region (Table 1).

The relationship between the relative areas of the first two peaks and BBF concentration was linear related by regression analysis $y=54.6380+3.0695\cdot$(correlation coefficient=0.993, $p<0.05$; Fig. 5). However, in case of margarine adulteration, this relationship was not linear (correlation coefficient=0.788, $p>0.05$). In BBF adulteration this area was increased markedly from 10 to 20%. This comes from the difference between margarine and BBF enthalpies.

## Conclusions

It can be concluded that the thermal properties of various oil and fat samples from the DSC melting and crystallization curve can be characterized by various transition temperatures. It provides useful information on the nature of the thermodynamic changes that are associated with the edible oils and fats transforming from one physical state to another. These thermodynamic characteristics are sensitive to the general chemical composition of edible oils and fats and thus can be used in qualitative and quantitative ways for identification of edible oils.

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