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# Differential scanning calorimetry for authentication of edible fats and oils—What can we learn from the past to face the current challenges?

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## Abstract

Fats and oils authentication has become an important issue recently, due to the growing interest in consumption of cold-pressed oils. Therefore, it is becoming more and more difficult to maintain official control over the growing assortment of new cold-pressed oils. Authenticity of plant oils is also an important issue for religious or cultural reasons. This review article focuses on the application of differential scanning calorimetry (DSC) in the field of assessing authenticity of various fats and oils (e.g. olive oil, palm oil, confectionery fats, butter). Extra virgin olive oil (EVOO) is the most comprehensively tested oil by means of the DSC technique in terms of the authenticity of origin as well as the adulteration with foreign oils. In most of the studies on DSC applicability for authentication, crystallization and melting curves were analyzed by the conventional DSC, although other modified DSC methods were also applied, such as isothermal freezing, modulated temperature DSC (MT-DSC) and fast DSC. However, the most promising are the melting profiles, which, due to the complexity of transitions, need advanced chemometric tools as well as tools for peaks deconvolution. The future prospect of using DSC in the authenticity assessment lies also in the use of DSC techniques along with other complementary chromatographic or spectroscopic techniques.

**Keywords:** Crystallization profiles, Differential scanning calorimetry, Edible oils, Food authenticity, Melting profiles

## 1. Introduction

Innovative food formulation research is coherently connected with the study of its thermal properties, since temperature is one of the most important parameters used in the production. Thermal analysis (TA) techniques have always been extensively employed in research and development and have been found useful in many wide-ranging applications from inorganic to organic materials, providing valuable thermodynamic and kinetic data simultaneously [1,2]. As the most widely used among the various thermal techniques, DSC was initially used to test inorganic materials such as polymers or construction materials, e.g. cement, gypsum and clays and later in the studies of biomolecules like

proteins or biomembranes [3]. Nowadays, this technique is also widely used in the routine analysis of drugs compounds [4], biomolecules and nano-sized materials [5], energetic materials [6] as well as petroleum products [7]. Gradually over time, food research using DSC techniques has undergone intensive development, mainly focused on the study of protein denaturation and protein unfolding [8], starch gelatinization or retrogradation in cereal based products technology, evaluation of physical characteristics and authentication of oils and fats based on their thermal behaviors like the phase transition of melting and crystallization, as well as oxidative stability. There are numerous review articles showing the spectrum of application and the importance of DSC in various food compounds

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analysis [1,9–12]. Differential scanning calorimetry (DSC) has been applied in oils and fats research for various aspects, providing thermodynamic data for their description, as thermal properties were found to be related to the chemical composition [13]. According to the study, it has been shown that the crystallization and melting profiles of fats and oils are regulated by heat-related phenomena and associated mainly with their triacylglycerols (TGA) and fatty acids (FA) compositions. Thus, analysis of their melting and crystallization behavior using the DSC technique has made a significant contribution to the authenticity assessment of fats and oils. The pioneering research on the use of the DSC technique for authenticating oils was published by Dyszel and Baish [14]. This was the first attempt at creating a data bank with the calorimetric “fingerprints” of the main edible oils. Scientific literatures framed from last decade showed plenty possibilities of DSC usage in qualitative and quantitative analysis of fats and oils from wide array of resources, i.e. cold-pressed oils, extracted oils, refined oils, as well as interesterificated or fractionated fats [15–20]. Latest scientific papers with application of DSC for oils and fats authenticity assessment showed that this technique has been used in wide range of fats and oils from both animal and plants sources, however it is not used as only one technique, but usually in combination with other complementary chromatographic or spectroscopic techniques [21–26].

## 2. Differential scanning calorimetry

### 2.1. Principles of differential scanning calorimetry

According to the terminology of ISO standard, differential scanning calorimetry (DSC) is a technique in which the difference between the rate of heat flow into a specimen and into a reference is derived as a function of temperature and/or time, while the specimen and reference are subjected to the same controlled temperature program in a specified atmosphere using a symmetrical measurement system [27]. The DSC technique is a development of the earlier method of DTA (Differential Thermal Analysis), where the basic principle is measuring temperature changes. A DTA curve depicts temperature difference between sample and reference material ( $\Delta T$ ) as a function of temperature. The earliest DSC (Model DSC-1) was originally introduced in 1964 by Watson and his co-workers at Perkin-Elmer Co [28]. The basic novelty of DSC involves measuring the temperatures and heat flows associated with transitions in materials as a function

of time and temperature in a controlled atmosphere. In contrast to DTA, DSC is a technique where it is presumed that the sample and the reference are in similar conditions at a pre-programmed heating/cooling rate, and thus measures the energy (in the form of heat) necessary to achieve a zero-temperature difference between them, as a function of temperature or time. With the DSC technique it is possible to detect the amount of heat absorbed or released during phase transition by measuring the difference in heat flow between the sample and reference [29]. The resulting plot is known as the DSC curve, which expresses heat flow as a function of temperature.

Based on the principle of the measure, DSC instrumentation uses two different methods of measurement, which can be distinguished as follows:

- The heat flux DSC (heat flow)
- The power compensation DSC (heat compensation)

The difference between two these two types of DSC is presented in Fig. 1. The heat flux DSC comprises two crucibles, sample and inert reference material, placed symmetrically on a thermoelectric disc, surrounded by a single furnace. The sample and reference material are subjected to the same temperature-control program by a single heater. If there would be a temperature difference between the sample and reference, which is measured by thermocouples, the consequent heat flow is determined by the thermal equivalent of Ohm's law:  $q = \Delta T/R$ , where  $q$  is “sample heat flow”,  $\Delta T$  is “temperature difference between sample and reference”, and  $R$  is “resistance of thermoelectric disk” [30]. In fact, in heat flux DSC the heat flow is derived from the temperature difference.

The most noticeable difference between the heat flux DSC and the power compensation DSC is the presence in the latter technique of two identical and isolated heaters, one for the sample and one for the reference pan [31]. Through these two independent heating sources, the temperature generated to the sample and reference pans are kept equal (i.e. increased or decreased linearly). Hence, the power necessary to achieve identical temperature for the sample and reference is measured and the temperature difference is kept close to zero by adjusting the heat flow between both pans. The temperature of the sample and reference are continuously monitored by a platinum resistance thermometer. By following the principle of this instrument, the quantitative measurement is performed in the form

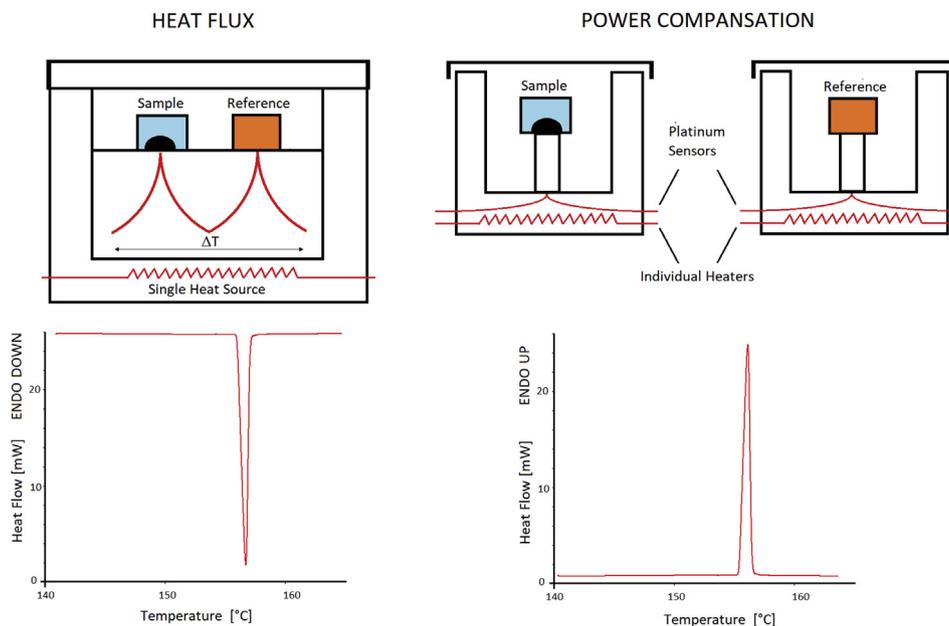


Fig. 1. Comparison of the Heat flux and Power compensation DSC.

of power expressed in watts [31]. The difference between heat flux and power compensated DSC is also visible in the form of DSC curves, as endothermic power compensated DSC curves are directed up and in heat flux DSC directed down (Fig. 1).

## 2.2. Modifications of conventional DSC

Besides traditional DSC, there are some modifications used in thermal properties research, such as High Pressure (HP DSC), Modulated Temperature DSC (MT-DSC) or Fast Scan DSC. HP DSC is preferable when an oxidative stability test takes too long time at atmospheric pressures or when it is important to suppress the volatile compound present in the sample or when water or methanol is produced as a by-product, leading to foaming in the sample. The HP DSC as an alternative method to conventional DSC, is used mostly for oils oxidative stability studies [32]. Modulated Temperature DSC (MT-DSC) is the term for DSC techniques where a non-linear heating or cooling rate is applied to the sample by applying a series of heating (or cooling) micro-steps followed by an isothermal hold to separate the kinetic from the thermodynamic data. MT-DSC has been also used in the study of fats and oils [33]. Samyn et al. presented the utility of MT-DSC for analysis of vegetable oils as they assessed the heating curves of palm, soybean, sunflower, corn, rapeseed and castor oils to determine their thermal behavior [34]. The seed flours and oils of

blackberry and raspberry were analyzed by means of conventional DSC and MT-DSC [35]. Leyva-Porras showed application of using Supercooling Modulated Differential Scanning Calorimetry (SMDSC) in the studies of food materials presenting phase transitions at relatively low temperatures [36].

A Fast Scan DSC applies very high heating rates from 30 to 2 400 000 °C/min and cooling rates from 6 to 240 000 °C/min to a sample to increase the sensitivity of a DSC or to trap kinetic behavior. In the case of a high scanning rate, it is possible to enhance weak transitions and to analyze, for example, at very low levels of amorphous materials in pharmaceuticals [37,38]. At fast scanning rates, high or low temperatures are reached in a very short time causing that triacylglycerols are melting simultaneously even though their melting temperatures are different, which results in broader peaks. In fact fast DSC provides true information of the sample without introducing any additional interference, such as recrystallization or decomposition [39], but it should not be used to study polymorphism of fats.

## 2.3. DSC hyphenated with other analytical techniques

During last decades the instrumentation of DSC has been upgraded and hyphenated with other analytical instruments to exploit the advantages from different techniques i.e. mostly popular DSC-Raman (differential scanning calorimetry and

Raman spectroscopy), DSC-FTIR (differential scanning calorimetry and Fourier transform infrared spectroscopy), TG-MS-DSC (thermogravimetry-mass spectrometry-differential scanning calorimetry). Along with the advanced DSC models, there has been remarkable progress in the field of DSC and hyphenated techniques application in characterization of food compounds i.e. DSC combined with mass spectrometry and thermogravimetry analysis (TG-MS-DSC) was used to assess pyrolysis conversion process of food wastes [40] by analyzing thermal decomposition of samples or in the study of the thermal behavior of 4,4-dinitrocarbanilide (DNC) decay in chicken meat [41]. In another research, researchers coupled TG-DTG-DSC (thermogravimetry-derivative thermogravimetry-DSC) to study the thermal behavior and structure analysis by FTIR. DSC with FTIR was used to analyze the optimal reaction conditions for transesterification of frying and fish oils residues to obtain promising yield of biofuels [42]. TG-DSC-FTIR (DSC coupled with and thermogravimetry and Fourier transform infrared spectroscopy) was applied to investigate the thermal behavior of inulin by analyzing the enthalpy of the endothermic and exothermic events) [43]. DSC-FTIR techniques can provide parallelly the spectroscopic and thermodynamic information of solid or liquid phase of various materials [44]. This techniques have been effectively used for the investigation of encapsulated squid oil stability [45].

Another hyphenated technique called MEMS-DSC (Micro electromechanical systems combined with differential scanning calorimetry) consists of integrated microfluids. It's applicability has been reported for assessing protein denaturation by measuring samples thermodynamic parameters through transition in liquid phase [46]. The most promising coupled technique is Raman-DSC because of its applicability to characterize polymorphic materials. This technique allows to investigate the combination of chemical and structural properties through Raman laser, and thermal properties through DSC, which has been demonstrated by the work of Zhou et al., where they characterized the protein denaturation in the rice bran protein and structural changes, occurred during different extrusion temperatures (by assessing the peak and enthalpy of denaturation curve) [47]. Another study showed the applicability of simultaneous FT-Raman-differential scanning calorimetry technique, to study the polymorphism of mono-unsaturated triglyceride SOS (*sn-e 1,3-distearoyl-2-oleoylglycerol*), which is abundant in many fats and oils i.e. cocoa butter [48].

In recent research, another powerful coupled instrument has become popular amongst researchers, which is DSC-XRD (DSC coupled with X-ray diffraction technique). Though the usage has been reported more in pharmaceutical science and materials industry, food analysts are also using this coupled technique in the laboratory, i.e. for assessing the *in situ* polymorphic forms and thermal transitions of palm oil products [49]. Also Barba et al. showed the applicability of DSC analysis in combination with synchrotron XRD technique to identify and characterize the crystal polymorphism of extra virgin olive oil by analyzing the cooling and subsequent heating curves [50].

### 3. Authenticity assessment of olive oil by DSC technique

According to the report published by Moore et al. olive oil is one of the most likely targets for intentional, economically motivated adulteration [51]. Olive oil of the highest quality, named extra virgin olive oil (EVOO), has a long history of being adulterated with cheaper vegetable oils like sunflower or hazelnut oils but also with olive oils of lower quality i.e. refined olive oil, lampante or pomace olive oil. The only oils in commerce that have a legal definition backed by officially sanctioned methods of analysis are the various grades of olive oil [52].

One of the first papers on the application of DSC for detecting olive oil authenticity was published by Márquez and Maza [53] and Márquez et al. [54]. They analyzed the thermal profiles of virgin olive oils from six different Spanish cultivars and correlated their thermal properties with triacylglycerol and fatty acid compositions. A good correlation was found between the temperature of transition (crystallization and melting) and the triacylglycerol composition for the different cultivars. They showed the possibility to differentiate monovarietal virgin olive oils based on temperature (onset and transition range) of crystallization and melting profiles, which were well correlated with oleic and linoleic acid contents. The next studies on olive oil and DSC were performed by Angiuli et al. [55], in which Tuscan oils, defective olive oils, commercial edible seed oils and commercial olive oils were analyzed by modulated differential scanning calorimetry (Table 1). The nucleation and growth rate of the polymorphous crystalline phases of the triacylglycerols and the melting process was investigated. The calorimetric method was also used to analyze the solid-liquid phase transitions in a Tuscan extra-virgin olive oil in order to evaluate the

Table 1. The studies of DSC application for the authenticity (origin and category) assessment of extra virgin olive oil (EVOO).

Oils analyzed	DSC parameters used for analysis	Thermal Program	Scanning rate [°C/min]	Source
Tuscany, defective olive oils, commercial edible seed oils, commercial samples of olive oils	Temperature, enthalpy ( $\Delta H$ , J/g), Induction time IT, Peak time (PT)	Cooling to $-40^\circ\text{C}$ Heating to $50^\circ\text{C}$	10	[55]
Tuscan extra-virgin olive oil	Enthalpy ( $\Delta H$ , J/g), waiting time (WT), Peak minimum (PM)	Cooling to $-40^\circ\text{C}$ Heating to $50^\circ\text{C}$	10	[56]
Tuscan extra virgin olive oil	Induction time (IT), enthalpy ( $\Delta H$ , J/g), temperature	Cooling from $50^\circ\text{C}$ to $-30^\circ\text{C}$ Crystallization isotherm at $-40^\circ\text{C}$	2, 10, 20	[57]
Tuscan and Apulian EVOOs	Enthalpy ( $\Delta H$ , J/g), peak height (Hp w/g), peak area (%), transition range ( $T_{\text{on}}$ , $T_{\text{off}}$ )	Cooling to $-40^\circ\text{C}$ Heating to $50^\circ\text{C}$	10	[58]
Different commercial categories of olive oil	Enthalpy ( $\Delta H$ , J/g), peak height (Hp W/g), transition range ( $T_{\text{on}}$ and $T_{\text{off}}$ )	Cooling to $-80^\circ\text{C}$ Heating to $30^\circ\text{C}$	2	[63]
Virgin olive oil, refined olive oil, soybean, canola, and sunflower oil	Peak height (Hp w/g), transition temperature range $T_{\text{on}}$ ( $^\circ\text{C}$ ) and $T_{\text{off}}$ ( $^\circ\text{C}$ )	Cooling to $-100^\circ\text{C}$ Heating to $25^\circ\text{C}$	5	[64]
18 monovarietal EVOOs from the Apulia region (Italy)	enthalpy (J/g); induction time (s), peak width, width at half peak height ( $^\circ\text{C}$ ) peak temperature ( $^\circ\text{C}$ )	Cooling to $-30^\circ\text{C}$ Heating to $15^\circ\text{C}$	2	[65]

changes induced by light exposition. An isothermal freezing curve and melting profile were applied for assessing extra-virgin olive oils aging and storage effects during 31 weeks in the dark and light exposure [56]. Significant effects caused by the deterioration of olive oil were observed on the isothermal freezing curve after 8 weeks of light exposition. They continued the study of using the isothermal freezing curve for olive oil authentication [57]. Protocols to obtain reproducible DSC thermograms and experiments to understand the origin of the often-observed non-reproducibility were described (Table 1). They observed also that any physical or chemical alteration in the oil composition can interfere significantly with the crystal nucleation and transition enthalpies affecting the isothermal freezing curve. In a subsequent article, the same team [58] explained how analyzing melting curves enabled detection of adulterations in EVOO with peanut, refined hazelnut, crude hazelnut, high oleic sunflower, refined olive oil. They showed in the running program with a temperature range from  $-40^\circ\text{C}$  to  $50^\circ\text{C}$  and a  $10^\circ\text{C}/\text{min}$  scanning rate, melting curves of Tuscan and Apulian EVOO samples (Table 1) and EVOO adulterated with various oils (Table 2). The parameters analyzed from the DSC curves were: peak height, and transition ranges  $T_{\text{on}}$  and  $T_{\text{off}}$ . The authors stated that calorimetric tests could be a useful preliminary stage for quality testing. They also concluded that in order to check the addition of seed oils or refined olive oils to EVOO, the sensitivity limit was about 2% [58]. This research team proved that the calorimetric technique allowed the detection of the adulterant (seed oils or refined olive oil), oil origin, and possible photo-oxidation degradation processes of extra virgin olive oil [56–58].

At the same time, similar research on olive oil by using the DSC technique was carried out by Chia-varo et al. [59]. Crystallization [59] and melting [60] behavior of three monovarietal (*Biancolilla*, *Cerasuola*, and *Nocel-lara del Belice*) extra virgin olive oils from Palermo (Sicily, Italy), were studied by DSC. Two exothermic peaks on the crystallization curve were observed, a minor from  $-17$  to  $-13^\circ\text{C}$  and a major from  $-40$  to  $-38^\circ\text{C}$  [59]. For melting of pure EVOO, multiple transitions were detected: first, an exothermic peak at around  $-20^\circ\text{C}$ , followed by two endothermic peaks, at around  $-4^\circ\text{C}$  and  $10^\circ\text{C}$ , respectively [60]. The thermal properties measured both in cooling and heating regimes of monovarietal EVOO samples were found to correlate well with the chemical composition [59,60].

DSC technique was also employed to detect adulteration of EVOO with refined hazelnut oil (HaO) [61] and high oleic sunflower oil (HOSO) [62]

Table 2. The studies of DSC application for detection of extra virgin olive oil (EVOO) adulteration with others oils.

Types of adulteration	Concentration of adulterant [%]	DSC parameters used for analysis	Thermal Program	Scanning rate [°C/min]	Source
Refined hazelnut oil (HaO)	5, 10, 20, 30, 40	Enthalpy ( $\Delta H$ , J/g), $T_{on}$ (°C), $T_{off}$ (°C), peak height ( $H_p$ W/g)	Cooling to $-80$ °C Heating to $30$ °C	2	[61]
Refined Olive Oil, peanut, refined hazelnut, high oleic sunflower oil	5, 10, 20, 40	Enthalpy ( $\Delta H$ , J/g), peak height ( $H_p$ w/g), peak area (%), transition range ( $T_{on}$ , $T_{off}$ )	Cooling to $-40$ °C Heating to $50$ °C	10	[58]
High oleic sunflower oil (HOSO)	5, 10, 20, 30, 40	Enthalpy ( $\Delta H$ , J/g), peak height ( $H_p$ W/g), transition range $T_{on}$ and $T_{off}$ , transition range.	Cooling to $-80$ °C Heating to $30$ °C	2	[62]
Refined olive oil	5, 10, 20, 30, 40, 50	Onset temperature ( $T_{on}$ ), offset temperature ( $T_{off}$ ), enthalpy ( $\Delta H$ , J/g), peak height ( $H_p$ W/g)	Cooling to $-80$ °C Heating to $30$ °C	2	[66]
Sunflower oil	10, 20, 30	Enthalpy ( $\Delta H$ , J/g), peak height ( $H_p$ W/g), peak temperature	Cooling to $-80$ °C Heating to $30$ °C	Cooling: 2, 100 [°C/s] Heating: 100 [°C/s]	[68]

at a concentration of 5, 10, 20, 30, 40% (Table 2) using cooling and heating procedures at a scanning rate of 2 °C/min. Amongst vegetable oils, HaO has been used frequently to adulterate EVOO, due to its noticeable similarity in chemical composition with EVOO. As the results of DSC studies, it was stated that the addition of HaO significantly enhanced crystallization enthalpy (at hazelnut oil  $\geq 20\%$ ) and altered the minor peak position, shifting towards the lower temperature (at hazelnut oil  $\geq 5\%$ ). In the case of melting curve, peaks were shifted towards a lower temperature with an increasing amount of HaO. Despite the distinct differences established in the shape of melting curves of pure EVOO and pure HaO, enthalpy and temperature data showed that the addition of 5–30% of HaO did not result in any significant differences. The authors concluded that both cooling and heating curves undergo significant changes as a result of the addition of HaO to EVOO, although only the values of the peak height of exothermic and endothermic curves may be used for the quantitative detection of EVOO adulteration with HaO [61].

Similarly, in the studies with adulteration of EVOO with HOSO it was concluded that addition of HOSO did not significantly alter cooling profiles of EVOO except for onset temperature of crystallization, which was significantly shifted toward lower temperature at 40% of adulterant addition (Table 2). At the same percentage of adulteration, the heating profile of EVOO was significantly changed as the major endotherm broadened and the minor event became smaller and less evident. Despite the differences being observed, the authors concluded that detection of HOSO at levels lower than 20% was not possible, even by applying deconvolution analysis [62].

Differential scanning calorimetry was also tested as a potential tool for discriminating five olive oil commercial categories i.e. extra virgin olive oil (EVOO), refined olive oil (ROO), olive oil (OO), olive pomace oil (PO), and refined crude olive-pomace oil (RPO) [63]. The authors verified the possibility of discriminating different commercial categories of olive oil, evaluating the relationship between thermal properties (obtained upon cooling and heating with a scanning rate of 2 °C/min) and chemical composition (major and minor components). The parameters analyzed includes enthalpy, peak height and the transition range calculated as temperature difference  $T_{on}$  and  $T_{off}$  (Table 1). Deconvolution analysis was applied to better describe the complex nature of the transitions. An analysis of heating curves with a deconvolution assessment provided an idea of how mono- and polyunsaturated

triacylglycerols may influence DSC peaks. By analyzing the data, the authors proclaimed that observing the heating profile it was difficult to differentiate the categories for oil samples [63].

Accordingly, in the same year, from a different horizon, team of researchers Jafari et al. experimented with the possibilities of detecting adulterations in olive oil collected from the local market [64]. Samples of different varieties of virgin olive oil, refined olive oil and genuine olive oil were tested with the addition of potential adulterants of soybean, canola, and sunflower oil. In the thermal program, the temperature range used was from  $-100\text{ }^{\circ}\text{C}$  to  $25\text{ }^{\circ}\text{C}$  at a  $5\text{ }^{\circ}\text{C}/\text{min}$  scanning rate (Table 1). To explain the changes in the crystallization curves, the authors also analyzed fatty acids and triacylglycerols composition. For example, oils containing the higher amount of oleic fatty acid were crystallized with major exothermic peak at  $-55\text{ }^{\circ}\text{C}$  (canola oil) or at  $-45\text{ }^{\circ}\text{C}$  (olive oil), whereas oils with more polyunsaturated fatty acids like soybean and sunflower oil had the crystallization peak temperature at  $-75.5\text{ }^{\circ}\text{C}$  and  $-73.5\text{ }^{\circ}\text{C}$ , respectively. The melting data showed that for soybean and sunflower oil the major peak appears at  $-15\text{ }^{\circ}\text{C}$  to  $-25\text{ }^{\circ}\text{C}$ , for canola oil at  $-16\text{ }^{\circ}\text{C}$  and for pure olive oil from  $-4\text{ }^{\circ}\text{C}$  to  $-6\text{ }^{\circ}\text{C}$ . Analyzing these results, the authors stated that DSC melting curves are good tools to detect adulteration in olive oil with soybean and sunflower oil. In 2013, Laddomada et al. analyzed the thermal properties of eighteen monovarietal extra virgin olive oils from the Apulia region of Italy by means of a modulated adiabatic scanning calorimeter (MASC) in relation to their chemical properties and composition i.e. acidity, UV absorbance, fatty acid composition [65]. MASC was used to study oil sample phase transitions in a temperature scanning mode by using a tailor-made time-temperature protocol (Table 1). They observed that the crystallization curves were significantly shifted, and occurred over longer time ranges as a function of higher peroxide index and linoleic acid content. Significant correlations were observed between the melting profiles and single fatty acids, unsaturated/saturated fatty acid ratio and oleic/linoleic acid ratios.

In turn, team of researchers, Karbasian et al. [66] used the DSC technique to detect the adulteration of EVOO with refined olive oil (ROO) at concentration of 5, 10, 20, 30, 40 and 50 w/w % (Table 2). The thermal program operated in the temperature range of  $-80\text{ }^{\circ}\text{C}$  to  $30\text{ }^{\circ}\text{C}$  with a scanning rate of  $2\text{ }^{\circ}\text{C}/\text{min}$ . The authors analyzed the following parameters: onset temperature ( $T_{\text{on}}$ ), offset temperature ( $T_{\text{off}}$ ), enthalpy, peak height from the exothermic and

endothermic curves. The experiment showed a significant increase in  $T_{\text{on}}$ , and a decrease in  $T_{\text{off}}$  with the increasing ROO. A different approach was taken by Van Wetten et al. to detect adulterations in olive oil by using a fast DSC instrument [67]. To perform the experiment, they collected samples of EVOO and sunflower oil (SFO) from a local market. A mixture of EVOO: SFO was prepared in ratios of 90 : 10, 80 : 20 and 70 : 30 w/w (Table 2) [68]. The program temperature range was from  $-80\text{ }^{\circ}\text{C}$  to  $30\text{ }^{\circ}\text{C}$  with different cooling rates  $2\text{ }^{\circ}\text{C}/\text{s}$  and  $100\text{ }^{\circ}\text{C}/\text{s}$  and a heating rate of  $100\text{ }^{\circ}\text{C}/\text{s}$ . To detect the adulteration parameters of enthalpy, peak height, transition range and peak temperatures were analyzed. In the conclusions, the authors suggested not considering cooling curves to detect adulteration. For the heating curves, an increasing amount of SFO in EVOO decreased the enthalpy, peak height and peak temperature of the peak appearing at the lowest temperature, which disappeared with the addition of 40% SFO. With this observation, the authors also calculated a detection limit of 2–10% of SFO in EVOO [68].

As presented in the above chapter, extra virgin olive oil (EVOO) is the most comprehensively tested of all edible oils by means of the DSC technique in terms of the authenticity of origin i.e. various varieties of EVOO from Spain [53,54] and from Italy (Tuscan and Apulian [58], Sicilian [59,60], Apulian [65]) as well as the authenticity of the composition i.e. EVOO adulterated with refined HaO [61], HOSO [62], ROO [66], SFO [68]). In most of the studies performed, the crystallization and melting curves of EVOO were analyzed, although other modified DSC techniques were also used, such as isothermal freezing [57], modulated adiabatic scanning calorimeter (MASC) [55,65] and a fast DSC [67,68].

#### 4. Palm oil authenticity determined by the DSC technique

Surprisingly, the authenticity of palm oil is also a complex issue in food industry, as mentioned by Okiy et al. [69]. There have been several approaches to investigate the authenticity of palm oil. Primarily, scientists carried out experiments on the crystallographic properties of palm oil and modified palm oil using DSC and X-ray diffraction [69–71]. During the late 1970's, Kawamura [72,73] stated the supremacy of DSC in detecting the polymorphic changes in palm oil as well as in modified palm oil, which was supported by Yap et al. [71]. Being a primary ingredient for the production of fat spreads, the TAG properties of palm oil have always been a vital issue in the food industry. Authors from Malaysia have performed

several experiments based on DSC to characterize and facilitate the authenticity of palm oil [13,20,74]. Che Man et al. experimented on crude palm oil, RBD (refined, bleached, deodorized) palm oil, refined palm olein, super olein, RBD palm stearin with a thermal protocol from a  $-80^{\circ}\text{C}$  to  $80^{\circ}\text{C}$  temperature range with  $5^{\circ}\text{C}/\text{min}$  scanning rate [75]. Their results suggested analyzing the cooling curves than heating profiles, as by the melting peaks overlapped one another. For unadulterated crude palm oil (CPO), the temperature  $T_{\text{onset}}$  was detected at  $16.64^{\circ}\text{C}$  and  $T_{\text{offset}}$   $7.84^{\circ}\text{C}$ , whilst for RBD palm oil, the temperature shifted higher to  $17.74^{\circ}\text{C}$  ( $T_{\text{onset}}$ ) and  $10.04^{\circ}\text{C}$  ( $T_{\text{offset}}$ ). Tan and Che Man continuously analyzed palm oils and products with different scanning rates, i.e. 1, 5, 10,  $20^{\circ}\text{C}/\text{min}$  [76]. This studies showed that in melting curves different scanning rate affects the shape and number of the peaks and their transition temperature. With increasing heating rates, the relative magnitude temperature of the peaks increased and the curve became smooth. These results were analyzed by shedding light on changes in enthalpy, onset and offset temperature.

At the same time Marikkar et al. examined the adulteration of refined-bleached-deodorized (RBD) palm oil with lard (genuine and randomized) and other animal fats i.e. mutton tallow (MT), beef tallow (BT), chicken fat (CF) [77]. Two major exothermic peaks (at  $17.8^{\circ}\text{C}$  and  $1.3^{\circ}\text{C}$ ) with two shoulder peaks (at  $-6.8^{\circ}\text{C}$  and  $-43.9^{\circ}\text{C}$ ) were observed for the unadulterated RBD palm oil cooling curves, and in adulterated samples with lard/randomized lard (1–20%) a small peak at  $-43.9^{\circ}\text{C}$  appeared, increasing in size and shifting to higher temperature values. This peak was confirmed as an indicator of the presence of lard in RBD palm oil and a detection limit of 1% lard/randomized lard was reached. In the following year, they also experimented on the adulteration detection of RBD palm oil with lipase catalyzed interesterified lard denoted as enzymatically randomized lard (ERLD) [20]. To perform the analysis, RBD palm oil was adulterated with both ERLD and genuine lard (GLD). It was established that with an increasing amount of adulterant, the peak height increased and its position shifted to a higher temperature. To perform qualitative adulteration analysis, peak area, peak height and peak onset values were calculated. In addition to RBD palm oil, a fraction of palm olein was also tested for adulteration [78]. Detection of adulteration was tested on RBD palm olein with different animal fats such as BT (beef tallow), CF (chicken fat) and GLD (genuine lard). An adulteration mixture was prepared with a proportion range of 1–20% animal fat. The DSC cooling curve of unadulterated RBD palm

olein depicted one major peak at  $1.5^{\circ}\text{C}$  and three shoulder peaks at  $-4.9^{\circ}\text{C}$ ,  $-23.3^{\circ}\text{C}$  and  $-54.8^{\circ}\text{C}$ . The last peak at  $-54.8^{\circ}\text{C}$  started disappearing when adulteration exceeded 3%. On the other hand, with the addition of BT, an additional new peak was observed at the temperature  $8.5^{\circ}\text{C}$ . To detect the adulteration effects of GLD and CF, a subtraction procedure provided by DSC software was applied, which showed that GLD adulteration results in a notable expansion of the major peak with an additional small peak in the lower temperature region. Meanwhile, adulteration with CF caused a tiny reduction in the major peak and a new shoulder peak appeared in the lower temperature region. Good correlation between the parameters (peak area, peak height and peak onset) and adulterant concentration was observed. Authors from the same region Noor Lida, Sundram, and Idris [18] considered analyzing the melting curves to characterize the blends of palm oil (PO), sunflower oil (SFO) and palm kernel olein (PKOo) using a combination of blending and chemical interesterification (CIE) techniques and determining total melting and partial melting enthalpies. A scanning rate of  $10^{\circ}\text{C}/\text{min}$  was used, while a melting thermal profile was obtained from  $-50^{\circ}\text{C}$  to  $60^{\circ}\text{C}$ . It was shown that for non-blended samples, PKOo melted at  $25\text{--}30^{\circ}\text{C}$  and PO at  $35\text{--}40^{\circ}\text{C}$ . Blending and CIE significantly modified the DSC melting properties of the PO/SFO/PKOo blends. The authors also stated the melting behavior was affected by the proportional variety of oils in their ternary blends.

Summarizing, in the entire literature on the authenticity of palm oil tested using the DSC technique, two groups of researchers from Malaysia made a significant contribution in research ([75–78]), which was focused on the differentiation of crude palm oil, refined, bleached, deodorized (RBD) palm oil, refined palm olein, super olein, RBD palm stearin or on detection of adulteration of crude palm oil with animal fats.

## 5. Authentication of other plant oils using DSC

### 5.1. Authenticity of cocoa butter

Cocoa butter (CB) is one of the most expensive edible fats and is a basic raw material for the confectionery industry. Cocoa butter is also one of the few fats for which artificially manufactured substitutes of similar composition have been constructed and openly marketed. Because of this, analysis of the adulteration of cocoa butter probably has greater importance than that of any fat other

than olive oil [52]. The DSC crystallization and melting curves of typical confectionery fats were presented by Cebula and Smith [79]. Their results showed the sensitivity and reproducibility with which DSC data can be used to classify the types of confectionery fat. The effects were shown on the DSC profiles of progressive changes in formulation to a typical cocoa butter equivalent (CBE). Additionally, the effects of the presence of minor constituents, such as diacylglycerol (DAG) and tripalmitin (PPP), which are common in confectionery fats, on the changes in the crystallization properties of the overall fat were described and quantified. It was found that even the presence of approximately 4.4% DAG and 3.9% PPP in the CBE caused a distinct increase in peak sharpness. In addition, the crystallization of DAG caused retardation in polymorphic transitions of TAG on melting transition. Different approach was presented in the studies of Kerti, in which thermal behaviors of cocoa butter and representatives of the 3 classes of cocoa butter alternative fats were investigated using isothermal crystallization DSC method. Using a new parameter,  $t_{max}$  related to the speed of the crystallisation, 100% success in classification of the investigated confectionery fats ( $p < 0.05$ ) was obtained [80,81]. Possibility of detection of lard as adulterant in cocoa butter was investigated by means of DSC by Azir et al. [23]. In this study cocoa butter was mixed with 1% to 30% (v/v) of lard and heating and cooling curves were used for the analysis.

## 5.2. Authenticity of cold-pressed oils

Recently, there has been growing interest in consumption of cold-pressed oils. The range of products is expanding regularly, because producers are looking for new, hitherto unknown plant raw materials for oil pressing. Therefore, it is becoming more and more difficult to maintain official control over large quantities of products, because food law, which should regulate the issues of authenticity and freshness of oils, does not keep up with new products appearing on the market. In 2002, Marikkar et al. undertook the study with adulteration analysis of canola oil with lard, beef tallow and chicken fat [82]. While beef tallow addition seemed to be possible to identify, even when only 2% was added to the blend, for lard in canola oil the concentration over 8% was feasible to detect and a heating curve was preferred over a cooling curve. A few years ago, coconut oil enjoyed enormous popularity among consumers, a trend which was additionally boosted by nutritionists. The adulteration study of virgin

coconut oil by using enthalpy analysis of the melting and crystallization curves was assessed by Marina et al. [83]. Palm kernel oil and soybean oil were added to prepare the adulteration mixtures. While the adulterated mixture contained soybean oil, melting curves showed the new peak appearing at the lower temperature region at 10% adulteration level. Stepwise multiple linear regression analysis was used to predict the percentage of soybean oil in virgin coconut oil with correlation coefficient of 0.949. In turn for the palm kernel oil, a gradual decrease in the peak height of the major exothermic peak was observed. In another study Mansor et al. observed a peak broadening for the heating curves with the increasing concentration of lard in virgin coconut oil [84]. Based on stepwise multiple linear regression analysis, two independent variables were found for prediction of lard content in virgin coconut oil, one was the offset temperature of main melting peak and the second parameter was the range of thermal transition for the first crystallization peak. The authenticity of virgin coconut oil was also investigated by Marikkar et al. [25]. The effect of adulteration by palm olein on DSC heating and cooling profiles of virgin coconut oil.

Experiments with DSC on the composition and thermal properties were continued for other oils like avocado oil, where samples were collected from Malaysia and Australia [85]. It was observed that differences in thermal behavior between three local types (Malaysian) and the Australian Hass variety can be explained based on unsaturated fatty acid and TAG composition. The crystallization curves showed the initial transition for the Malaysian variety between 22–29 °C, whereas for the Australian variety it was –13.67 °C. Similarly, for the final transition in the melting curves, temperatures of 41–45 °C for the Malaysian variety and 2.3 °C for the Australian variety were recorded [85]. Yanty et al. also drew attention to the problem of the purity of oils in the context of halal food, where the use of lard as an ingredient is not permitted. Owing to growing public concern about the halal status of food in many parts of the world, producing safe and high-quality halal food is a prerequisite to ensure consumer health and successful domestic and international trade [86]. A comparison of the thermo-physical properties of lard and plant fats, namely avocado butter (*Persea americana*), cocoa butter (*Theobroma cacao* L.), palm oil (*Elaeis guinensis*) and mee fat (*Madhuca longifolia*), was presented with respect to the basic physico-chemical parameters, fatty acid and triacylglycerol (TAG) compositions, and melting and solidification behaviors. The authors established that although plant fats are

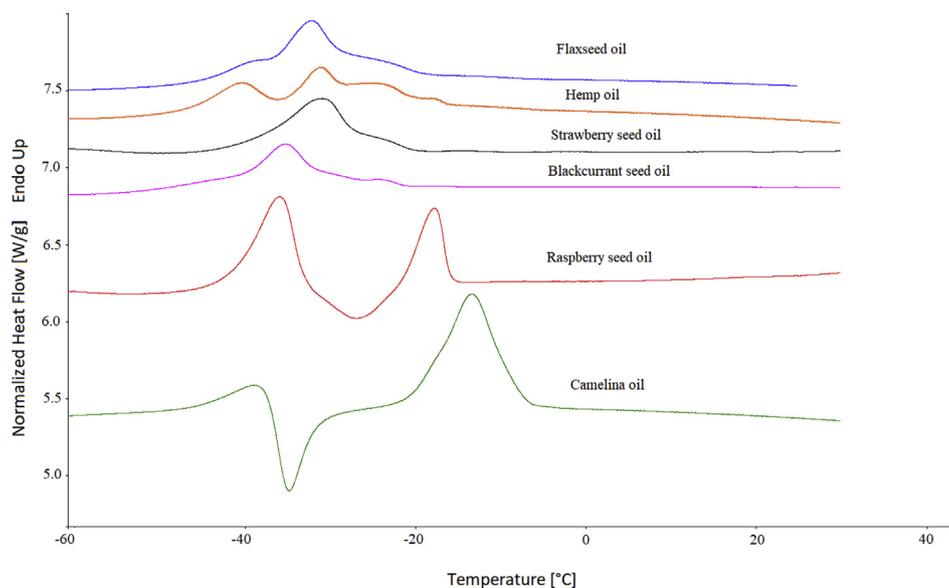


Fig. 2. DSC melting curves of selected cold pressed oils (flaxseed, camelina, hemp, strawberry, raspberry, blackcurrant seed oils). Scanning rate 5 °C/min.

completely different from lard with respect to fatty acid and TAG compositions, they share some common thermal features with lard. The authenticity of avocado oil was also investigated by using data from DSC heating and cooling curves of avocado oil adulterated with refined bleached deodorized palm superolein [21].

The wide variety of oils on the market today requires continuous research and the development of a database of DSC profiles specific to the oils in order to authenticate them. Tomaszewska-Gras et al. used different varieties of flaxseed to show that irrespective of the variety of seeds, the melting and crystallization profiles of flaxseed oil were reproducible [87]. Another booming concern for today's oils industry is the use of more and more waste materials for the extraction of oils. This is dictated by the idea of maximum utilization of raw

materials and reduction of waste, in accordance with the circular economy model [88]. Figure 2 shows the DSC profiles for newly marketed oils pressed from fruit seeds which are by-products of juice pressing. It can be seen that the DSC profiles differ significantly from the profiles presented for flaxseed, camelina or hemp oils (Fig. 1) as well as from the refined oils, which are often used as adulterants (Fig. 3). For the comparison with melting curves showed in Fig. 2, also the crystallization curves of various oils are presented in Fig. 4. In Table 3 it was compiled from a huge number of available publications, the temperature parameters of the main peaks of the crystallization and melting curves, allowing to see that each oil has its unique profile and different phase transition parameters.

Concluding, there is still lack of data on the composition and physico-chemical properties of

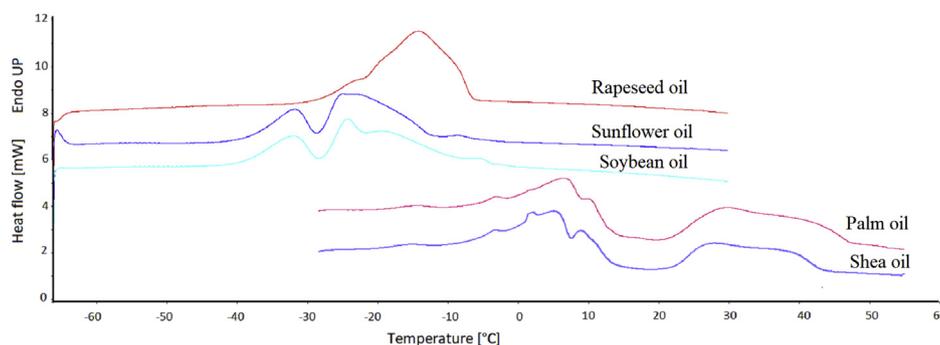


Fig. 3. DSC melting curves of refined oils (rapeseed, sunflower, soybean, palm, shea). Scanning rate 5 °C/min.

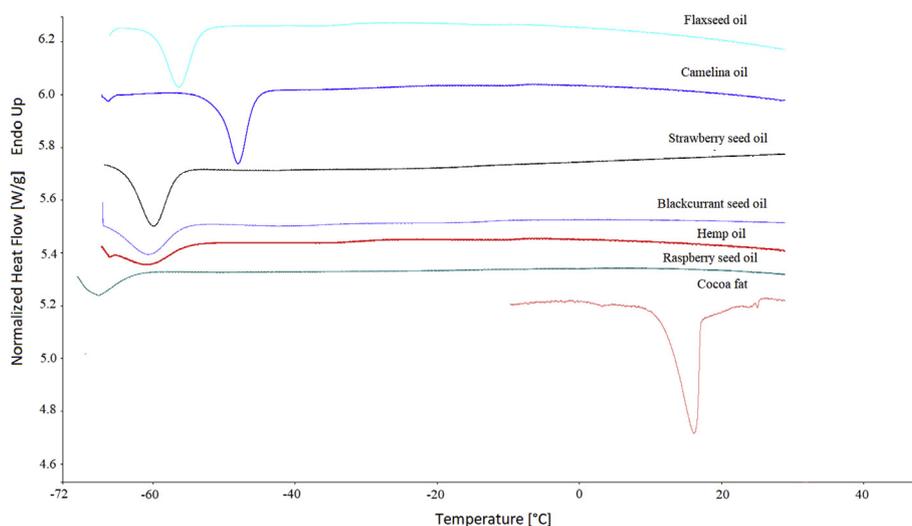


Fig. 4. DSC crystallization curves of plant oils (flaxseed, camelina, strawberry, raspberry, blackcurrant seed oils, cocoa fat). Scanning rate 2 °C/min.

new marketed cold-pressed oils, which should be tested under the same conditions to allow comparison and differentiation of various oils based on specific markers obtained, among others, from DSC parameters.

## 6. Authenticity assessment of animal fats by DSC

### 6.1. Methods for butter authenticity assessment

Among all animal fats, the most frequently adulterated fat is butter, due to its relatively high price

compared to vegetable oils and because of its high consumption of 4.3 kg per capita in EU in 2017 [89]. Recently, the worldwide butter crisis, which started in November 2016 and manifested in rising prices, has exacerbated this problem. In the European Union, the composition of butter is protected by Regulation No. 1308/2013 [90]. Butter is defined as a product which can be made solely from milk and can contain a milk fat of not less than 80% but less than 90%. Any product containing foreign fats of vegetable or animal origin may not be labelled as butter. For many years, efforts have been made to develop a method to detect adulterated butter.

Table 3. DSC markers of peak temperature for melting and crystallization curves at 5 °C/min scanning rate for the authentication study of mostly popular fats and oils.

Fats/Oils	Melting, Peak Temperature [°C]				Source	Crystallization, Peak Temperature [°C]			Source
	T 1	T 2	T 3	T 4		T 1	T 2	T 3	
Virgin olive oil	-17.2	-6.0	6.5	—	[64]	-12	-32.5	-45.5	[64]
Refined Olive oil	-15.7	-4.7	7.0	—	[64]	-14.2	-30.5	-42.0	[64]
Coconut oil	-2.64	12.42	22.45	—	[13]	-19.59	-49.29	—	[13]
		14.20	23.78	—	[25]	3.56	-0.89	—	[25]
Refined, bleached, deodorized palm oil	-18.4	-6.22	0.23	5.25	[13]	15.43	6.11	-2.85,	[13]
			21.9	35.35	—	—	-11.09	-46.92	—
			(T5)	(T6)			(T4)	(T5)	
Palm kernel oil	-19.12	1.31	13.13	26.03	[13]	-1.09	-2.49	—	[13]
Butter fat	6.84	15.88	37.09 (T <sub>end</sub> )	—	[101]	15.17	8.45	—	[98]
Soybean oil	-24.5	-15.7	-8	3.25	[64]	-15.5	-38.2	-75.5	[64]
	-39.6	-25.57	-9.9	-2.47	[13]	-19.49	-43.24	-73.7	[13]
					[108]				
Sunflower oil	-24.7	-17	—	—	[64]	-15	-41.2	-73.5	[64]
	-41.25	-32.82	-26.12	-14.3	[13]	-19.87	-45.99	-72.12	[13]
Canola oil	-29.15	-19.25	—	—	[13]	-23.44	-43.57	-60.02	[13]
Flaxseed oil	-30.30	—	—	—	[87]	-59.52	—	—	[87]
Avocado oil	1.13	5.32	9.21	—	[21]	5.49	-6.78	—	[21]

The first methods developed were classical, such as determining the iodine number, Reichert-Meissl value (RMV) and Polenske value (PV). However, this has been proved to be insufficient, particularly at low concentration levels of adulterants [91,92]. So far, the instrumental methods used to detect adulteration of butter are based on detecting various milk fat components. One of the earliest tested methods was determining the fatty acids (FA) composition using gas chromatography. This is a relatively simple method, although burdened with certain limitations connected to changes in acid composition, depending on the season of the year and on animal feeding. A further disadvantage is the similar boiling temperatures of the methyl ester of butyric acid and of hexane. Another analytical problem is FID detection, because of the lower mass fraction of carbon in the molecular mass of butyric acid than of long chain acids. Detecting butter adulteration is also possible by using gas chromatography to determine phytosterols such as  $\beta$ -sitosterol, stigmasterol and campesterol, which are specific to vegetable oils. However, one drawback of this method is the use of sitosterol and stigmasterol in the EU as markers for subsidized processed foods. The detection method for these substances is described in Annex VIII of the Regulation (EC) no. 273/2008 [93]. Another method for detecting vegetable oils added to butter may be determination of tocochromanols using liquid chromatography [94]. The content of  $\alpha$ -tocopherol in the overall composition of tocopherols in milk fat accounts for approx. 95%, while in oils, mainly  $\beta$ - and  $\gamma$ -tocopherols are present. Moreover, palm oil, used very often as adulterant, contains tocotrienols (T-3), which are not found in milk fat. However, this method does not facilitate detection of added animal fats. The currently official analytical method for the assessment of butter purity, established in the EU by Annex XX of the Regulation [93], is to determine triacylglycerols using gas chromatography. This method, which is characterized by high accuracy, enables the detection of both vegetable and animal fats. However, it is relatively time-consuming, and involves chemicals, as well as being expensive. For this reason, in the scientific literature, there are numerous reports concerning the search for alternative methods, one of which is differential scanning calorimetry. Butter can be adulterated with animal (chicken, lard, beef tallow) as well as with plant fats and oils (palm oil, shea butter). On Fig. 3 DSC melting curve of milk fat with animal fats is compared.

## 6.2. Butter authenticity assessment by DSC

One of the first papers on applying the DSC technique to detect adulteration of ghee (clarified butter) was published by Lambelet et al. [95]. In this study, the authors tested the possibility of detecting goat body fat in cow and buffalo ghee by using differential thermal analysis (DTA). Melting and crystallization curves, obtained using a cooling/heating rate at 2 °C/min, showed pure goat body fat, cow and buffalo ghee, as well as samples containing the addition of 5, 10, 20% of goat body fat to cow and buffalo ghee. The calculations were based on the temperatures of the melting and crystallization peaks. The authors concluded that adding 5% goat fat did not lead to significant differences in temperatures. However, they proved that by adding 10% goat fat, qualitative detection was possible by means of the melting curve and quantitative by means of the crystallization curve. In a subsequent paper, Lambelet and Ganguli proved that using the crystallization curves obtained by cooling at 1 °C/min, it was possible to detect 5% buffalo body fat in cow and buffalo ghee, based on the temperature and peak area [96]. They also stated that pig body fat was more difficult to quantify. After the addition of 5% pig body fat to cow and buffalo ghee, they only observed changes in the crystallization curves, although quantitative measurements were not obtained.

In 1980, Von Ruegg et al. published model melting curves for butter and anhydrous milk fat, showing reproducibility of the measurement of the onset temperature, peak temperature and final temperature of the transition, as well as for the enthalpy of melting [97]. They obtained relative standard

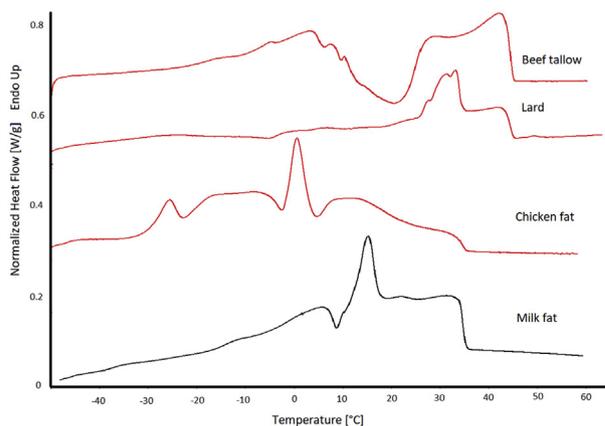


Fig. 5. DSC melting curves of animal fats (chicken, beef tallow, lard, milk fat). Scanning rate 5 °C/min.

deviation (RSD) for the temperature measurements that were on average 2.4% and for the enthalpy measurement 1.1%. Coni et al. tested the suitability of the DSC method for monitoring the authenticity of butter by examining samples of butterfat with the addition of 2, 5, 10, 15, 20% chicken fat [98]. They carried out both the crystallization and melting analyses at a scanning rate of 5 °C/min. After analyzing the curves, the authors decided to focus only on crystallization curves, due to the easier interpretation of cooling curves, which are not affected by the thermal history of the sample. It was found that there was a good linear correlation ( $r = 0.998$ ) between the  $-12$  °C exothermic peak area and the chicken fat content in butter in the range of composition from 2 to 20%. Aktaş and Kaya conducted a similar experiment, in which they investigated the possibility of detecting the addition of beef body fat and margarine to butterfat [99]. In the same way, they used crystallization peak areas to calculate added nonmilk fats at a cooling rate of 1 °C/min. A linear relationship between the crystallization peak areas and beef fat content ( $r = 0.993$ ) was obtained. In the case of margarine, the addition relationship was not linear. The detection of animal body fat added in ghee individually and in combination with vegetable oil using the DSC technique was also examined [100]. The authors stated that the addition of different levels of caprine body fat did not increase the number of peaks in the DSC curve, but displaced the crystallization region and the transition region.

In order to avoid problems connected with the polymorphism of fats, which is a common phenomenon in fats that is observed during the melting process, in all the studies described above only the cooling curves were analyzed for butter authenticity assessment. However, as shown by a study by Tomaszewska-Gras [101] milk fat crystallization parameters are characterized by worse reproducibility than for melting. There are limited data in the literature on using melting curves for the assessment of butter authenticity. In Fig. 5, the DSC melting profiles of butterfat are compared with palm oil, chicken fat, lard and tallow. Tunick et al. used melting curves to detect recombined butter, for which the main ingredients are skimmed milk powder, anhydrous milk fat and water [102]. They applied the method of heating the sample from  $-50$  °C at a scanning rate of 5 °C/min to differentiate natural butter from recombined butter. The calculation was based on the peak temperature and enthalpy. Tomaszewska-Gras used both the crystallization and melting curves with scanning rates of 5 °C/min for the quantitative detection of butter

adulteration with refined palm oil in the range of concentration from 2 to 35% [103]. In this study, not only the temperature and enthalpy ( $\Delta H$ ) of the melting curve were analyzed but also the peak heights ( $h$ ), and complex variables determined for three peaks like  $\Delta H1/\Delta H2$ ,  $\Delta H1/\Delta H3$ ,  $h1/h3$ ,  $h1/h2$  were used for simple and multiple linear regression analysis. The equations obtained were used for predicting palm oil content in six adulterated butterfat samples. Regression equations were found with a high correlation coefficient between the true and measured values, amounting to 0.999, as well as a low average value of bias (0.84). The accuracy of the DSC method, expressed as bias ( $b$ ) and recovery ( $R$ ), was compared with the results recorded by the Official method, for which the bias value was 2.2. When comparing  $R$  values, for the Official method it was 89.2%, whereas for the DSC method it was 90.7%. This study also comprised aspects of method validation not included in previously published studies in this field. The accuracy of determination using the developed DSC method was verified on the basis of adulterated samples of butterfat and compared with the Official method, consisting in determining triacylglycerols using gas chromatography.

Another alternative approach to assessing butter authenticity was presented in the paper by Tomaszewska-Gras, in which the suitability of the chemometric method i.e. principal component analysis (PCA) for distinguishing genuine from adulterated butterfat was tested [104]. DSC parameters such as temperature, enthalpy, peak height and partial surface area were selected for the best differentiation of butterfat samples with various levels of palm oil added as an adulterant. PCA analysis revealed that the best separation of samples with different palm oil concentrations was achieved using DSC melting parameters of enthalpies and peak heights of low and medium melting fractions. PCA of crystallization parameters did not produce such a distinct separation. The results of this study confirmed that the DSC technique coupled with PCA can be applied successfully to detect adulterants in butter with a high sensitivity of 2%.

### 6.3. Detection of water content in butter by DSC

Besides the addition of vegetable and animal fats, another way to adulterate butter is to add more water than is allowed. According to Regulation No. 1308/2013, this permitted addition is a maximum of 16%. Tomaszewska-Gras evaluated the applicability of differential scanning calorimetry for detecting water content in butter [105]. High correlation

coefficients were found between the water content of 5, 10, 15, 20, 25, 30% and the enthalpies of the ice melting/water crystallization. The correlation equations were used to calculate the water content in seven butter samples with various water contents. The results were compared with the values obtained by the reference method. It was found that there was higher accuracy of water determinations based on the measurement of enthalpy of ice melting (at 1%) than the measurement of crystallization enthalpy (at 3%), so the parameter of ice melting enthalpy can be used in the quantitative determination of water content in butter.

#### 6.4. Lard authenticity assessment by DSC

Alongside butter, other animal fats have also been investigated for adulteration by means of DSC. Kowalski tested the DSC method to detect adulteration of lard with tallow [106]. The crystallization process of lard and its mixtures with tallow with different cooling rates (1, 1.6, 2, 3, 4, 5 °C/min) was analyzed. A linear relationship was established between crystallization peak temperature and tallow content in lard. Other animal fats (lard, beef tallow, chicken fat) were monitored for adulterations by Dahimi et al. by using the parameters of temperature and enthalpy from cooling and heating curves [107]. Nina Naquiah et al. published research on the discrimination of lard olein and lard stearin from other animal fats (beef fat, mutton fat, chicken fat) [22]. Principal component analysis was used to compare the results obtained from DSC cooling curves of all fats.

To summarize the studies regarding animal fats, the main concern of researchers about authenticity was focused on butter from cow milk, which can be adulterated with animal sourced (chicken, lard, beef tallow) as well as with plant sourced fats and oils (palm oil, shea butter). Researchers were working also on applying DSC technique to the quantitative determination of water in butter. Mostly melting and crystallization profiles were used for butter authenticity assessment with such parameters as peak temperature, peak height and enthalpy.

## 7. Conclusions

In presented review a stepwise approach has been attempted to explain the fundamental concept of using DSC for characterization of various fats and oils in terms of adulteration detection to prevent food fraud. As the research presented in this paper shows, olive oil is one of the most studied edible oils

using differential scanning calorimetry. The applicability of several calorimetric techniques like DSC, modulated DSC, isothermal or fast calorimetry were used to evaluate the authenticity as well as the deterioration of quality during storage of oils. Presented studies proved that the calorimetric technique allowed the detection of the adulterant (various seed oils or refined olive oil), oil origin, and oxidation processes of oil. Moreover, in this paper palm oil has been presented from two sides i.e. as a cheap oil used as an adulterant and as an adulteration object, mainly with fats of animal origin. Possibilities of detection adulteration of palm oil and assessing authenticity of different categories and fractions of palm oil by DSC technique was discussed. The review also presents what has been done in the area of using DSC to analyze the authenticity of milk fat, which is very often subject to fraud due to high production costs. It was shown that quantitative detection of butter adulteration with palm oil or water was possible by using DSC technique with regression models based on the DSC parameters like peak temperature, peak height, enthalpy of melting or crystallization phase transition.

Due to the growing market of cold-pressed oils there is still need of substantial work on the creation of a wide database on the composition and physico-chemical properties of new marketed oils, which should be tested under the same conditions. This approach will allow differentiation of various oils based on specific markers, provided by the DSC technique. Next issue to face with DSC analysis of fats and oils is the complexity of melting curves, caused by the proneness of fats and oils to polymorphism. For the further development of the thermal analysis of oils and fats towards authenticity assessment, it is necessary to use deconvolution procedures for the complex peaks, formed during melting transition or to use of modified DSC such as fast DSC to avoid any additional interference.

#### Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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