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Thermochimica Acta 459 (2007) 58–63
thermochimica acta
www.elsevier.com/locate/tca

Promoting calorimetry for olive oil authentication

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Received 7 March 2007; received in revised form 30 March 2007; accepted 1 April 2007
Available online 5 April 2007

Abstract

The aim of this paper is to promote calorimetry for olive oil authentication. It is our belief that the melting and freezing curves by DSC of olive oil and other edible oils could be correlated with quality, origin and storage history of the oil in a simple way, suitable for oil industry and market. Nucleation, crystallization kinetics and transition enthalpies of oil are indeed strongly dependent on molecular interactions, that is any change of oil acidic composition and minor components can be detected, as we have recently demonstrated. In particular, addition of low-cost oils to extra virgin olive oil (EVOO) and thermal and/or mechanical treatments (refinement, deodorization, filtration, etc.) of EVOO can be assessed by a first-sight analysis of the thermograms.

Protocols to obtain reproducible DSC thermograms and experiments to understand the origin of the often-observed non-reproducibility, which prevented until now the use of calorimetry for oil authentication, are here described.

An explanation of the melting curves even limited to the main features is a hard task owing to the polymorphism of the numerous triacylglycerols (TAG), the main component of the oil, the complexity of their mutual interactions and the effects of the minority components. To outline the level of this challenge we report: (i) the evidence that thermodynamic and kinetic processes overlap during heating of solid EVOOs and (ii) the melting curve of one pure TAG with its polymorphic transitions.

Keywords: Isothermal crystallization; Melting; Differential scanning calorimetry; Olive oil authentication; Polymorphism

1. Introduction

Among the numerous experimental techniques currently used for testing nature, quality and geographic origin of edible oils, calorimetry is not present. DSC has been applied mainly to the study of the thermo-oxidative process against temperature, with the purpose of selecting oil type and improving utilization conditions [1–7]. The oxidative stability was also monitored by pressure DSC [8]. The liquid ↔ solid phase transitions of the oil have been particularly studied to this end, as they are affected by molecular composition changes. Therefore freezing and melting are promising also to assess oil nature, quality and origin. Few attempts in this direction can be found in the literature. About 10 years ago, Dyszel and co-worker [9,10] worked to create a data bank with the calorimetric "fingerprints" of the main edible oils. More recently, the effects of heating and cooling rate on the melting and freezing thermograms have been studied with DSC [11,12] and the potentiality of calorimetric methods for oil quality control discussed [13].

The experiments in Refs. [11,12] outlined the strong dependence of the thermograms on the temperature-scanning rate, which was found much larger than that observed for monocomponent molecular liquids [14]. The authors attributed this effect to the complexity of the oil and the polymorphism of the crystalline phase of triacylglycerols (TAG), the main components of the oil [11,12]. They also outlined the difficulty in obtaining experimental reproducible conditions. Our attempts to use calorimetry for edible oil authentication started just from this results with the aim of: (i) understanding the origin of the observed non-reproducibility; (ii) defining a suitable measurement protocol; (iii) studying the physical processes occurring at the oil liquid ↔ solid transitions.

The experiments reported in Refs. [11–14] suggested that the sensitivity of the calorimetric method to oil composition is due, besides to solid TAGs polymorphism, also to the presence of many minor components, generally responsible for the positive or negative organoleptic and healthy features of the oil. The numerous molecular components interfere indeed with

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0040-6031/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.tca.2007.04.002

crystal nucleation and growth kinetics, so affecting the solidification and melting thermograms in a characteristic way, strictly related to oil identity. Therefore the solidification and melting thermograms can be used for assessing: (i) commercial frauds, if these molecules have been introduced illegally in the genuine oil; (ii) the geographic origin of the oil, inasmuch as it is reflected by typical cultivars and/or production procedures; (iii) the storage history of the oil, clearly depending on ageing and storage conditions [15–17].

We report here in details the foundations of the calorimetric method we have developed and two measuring protocols tested on edible oils, together with preliminary results of a calorimetric study on the physical processes active in the olive oil melting.

Finally, to point out the complexity of the processes occurring during the EVOO heating, the triolein melting is studied and discussed.

2. Experimental

2.1. Materials

A Tuscan extra virgin olive oil (EVOO), crop season 2005–2006, produced at a traditional plant (stone mill and hydraulic press), was used in these experiments without further treatments. The main characteristics of the sample were acidity (oleic acid%) 0.15; peroxides ($mEO_2\ kg^{-1}$) 10.85; biophenols (mg/kg gallic acid) 110.00. The sample was sealed in clear glass jar (200 cm^3 in volume) and stored in dark at the laboratory room temperature of $22 \pm 3\,^{\circ}C$. Triolein was purchased from Fluka (99% purity) and used as received.

2.2. The calorimeter

Calorimetric measurements were performed with a Perkin-Elmer DSC7 equipped with an Intracooler II. Dry nitrogen was used as purge gas at a rate of 30 ml/min. The instrument was calibrated in temperature and energy with high purity standards (indium, naphthalene and cyclohexane) at 10 °C/min, according to the procedure for standard DSC. The temperature was known at ±0.1 °C; the samples weighted to ±0.01 mg. In order to reduce temperature gradients, the sample mass was kept small, approximately equal to 6–8 mg. A blank run with two empty aluminium pans was subtracted from all the experimental curves.

3. Results and discussion

3.1. Preliminary considerations

The applicability of calorimetry to edible oil authentication imposes the fulfilment of two basic conditions: (i) the attainment of a homogeneous liquid state at the high temperature side of the calorimetric cycle and a complete reproducible solidification of the sample at the low temperature side; (ii) a short measuring time, as requested by oil industry and commerce.

Condition (i) is the physical prerequisite to have reproducible thermograms. It can be fulfilled if one takes into account two subtle features of olive oil, which we have experimentally put in evidence:

(a) An oil sample maintains "memory" of its solid phase in the liquid phase. This is supported by measurements of crystallization induction time (IT) performed at 2.9 °C with a TAM isothermal calorimeter and a large sample (about 2.5 cm^3) [15]. In the first isothermal freezing the IT varied in the range of 6–10 h, depending on the oil type and composition. The IT value of the same sample, measured during freezing after melting at room temperature, decreased to less than 1 h and its value was reproduced in successive identical melting–freezing cycles. This behaviour recalls the homogeneous nucleation, which occurs at high undercooling in dynamic conditions or after a long IT in isothermal conditions. On the contrary, the heterogeneous nucleation, due to impurities and/or crystal seeds present in the liquid, occurs at low undercooling or after a short IT. In our case, being crystallization nuclei, if present, fixed, the observed thermal effects suggest that the melting of the oil at room temperature is not complete and structures able to nucleate efficiently the crystalline phases can survive in the liquid.

This "memory effect" was not observed in much smaller samples, as those used for DSC experiments (∼10 mm^3). Indeed an experiment performed with DSC on the same EVOO sample, subjected twice to a temperature cycle from 50 to −30 °C and successively twice to a cycle from 20 to −30 °C, gave four crystallization and melting thermograms practically coincident.

(b) A liquid oil volume can be heterogeneous at room temperature if it is sufficiently large.

This feature is related to the previous one. Its relevance for DSC reproducibility is related to the high probability to obtain samples substantially different even from the same source oil. This is confirmed by the thermograms depicted in Fig. 1. The panel B of the figure shows the melting thermograms of five samples, prepared from the EVOO stored

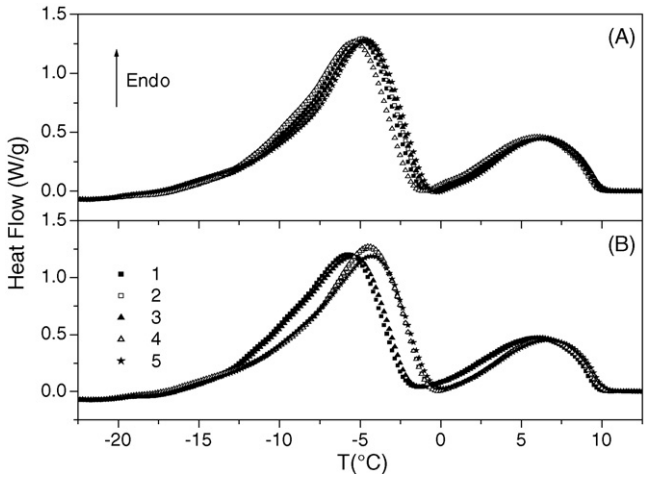

Fig. 1. Melting thermograms of five samples prepared with EVOO from the same container: (A) after thermal treatment of the oil in the container at 50 °C for 3 min and (B) before thermal treatment.

in the container at room temperature, following the Protocol 2 described below in Section 3. The differences among the curves are much larger than the usual experimental errors.

These results support the presence in oil volumes greater than few cube centimetres of macroscopic regions with different composition/structure, particularly if the oil underwent solidification during its storage history. Consequently, samples of few microliters, as those used with DSC, even if prepared with oil from the same container, may be different. After many attempts with both mechanical and thermal treatments, the best homogenisation of a large EVOO volume was obtained by heating the oil in the container to 50 °C for at least 3 min. As a proof of the effectiveness of this thermal method, the thermograms of five samples, prepared as those in Fig. 1B, but after the above described homogenisation procedure, are shown in Fig. 1A. The reproducibility of the curves is significantly improved. In conclusion, the thermal treatment at 50 °C destroys oil heterogeneities to a large extent and allows to obtain oil samples approximately identical. This oil feature was also observed by Adhvaryu et al. [18] in a study on the wax appearance temperatures of vegetable oils.

The second condition, i.e. a short time test, deals with the information contained in the melting thermogram against temperature-scanning rate. Fig. 2 shows the melting thermograms of the same oil sample at the rate of 2, 10 and 20 °C/min, after cooling according to Protocol 1, described in the following section. The profile of each melting peak becomes more and more rich of details by decreasing the temperature-scanning rate (see also Ref. [11]) but the measuring time increases. We assumed the scanning rate of 10 °C/min as a good compromise, also considering that at this rate the signal/noise ratio is high.

3.2. The measuring protocols

The two measuring protocols here described have been developed taking into account the above results and considerations. They both ensure thermogram reproducibility, so the researcher can select the method that at best fulfils the experimental needs and features of the oil and/or process under study.

(a) Protocol 1

For a clear description, the time–temperature profile and a typical thermogram or heat flow curve (HFC) of an extra virgin olive oil (EVOO) against time are shown in panel A and B of Fig. 3, respectively. The maximum temperature, +50 °C, allows the sample homogenisation; the minimum temperature of the cycle (−30 °C) is chosen in order to have during the isotherm at that temperature: (i) the oil solidification at a rate suitable for an accurate analysis of the exothermic peak, (ii) the isotherm duration compatible with the desired time test and (iii) a complete sample solidification.

During a calorimetric cycle of an EVOO sample no enthalpic process is observed during the isotherm at +50 °C and the successive cooling run down to −15 °C. At about this temperature, reported in literature as “wax appearance temperature” [18], an exothermic peak begins, which marks the onset of oil solidification. The relevant physical process occurring during the isotherm at −30 °C are the nucleation and the isothermal growth of the crystalline polymorphic phases of TAGs [19], marked by a large exothermic peak. The length of the isotherm (10 min) is larger than the time necessary for the completion of the solidification process of EVOOs, so the peak ends after few minutes and thereafter the heat flow becomes null. In the successive heating run the melting and/or transformations of the polymorphous crystalline fractions through solid–solid transitions and re-freezing of the melt are detected [19]. The curve shows two characteristic peaks just before and after 0 °C and other minor features. The heat flow jump at the beginning of each heating and cooling run is due to the power supplied to the sample for scanning the temperature. Its value is proportional to the heat capacity of the sample and to the temperature-scanning rate. It is negative during the

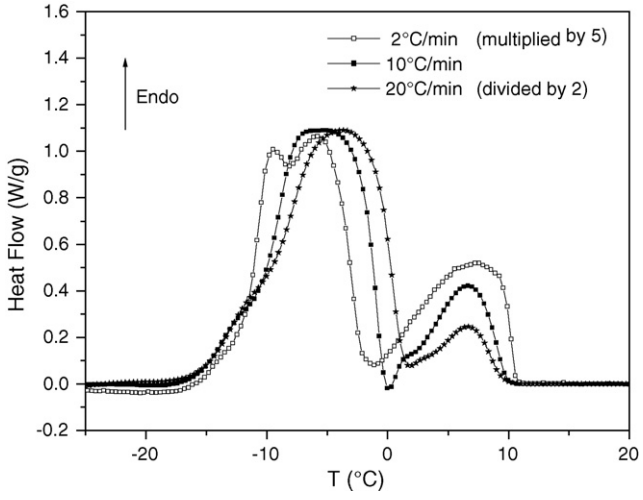

Fig. 2. The melting thermogram of the same EVOO sample at 2, 10, 20 °C/min, after cooling according to Protocol 1 (see text).

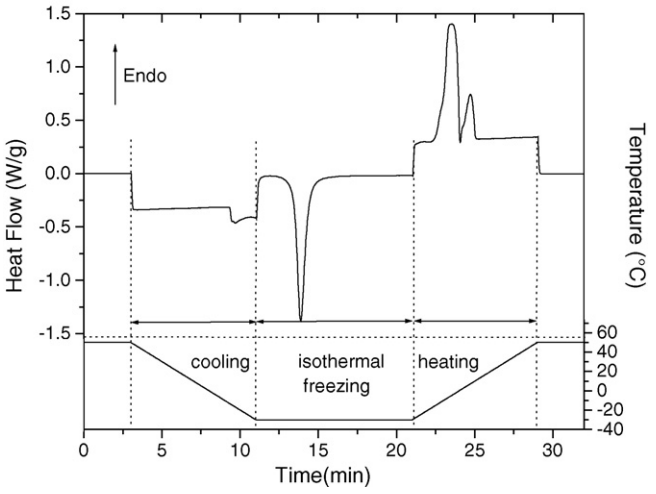

Fig. 3. The measuring protocol applied to EVOO and the resulting heat flow curve.

cooling, zero during the isotherm and positive during the heating.

(b) Protocol 2

In previous experiments [15–17] and also in this paper, when it resulted advantageous, a different protocol was applied. This protocol contains a crystallization isotherm at −40 °C for 6 min. Its application to EVOO gave good results, but for a quantitative analysis of the crystallization curves Protocol 1 is preferable, because it allows also to observe the beginning of the exothermic peak during the isotherm at −30 °C.

3.3. The crystallization and melting processes

The HFC measured on cooling down to −30 or −40 °C is not interesting for olive oil authentication. More relevant are the HFC obtained during the isothermal crystallization.

We have reported [16] on the high sensitivity of the exothermic peak to the quality degradation by light exposition. The products of the oxidative processes induced by light reduce the crystallization rate more and more and after a few weeks the 10 min isotherm is not sufficiently long for the complete solidification of the sample.

In general, any change of the oil composition, due to chemical or physical treatment, affects in a typical way the freezing HFC. In other words, the nucleation and growth of the polymorphous crystalline fractions of TAGs are dependent on oil molecular composition. For this reason, the freezing HFC can be correlated to oil quality, origin, production method, etc.

The melting HFC, on the contrary, shows the modifications and melting of the polymorphous phases in the solid oil during the heating run. The usefulness of the melting HFC for extra virgin olive oil authentication and its sensitivity to small addition of seed oils and refined olive oil has been studied and reported [17]. We observed that not only addition of seed oils but also any physical and/or mechanical treatments of EVOO, in other words any change of EVOO composition, affects area and height of the melting peak observed above 0 °C. These two quantities seem to be specific of the EVOO type (origin, cultivars, etc.) and about linearly dependent on its composition change [17].

For a better understanding of the large dependence of melting HFC on the temperature-scanning rate and the involved processes, we performed a few experiments described in the following.

Fig. 4 shows the standard HFC of an EVOO sample, obtained with Protocol 1. The other curves refer to melting runs of the same sample subjected to successive measuring cycles, during which the heating scan was stopped at 0 °C. The sample temperature was maintained constant at that value for increasing time intervals from 2 min (curve A) to 145 min (curve D); then the heating run started out again at the same rate and the last melting peak was monitored. No process is detected during the isotherm, but some kind of transformation must take place, since it is reflected in the shape and area of the successive peak. Indeed this experiment puts in evidence that the height and the area of this peak increases by increasing the time length of the isotherm at 0 °C.

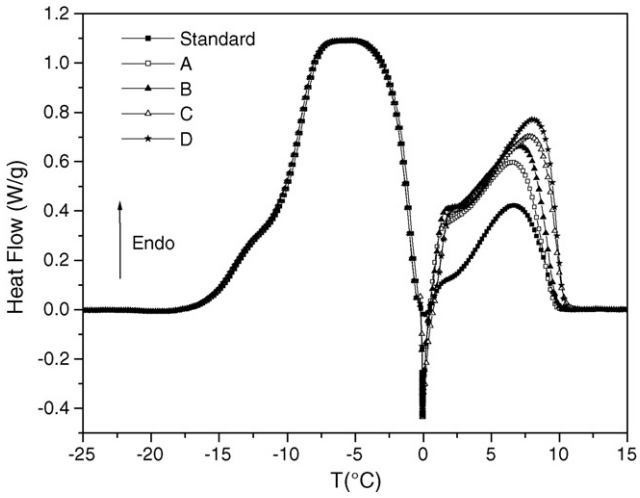

Fig. 4. Five melting thermograms of the same EVOO obtained with the standard cycle, the first, and by adding at 0 °C an isotherm of length increasing from A to D. The isotherm lengths are 2, 8, 30 and 145 min, respectively.

The total enthalpy variation, ΔH, of the melting process, measured as the integral of the HFC from −30 to +50 °C, divided by the scanning rate, is shown in Fig. 5.

The total ΔH for the solid–liquid transition increases by increasing the time length of the isotherm at 0 °C and it is always larger than the crystallization enthalpy (see Fig. 5), calculated as the sum of the integrals of HFC during the cooling and the isotherm at −30 °C (see Fig. 3). In Fig. 5 the negative value of the ΔH curve at temperatures lower than −12 °C, marks the occurrence of exothermic processes (refreezing and/or solid–solid transformation) during the sample heating [20]. This evidence explains the observed large HFC dependence on the temperature-scanning rate and supports the need of a well-defined measuring protocol for oil authentication by calorimetry.

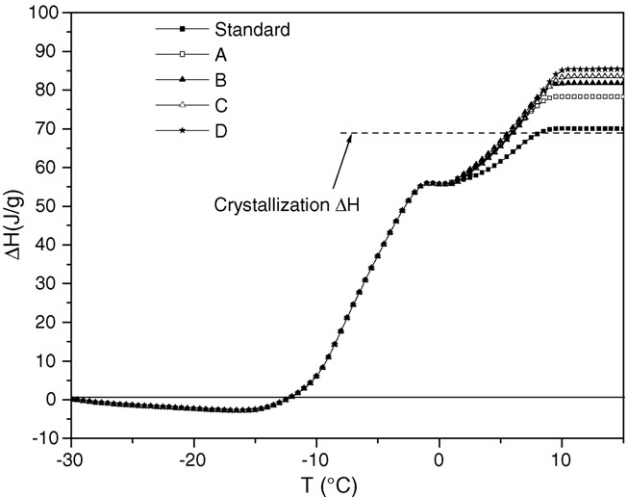

Fig. 5. The enthalpy calculated from the thermograms in Fig. 4. The value of the crystallization enthalpy is also shown. An exothermic process is present below −12 °C. The processes occurring during the isotherms at 0 °C are not considered and the residual melting enthalpy, from 0 to 20 °C, is added to the value calculated up to 0 °C.

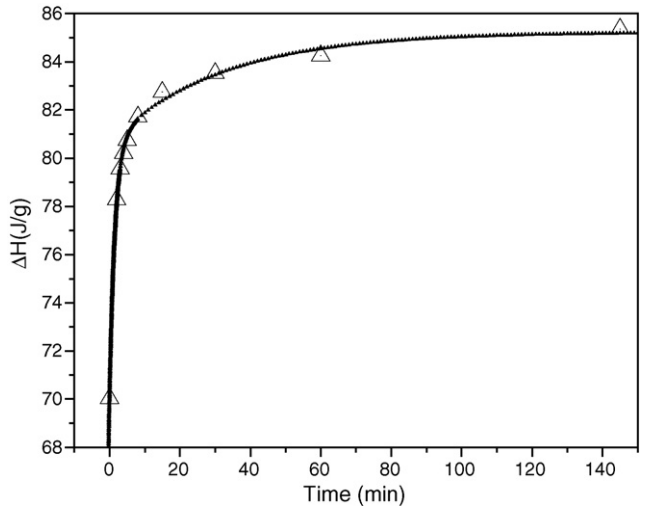

Fig. 6. The values of total melting enthalpy of the EVOO sample in Fig. 5 against the time length of the isotherm at 0 °C. The fitting curve (Eq. (1)) is also drawn.

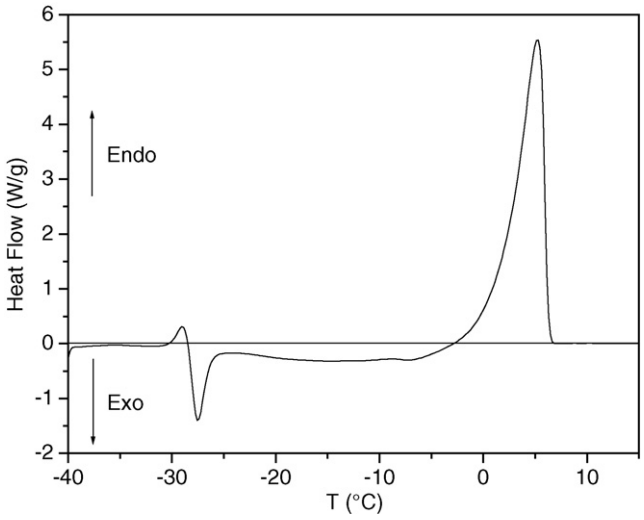

Fig. 7. The melting thermogram of triolein, obtained with protocol 2 (see text).

Fig. 6 shows the total ΔH of melting against the time length of the isotherm at 0 °C. The experimental points fit the following equation:

$$\Delta H = 14.378 + 10.618\left[1 - \exp\left(\frac{-t}{\tau_1}\right)\right] + 4.57\left[1 - \exp\left(\frac{-t}{\tau_2}\right)\right] \quad (1)$$

where $\tau_1 = 1.54$ min and $\tau_2 = 31.67$ min.

During the first 20 min a fast processes, such as phase transitions from metastable states, dominate ΔH behaviour; afterwards the ΔH rate change decreases sharply. The long characteristic time τ_2 could represent annealing and ageing processes.

In conclusion the melting HFC reflects processes that are simultaneously time and temperature dependent, with characteristic kinetic and thermodynamic properties.

For a better understanding of the structural isothermal transformations in solid EVOO, an adiabatic and modulated temperature-scanning calorimetry study at different temperatures is in progress.

3.4. TAG polymorphism and melting thermogram

The HFC of an EVOO sample mainly reflects the polymorphism of oil TAGs and an idea about the processes active during the melting can be gained by studying pure triolein, a TAG representing more than 30% of olive oil composition. Its melting HFC obtained with Protocol 2, is shown in Fig. 7. The main polymorphous phases of any TAG are named α, β′ and β [21]. In the just grown solid at −40 °C, owing to the relatively high cooling rate, the metastable α form is dominant with the respect to β′ [19]. During the heating the α form melts and converts into β′ and finally β form, which is the equilibrium modification. The HFC shows the melting onset of the α form at about −30 °C, then the exothermic modification to β is dominant at temperatures from −28.8 to −25.8 °C, with the peak centred at −27.5 °C. At higher temperatures the small and broad exothermic signal marks the occurrence of the β′ → β modification, which probably is a solid–solid transition, as the β′ melting takes place at about −10 °C [20] or −12 °C [22]. Finally, the melting of the β form gives a large endothermic peak with onset temperature at 1.5 °C and maximum at 5.1 °C. These values are comparable with those reported in literature: 4.8 °C [21] and 5 °C [22]. The enthalpy of fusion of the β form, calculated from the area of the peak above the baseline at zero level, in the interval $-2.5\,°C < T < +6.8\,°C$, is 98 kJ/mol, in good agreement with the value of 100 kJ/mol in Ref. [20].

In the case of EVOO, an explanation of the melting behaviour is not possible as the interaction among the numerous TAGs and among TAGs and minor components overlaps the transitions of each polymorphous phase in time and temperature in a characteristic way. This behaviour is likely to be responsible of the high sensitivity to oil composition, shown by the peak at the highest temperature [17], but a detailed description of the processes, if possible, will be attained only after systematic studies of such complex mixtures.

4. Conclusions

We can conclude that for practical utilization of calorimetry in edible oil authentication the following qualifications are necessary: (i) good thermograms reproducibility; the often observed non-reproducibility is due to the particular features of olive oil in the liquid state here discussed and to the kinetics of melting and crystallization processes [18,19]; (ii) large agreement on the method both at scientific and commercial level.

The calorimetric protocols here reported are simple, fast and low cost and fulfil the first request. They can be used for quality, origin, fraud detection and conformity tests in commercial practice.

The second request is the very aim of this paper. We expect that our results will promote: (i) calorimetric studies of edible oils, extra virgin olive oil in particular; (ii) coordinated activity for database formation and (iii) setting of correlations between

calorimetric and standard chemical, physical and sensorial data. The interest of oil sector operators will follow, but the market dimension and the oil varieties, also at national level, are so large that the contribution of the international calorimetry community is essential.

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