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Calorimetry for Fast Authentication of Edible Oils

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Abstract There are little data in the literature on how to authenticate edible oils through calorimetry techniques. However, oil melting curves can be used to represent correlations between calorimetric results and oil quality. A calorimetric method has been developed for studying the solid–liquid phase transitions of olive oil and seed oils, in which melting peak behavior is correlated to the type, quality, and composition of the oil. Good reproducible thermograms were obtained by defining precise protocols for use in testing, which take into account the specific characteristics of a particular oil. This approach does not replace classical analytical methods; nevertheless, it is believed that calorimetric tests could be a useful preliminary stage for quality testing. The calorimetric technique allows the detection of the adulterant (seed oils or refined olive oil), oil origin, and possible photo-oxidation degradation processes, before more complex and expensive procedures and analyses are applied.

Keywords Differential scanning calorimetry (DSC) · Edible oil authentication · Oil phase transitions · Olive oil · Polymorphism

1 Introduction

Sales of extra virgin olive oil (EVOO) have been growing rapidly in the world market as a result of its organoleptic features and health benefits. In Mediterranean countries, particularly Italy, Spain, and Greece, EVOO is intimately related to the culture and

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traditional cuisine, but it also involves significant financial aspects. Many attempts have been made in these countries to improve the quality of EVOO and to expand its market. Innovative technologies have been developed in each production phase, from cultivation to harvesting and oil extraction.

Despite these efforts, the genuineness and quality of EVOOs are sometimes difficult to define, and detecting non-genuine EVOO is still a challenge. The addition of seed oils and/or low cost olive oils is the most diffuse among the possible frauds [1] and is increasingly motivated by the increase in EVOO prices and in the market size. Seed oils generally have a different triacylglycerol (TAG) composition from that of EVOOs [2], so chemical analysis can detect if such seed oils have been added to EVOO [3,4]. However, a few seed oils, such as hazelnut and high oleic sunflower oil, have a TAG composition similar to that of EVOO; thus, the addition of these oils and refined olive oils is more difficult to detect. To identify the addition of refined oils to EVOO, stigmastadien determination (Reg.CEE 2568/91 all. IV) is usually applied, while the addition of hazelnut and high oleic sunflower oil is detected by Delta ECN42 (Reg.CEE 2568/91 all. VIII) and free and esterified sterols [5,6]. In some cases, the detection of molecular markers in the oil vapor phase [7,8] is also successfully used against frauds. But refined hazelnut oil has no volatile compound, and its presence cannot be detected by headspace sampling, even if combined with mass spectrometry [9]. Other techniques operating in the liquid phase, such as ^{13}C-NMR [10], ^{1}H-NMR [11], Raman [12,13], and FT-IR [14] spectroscopies and spectrofluorimetry [15], have to be used to discover the presence of hazelnut oil in EVOO, at least above a certain concentration value.

We believe that the EVOO sector would benefit from having some kind of initial screening that would immediately give some clear information on the quality of an oil. Calorimetry could represent such a technique. Calorimetry has already been used to analyze oil quality by monitoring liquid–solid phase transitions [16–20]. Recently [21] we performed exploratory measurements to test the quality and traceability of EVOO by means of different calorimetric methods. We found some level of correlation between the measured quantities (nucleation induction time, crystallization and melting enthalpy, crystallization and melting temperatures, heat capacity) and the identity of the oil (i.e., quality and origin). Both melting and freezing thermograms are strongly dependent on the temperature-scanning rate [19,22,23] and the thermal history of the sample [19]. Consequently, we defined a suitable time–temperature program for our calorimetric method, which for industrial applications needs to be simple and rapid. At the Italian CISETA Conference we presented some preliminary results of this new technique involving mixtures of EVOO with sunflower and hazelnut oils [24].

This paper reports the melting curves of selected EVOOs and mixtures of EVOOs with some seed oils. We discuss (a) the potential of calorimetry for the authentication of high quality EVOOs; (b) a calorimetric “identity card” of the oil to be used in commerce for rapid conformity tests; and (c) the detection of light and oxygen exposition effects to evaluate oil storage conditions and ageing processes.

2 Materials and Methods

2.1 Tuscan and Apulian EVOOs, Seed Oils, and Refined Olive Oil

The oil samples used in the experiments are listed in Tables 1 and 2, together with some of their characteristic properties (number of peroxides, acidity, and origin, if known). The EVOOs were used as received. The seed oils and the refined olive oil were bought at the market or supplied by other laboratories. The samples were stored in the dark at (20 ± 2) °C until the measurements were performed.

2.2 Differential Scanning Calorimeter (DSC)

Calorimetric measurements were carried out with a Perkin-Elmer DSC7 equipped with an Intracooler II. Dry nitrogen was used as a purge gas at a rate of $30\,\mathrm{cm}^3 \cdot \mathrm{min}^{-1}$. The instrument was calibrated on temperature and energy with high purity standards (indium, naphthalene, and cyclohexane) at $10\,^{\circ}\mathrm{C} \cdot \mathrm{min}^{-1}$, according to the procedure

Table 1 Parameters of extra virgin oils and refined olive oils

No.	Polyphenols ($\mathrm{mg} \cdot \mathrm{kg}^{-1}$ gallic acid)	Acidity (oleic acid %)	No. perox. ($\mathrm{mEO_2} \cdot \mathrm{kg}^{-1}$)	Origin
Extra virgin olive oils (EVOO)				
1	96	0.15	9.65	Pistoia
2	111	0.20	9.20	Florence
3	154	0.20	10.85	Florence
4				Florence
5	105	0.15	10.15	Grosseto
6	119	0.15	8.55	Siena
Refined olive oils (ROO)				
7		0.09	0.60	Italy

Table 2 Commercial oils and oils from different suppliers

No.	Type	Origin/Cultivar
Commercial oils (quality declared in the label)		
8	EVOO (IGP)	Tuscany/blend
9	Peanut	
10	EVOO	Liguria/Taggiasca
No.	**Type**	**Origin/Supplier**
Oils from different suppliers		
11	EVOO	Apulia/CNR-Bari
12	Refined hazelnut	Turkey/Carapelli-Florence
13	Crude hazelnut	Turkey/Carapelli-Florence
14	High oleic sunflower	Carapelli-Florence
15	Refined olive	ARPAT-Lucca
16	EVOO	Tuscany/private

for a standard DSC. The temperature was known within ±0.1 °C; and the samples were weighed to ±0.01 mg. In order to reduce temperature gradients, the sample mass was kept small, in the range of (6 to 8) mg.

2.3 Calorimetric Method

The sample pan was placed in the calorimeter and then subjected to the following time–temperature program: (1) loading of the sample at 50 °C; (2) isotherm for 3 min at 50 °C; (3) cooling at $10\,°\mathrm{C}\cdot\mathrm{min}^{-1}$ to −40 °C; (4) isotherm for 6 min at −40 °C; and (5) heating at $10\,°\mathrm{C}\cdot\mathrm{min}^{-1}$ to 50 °C. The cycle from phase (2) to phase (5) was usually repeated twice, even though reproducibility was so good that in the normal scale of the thermograms there were no differences between two successive runs. The length of the first cycle, phase (2) to phase (5), was 27 min. This time is suitable for online tests during production and any time during storage from the mill to the consumer. A reference run with empty pans was also required for an accurate quantitative analysis. Details of the measuring method, thermogram reproducibility, and the criteria for selecting the scanning temperature rate are reported in the literature [25].

3 Results

The melting curves of EVOOs (2004–2005 harvest) from some production areas in Tuscany (see Table 1) are depicted in Fig. 1a. The curves show the following features: (i) a large peak (P2) with the maximum at temperatures between −6 °C and −4 °C, and (ii) a smaller peak (P3) with the maximum between 4 °C and 6 °C. All the curves overlap at temperatures lower than −23 °C and higher than 10 °C. The ratio between the height of the two peaks, $R = h2/h3$, varies between 2.8 and 3.4. The similarity between the melting curves shown in Fig. 1a supports the possibility of defining a mean curve (MC), calculated as the mean of the melting curves of selected EVOOs, blends of the cultivars leccino, muraiolo, frantoio, and pendolino, which are present in the production areas of Tuscany considered. The calculated MC and the melting curves of three commercial EVOOs, one blend of other cultivars (from Apulia), one monocultivar (taggiasca) from Liguria, and a commercial IGP (blend) from Tuscany, are shown in Fig. 1b. There are clear differences from the MC and the curves of the Apulian and Ligurian oils, whereas the IGP Tuscan EVOO agrees very well with the MC. Moreover, a third peak (P1) is present at −20 °C in the melting curve of Apulian and Ligurian EVOOs.

Figure 2 shows the melting curves of samples of high oleic sunflower oil (HOSO), hazelnut and peanut oil, crystallized on cooling at $10\,°\mathrm{C}\cdot\mathrm{min}^{-1}$ down to −40 °C, an Italian refined olive oil (ROO), and one EVOO.

Since the TAG composition of HOSO, hazelnut oil, and peanut oil is similar to that of EVOO, the melting curves of these oils are found in the same temperature interval as the EVOO melting. Due to this similarity, the addition of these three seed oils to EVOO can only be detected with quite laborious, expensive, and time-consuming methods [1,3,9,15].

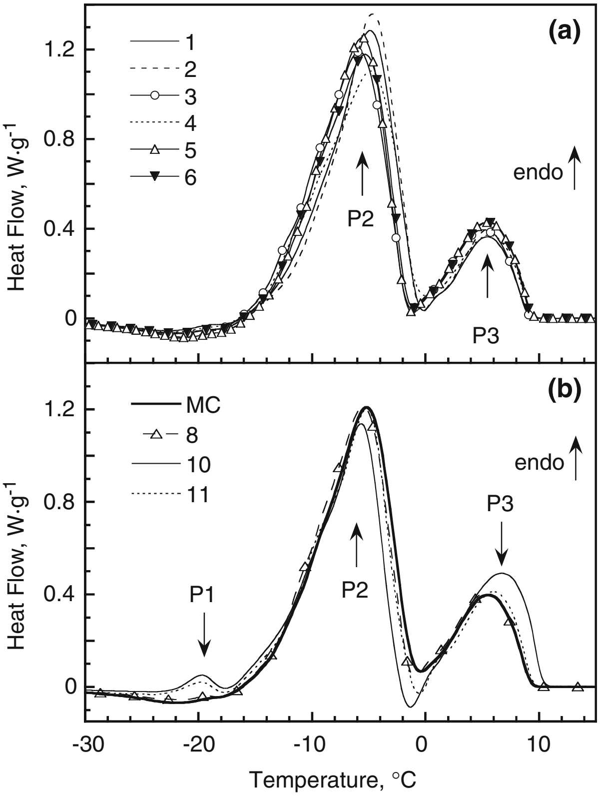

Fig. 1 (**a**) Melting thermograms of six extra virgin olive oils (EVOO) from Tuscany. The samples and their characteristics are listed in Table 1. (**b**) Mean curve (MC), calculated as the mean of the melting curves in panel (**a**), compared with the curves of two Italian EVOOs from different regions: one from Liguria (10) and one from Apulia (11). Curve of a commercial IGP EVOO from Tuscany is also shown (8). Numbers refer to samples listed in Table 2

The refined olive oil thermogram reported in Fig. 2 is representative of the ROO behavior, in line with many measurements not reported here. The key features are an endothermic peak at about −25 °C, a not very intense and flat P3 peak, and an R-value (>6) much higher than that of EVOOs.

In order to understand the effects produced on the melting thermogram by the addition of peanut oil to EVOO, mixtures of an Apulian EVOO with increasing amounts of this seed oil were studied. The results are shown in Fig. 3. The addition of peanut oil increases the intensity of peak P1 (see the inset of the figure) already present in pure Apulian EVOO, and decreases the height of peak P3. Figure 4 shows the effects produced by the addition of HOSO to the same EVOO. No change occurs in the melting curve up to −16 °C. At higher temperatures, the most significant effects are the change in height and shape of P3, which gradually goes down when the w% of added oil is increased. The melting curves of mixtures of the same Apulian EVOO and refined hazelnut oil are depicted in Fig. 5. The melting curves practically overlap up to −13 °C. By increasing the w% of refined hazelnut oil in the mixture, P2 gets higher

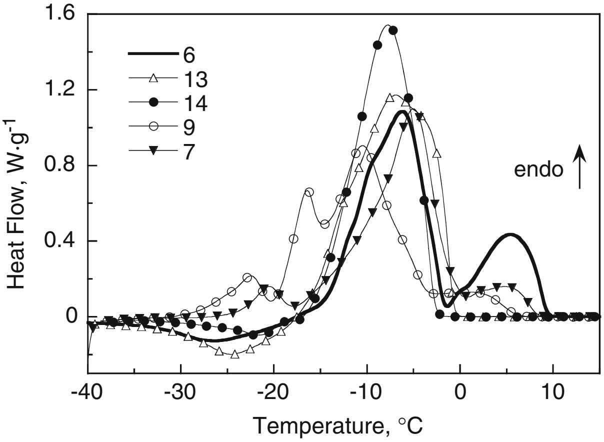

Fig. 2 Melting thermograms of some seed oils (crude hazelnut oil (13), high oleic sunflower (14), peanut oil (9), and refined olive oil (7)), compared with that of EVOO (6). Label numbers refer to samples listed in Tables 1 and 2

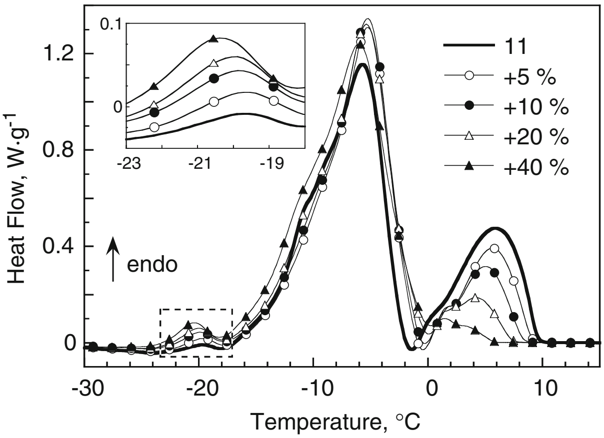

Fig. 3 Melting thermograms of mixtures obtained by adding increasing amounts (w%) of peanut oil (Sample 9 in Table 2) to Apulian EVOO (Sample 11 in Table 2)

and larger, and the height of P3 decreases. Mixtures of HOSO, peanut oil, and refined hazelnut oil with a Tuscan EVOO were found to give qualitatively similar results.

Figure 6 shows the melting curves of the same Apulian EVOO and some mixtures with a ROO. The addition of ROO above all affects P3, which decreases with increases in the added w% of ROO.

For a tentative quantitative analysis of the thermograms in Figs. 3, 4, 5, and 6, we considered the values of the height and the area of P3 (a quantity proportional to the enthalpy variation involved in the melting process) against the w% of the seed oil and ROO added to EVOO. The peak area was measured by integrating the thermogram between the minimum near 0 °C and the end of melting near 10 °C, with the base-

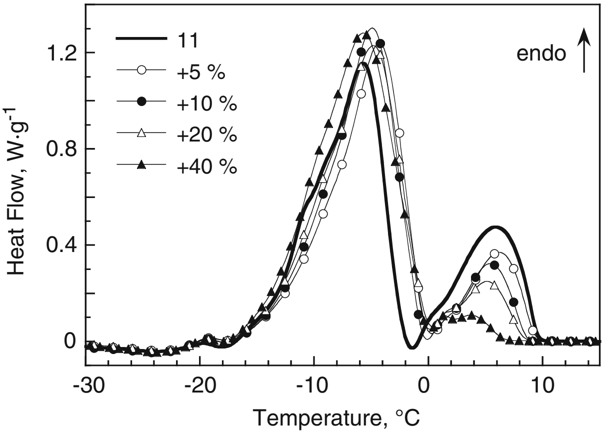

Fig. 4 Melting thermograms of mixtures obtained by adding increasing amounts (w%) of high oleic sunflower oil (Sample 14 in Table 2) to Apulian EVOO (Sample 11 in Table 2)

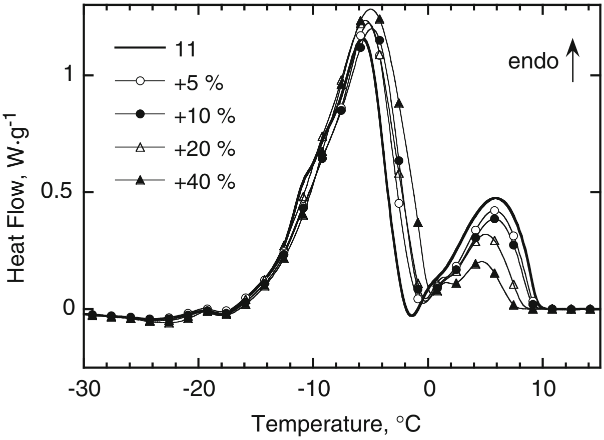

Fig. 5 Melting thermograms of mixtures obtained by adding increasing amounts (w%) of refined hazelnut oil (Sample 12 in Table 2) to Apulian EVOO (Sample 11 in Table 2)

line extrapolated from the straight line above the melting end. The data are shown in Fig. 7a–d, respectively. The effect of the addition of ROO is smaller than that produced by the seed oils; it reduces the height and the area of P3, but to a lesser extent. On the other hand, it increases the height of P1, although only slightly (see the inset of Fig. 6). The same behavior was also found when peanut oil was added to the same EVOO (see Fig. 3).

Figure 8 shows the light and oxygen exposition effects on the crystallization and melting thermogram of an EVOO. The crystallization exothermic peak position is very sensitive to the exposition time, which could be related to the degradation of the oil [26].

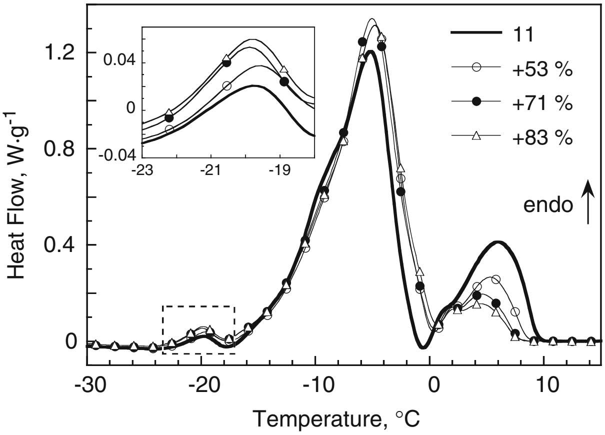

Fig. 6 Melting thermograms of some mixtures obtained by adding increasing amounts (w%) of refined olive oil (Sample 15 in Table 2) to Apulian EVOO (Sample 11 in Table 2)

4 Discussion

The melting of a solid EVOO is a more complex process than the fusion of a simple mixture of different crystalline structures. This is because the polymorphic phases of the numerous TAGs present in the oil undergo different modifications before melting. Moreover, even the minor components of EVOO affect the nucleation and growth kinetics of the various crystalline fractions. This means that a thermodynamic description of how the oil freezes and melts is practically impossible [27,28]. However, due to the high sensitivity of the calorimetric technique, the small changes observed in the thermograms could be explained in terms of oil composition.

In previous exploratory calorimetric work on virgin olive oils, it was shown that at least nine parameters can be obtained from melting, freezing, and isothermal freezing curves studied by DSC [21]. Moreover, the melting curve of the EVOOs only changes negligibly during the first 6 months from the production, if the oils are stored at room temperature in the dark [26]. Therefore, the parameters measured in this time interval can be considered as characteristic of the oil and could be correlated with its quality and origin. The use of a calorimetric method to test the quality of an oil, entails a wide and systematic study leading to a large data bank of all the most representative oils of each region. Monocultivar EVOOs should also be considered. These oils have a specific acidic composition and organoleptic features and could be identified by calorimetry, as suggested by our preliminary results and data in the literature [29].

We are well aware of the limits of our present data; however, we would like to give an idea of a possible calorimetric authentication procedure for EVOOs of a particular region (PDO or PGI indication). Let us thus assume that Tuscan EVOOs (2005–2006 crop season) are represented by the six samples, produced in three different provinces, as shown in Fig. 1. To say whether an unknown oil really originates from Tuscany, one could therefore check these points: the sample is Tuscan EVOO if its melting curve, obtained with the calorimetric method suggested in this paper, is wholly inside

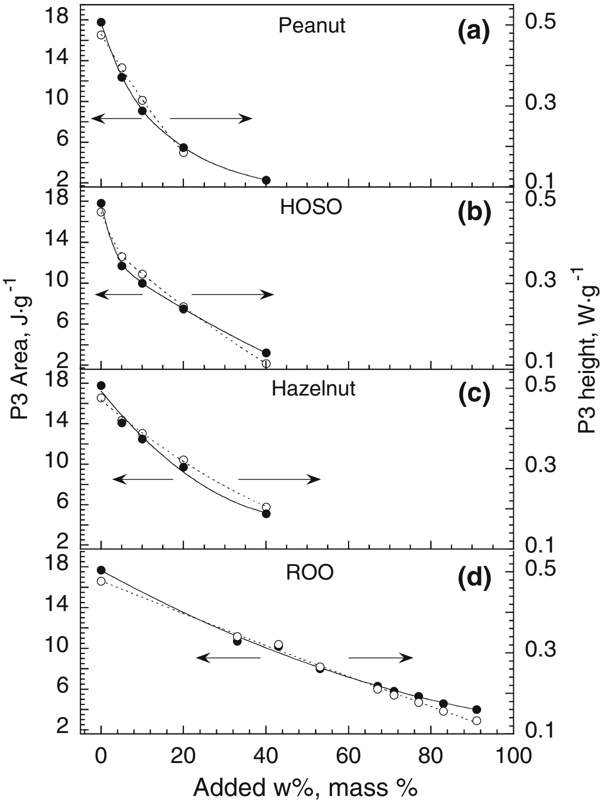

Fig. 7 Values of the area and the height of peak P3 calculated from the curves of Figs. 3, 4, 5, and 6 against the w% of added oil: (**a**) Apulian EVOO and peanut oil mixtures, (**b**) Apulian EVOO and high oleic sunflower oil mixtures, (**c**) Apulian EVOO and hazelnut oil mixtures, and (**d**) Apulian EVOO and refined olive oil mixtures. Enthalpy variation value for the melting process under peak P3 (in $\mathrm{J \cdot g^{-1}}$) was obtained by dividing the area by the temperature-scanning rate ($1/6\,^{\circ}\mathrm{C \cdot s^{-1}}$)

an agreed acceptance band, Δ, around the MC of Fig. 1, which acts as the standard melting curve, SMC, for the crop season considered. We have observed that the reproducibility of the melting curve, as a function of time, is within the assumed Δ band if the oil has been stored in the dark at room temperature. Consequently, a melting curve whose R value is too high could indicate that seed oils and/or ROO have been incorporated, or it might indicate a different origin of the sample. In addition, the simultaneous presence of a peak at about −20 °C in the melting curve could indicate the addition of ROO or peanut oil, or both.

Although such a check may seem rather naive, the results obtained by applying this procedure to six samples of Tuscan EVOOs and to numerous commercial EVOOs (whose origin is not declared on the label) are very promising: the six Tuscan EVOOs, four from private productions and two bought at the market with PDO declaration, fulfilled the above criterion for genuineness, while the commercial EVOOs frequently showed a peak at −20 °C and an R value that was too high. It is thus likely that

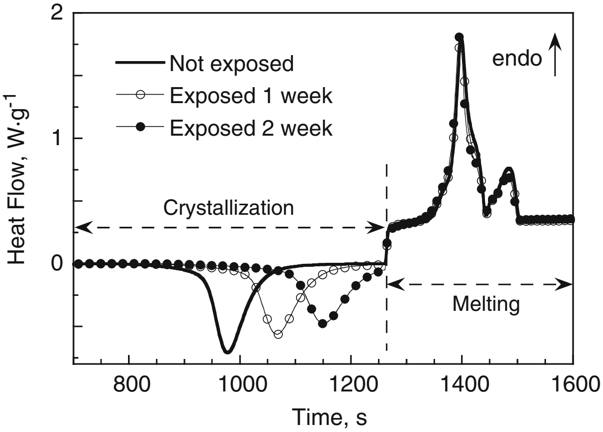

Fig. 8 Thermogram (crystallization followed by melting) of an EVOO (Sample 16 in Table 2) and its change when exposed to light and oxygen for 2 weeks

the commercial EVOOs were not produced with olives from the typical cultivars of Tuscany.

In order to check the addition of seed oil or ROO to a known EVOO sample, the sensitivity limit is about 2 % (see Fig. 7). In the authentication procedure described above, the sensitivity is reduced to about 10 %, resulting from the Δ band around the SMC. The uncertainty in the area of P3 due to the acceptance band corresponds to the effects produced by the addition of about 10 w% of seed oil or ROO. To identify the added adulterant oil, a more complex analysis needs to be performed. This becomes even more difficult if more than one oil has been added. For these reasons, the calorimetric method is not suitable for an absolute authentication test. However, we believe that this technique is suitable in each step of oil production and commercialization, as a fast and simple test for (a) detection of some fraudulent additions of other oils; (b) identification of storage history and ageing; (c) providing a "first glance" genuineness test for EVOO with PDO or PGI indication, if a significant data bank is organized for each region and/or producers' consortium; and finally (d) providing a conformity test.

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