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Application of a simplified calorimetric assay for the evaluation of extra virgin olive oil quality

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ABSTRACT

Thermal properties of eighteen monovarietal extra virgin olive oils from the Apulia region in Italy were evaluated by means of a modulated adiabatic scanning calorimeter (MASC) and related to their chemical composition (free acidity, UV absorbance, fatty acid composition, polyphenol and *o*-diphenol content, oxidation status). MASC was used to study oil sample phase transitions in a temperature scanning mode by using a tailor-made time–temperature protocol. Crystallization kinetics and transition enthalpies were found to be significantly correlated to single free fatty acids (palmitic, oleic and linoleic acids) and to unsaturated/saturated fatty acid ratio. The overall crystallization curves were significantly delayed and occurred over longer time ranges as a function of higher peroxide index and linoleic acid content. Significant correlations were observed between melting profiles and single fatty acids, unsaturated/saturated fatty acid ratio and oleic/linoleic acid ratio. No significant correlations were observed between thermal properties and free acidity, linolenic acid, polyphenol and *o*-diphenol content.
Compared with classical differential scanning calorimetry, MASC was simpler to use. The applied time–temperature protocol allowed the characterization of extra virgin olive oils (EVOOs) of different provenience with rapid measurements and giving rise to friendly outcome data. MASC, with its compact and portable equipment, may promote practical applications of calorimetric test along the olive oil supply chain.

1. Introduction

Extra virgin olive oil (EVOO) is the highest olive oil quality grade and one of the most important and traditional products of the Mediterranean basin, with Spain, Italy and Greece, contributing 38%, 37% and 23% to the world olive oil production, respectively (FAOSTAT, 2011). In Italy, the largest olive production occurs in the Apulia region that contributes to 37% of the national and 12% of the world EVOO production. As for many high-value products, EVOO is subjected to fraudulent practices, such as the addition of cheaper plant oils or the use of less valuable production methods (Woolfe & Primrose, 2004). Different olive oil grades and rules for commercialization have been established by IOOC (International Olive Council, 2012) and the European Community published a number of regulations to protect the authenticity of *EVOOs*. In particular the EC Reg. No. 2081/92 promoted territory-linked products by means of Protected Designation of Origin (PDO) and Protected Geographical Indications (GPI) labels. The present actions are particularly significant in Italy, where more than 400 autochthonous cultivars are grown in different climatic areas of the peninsula and 41 PDOs are currently recognized (Bartolini, Prevost, Messeri, Carignani, & Menini, 1998). Olive cultivar, geographical area and milling process are all involved in determining EVOO quality and several methods have been proposed to avoid frauds and validate product conformity.

Among the analytical methods available to EVOO authentication, NMR (Nuclear Magnetic Resonance) is sensitive, rapid and capable of simultaneous detection of a great number of components in the complex oil matrice (Del Coco, Schena, & Fanizzi, 2012; Papadia et al., 2011). Compared to classical methods based on chromatography (Cossignani et al., 2007), NMR is less time consuming, but its use is hampered by sophisticated equipment and, as for NIR (Near Infrared Resonance), it requires complex statistical analysis of spectral data (Casale et al., 2012). A novel real-time method of vegetable oil verification was recently proposed which is based on low-power microwave sensing principle and allowing to discriminate among different oil sample (Korostynska, Blakey, Mason, & Al-Shamma'a, in press).

Differential Scanning Calorimetry (DSC) application is largely recognized as a reliable tool for the characterization of oil thermal properties and can be used to test quality and to determine the genetic and the geographical origin of EVOO productions (Angiuli et al., 2012; Chiavaro, Cerretani, et al., 2011) and discriminate among olive oils of different commercial categories (Chiavaro, Rodriguez-Estrada, et al., 2008). DSC was proposed to assess time–temperature storage history of olive oils (Angiuli, Ferrari, Righetti, Tombari, & Salvetti, 2007) and to evaluate olive oil authenticity (Angiuli et al., 2006; Chiavaro et al., 2009). Crystallization and melting profiles obtained by DSC were shown to

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EVOO code	Production system	Cultivation area in Apulia	Olive Cultivar
Cel_SouthSS	SS	Carpignano (South)	Cellina di Nardò
Cel_SouthLS	LS	Carpignano (South)	Cellina di Nardò
Ogl_SouthSS	SS	Carpignano (South)	Ogliarola leccese
Ogl_SouthLS	LS	Carpignano (South)	Ogliarola leccese
Crn_SouthSS	SS	Carpignano (South)	Cornulara
Bam_SouthSS	SS	Carpignano (South)	Barone di Monteprofico
Usc_SouthSS	SS	Carpignano (South)	Usciana
Olr_SouthSS	SS	Carpignano (South)	Oliva rossa
Lec_SouthSS	SS	Lecce (South)	Leccino
Fra_SouthSS	SS	Carpignano (South)	Frantoio
Fs17_SouthSS	SS	Carpignano (South)	FS17
Noc_SouthSS	SS	Carpignano (South)	Nociara
Ogb_CentreSS	SS	Bitonto (Centre)	Ogliarola barese
Ogb_NorthSS	SS	Foggia (North)	Ogliarola barese
Cor_CentreSS	SS	Bitonto (Centre)	Coratina
Cor_CentreLS	LS	Bitonto (Centre)	Coratina
Kor_SouthLS	LS	Carpignano (South)	Koroneiki
Per_CentreLS	LS	Bitonto (Centre)	Peranzana

Fig. 1. Map of Italy showing the Apulia region where olive cultivars were grown and drupes were sampled. Sample codes, production system (SS: small scale and LS: large scale), cultivation area in Apulia and relative olive cultivar are also reported for all EVOOs.

depend on the chemical composition of olive oils, with particular regard to triacylglycerols (TAGs), diacylglicerols (DGs) and fatty acids and to be statistically correlated with major and minor components (Chiavaro, Rodriguez-Estrada, Bendini, & Cerretani, 2010; Chiavaro, Rodriguez-Estrada, et al., 2008; Chiavaro et al., 2007).

To further promote the application of calorimetry along the olive oil supply chain, we used a simplified calorimetric method based on the use of MASC (modulated adiabatic scanning calorimeter) in a temperature scanning mode with a tailor-made time–temperature protocol. MASC performances were tested upon 18 monovarietal extra virgin olive oils from the Apulia region in Italy. A reproducible calorimetric test was developed and thermal properties of monovarietal EVOOs were evaluated as a function of their chemical composition.

2. Material and methods

2.1. Samples

Eighteen monovarietal EVOOs from the Apulia region (Italy) were analyzed (Fig. 1). The olives used for oil production were hand-picked at technological ripening in 2010 and processed within 24–48 h. Thirteen monovarietal samples were produced by using a small-scale (SS) system (Spremoliva, Toscana Enologica Mori, Tavarnelle Val di Pesa, Italy) capable of milling, mixing and extracting extra virgin olive oil and discharging waste in one step through a single cylinder/decanter. Five monovarietal EVOOs were produced by using a large-scale continuous three phase apparatus (LS) which is most commonly used for olive oil production (Pieralisi, Jesi, Italy). At least two replicates were performed for each EVOO and subsequently subjected to calorimetric and chemical analyses.

2.2. Analytical indices

The EVOO samples were characterized for free fatty acid content (% of oleic acid), peroxide value (meqO_2/kg) and UV-absorption (K232 and K270), according to the EC Regution No. 2568/91.

2.3. Total phenol and o-diphenol content

The amount of total phenolics and *o*-diphenols in the oils was determined colorimetrically using the Folin–Ciocalteu procedure and results were expressed as ppm of gallic acid (Singleton & Rossi, 1965).

2.4. Oxidative stability

The oxidative stability of oil samples was evaluated following the Rancimat method (Gutierrez, 1989) and expressed in hours as OSI time (oxidation induction time). Measurements were carried out with a Rancimat 679 apparatus (Metrohm, Herisau, Switzerland), using 3.5 g of oil sample warmed to 100 °C and an air flow of 10 l/h.

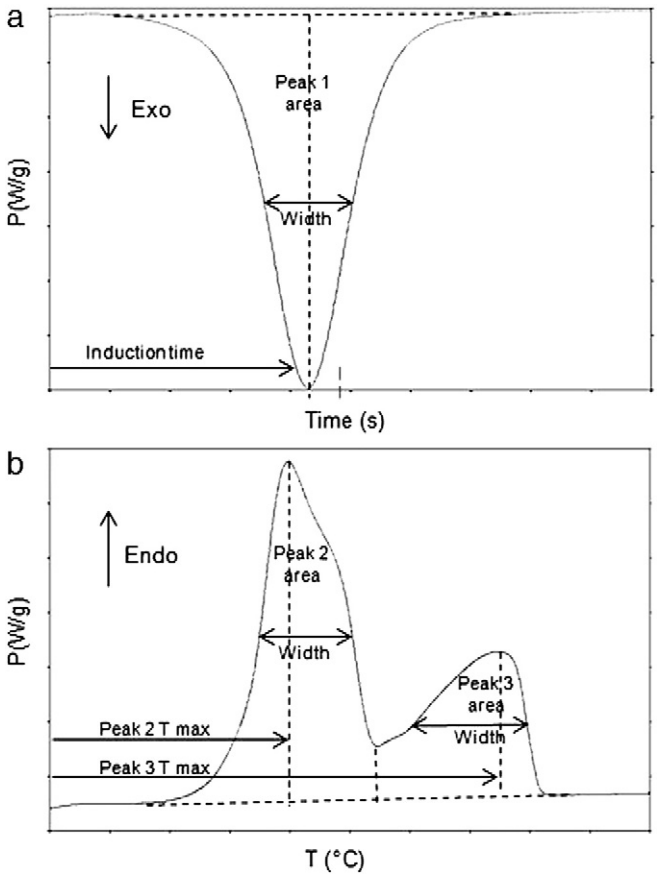

Fig. 2. (a): Crystallization curve (Peak 1) as described by area (ΔH, J/g), width (s) and induction time (s). (b): Melting curves (Peak 2 and Peak 3) as described by area (ΔH, J/g), width (°C) and temperature at maximum height (Tmax, °C).

Table 1
Quality indices of 18 EVOOs obtained from different olive cultivars grown in Apulia region (south, centre and north) with large (LS) and small continuous scale (SS) milling system.

EVOO sample	Free acidity (g/100 g oleic acid)	POV (meq/Kg)	Tot. Phen. (ppm gallic acid)	*o*-Phenols (ppm gallic acid)	OSI Time (h)	Palmitic acid (%)	Stearic acid (%)	Oleic acid (%)	Linoleic acid (%)	Linolenic acid (%)	Uns./Sat. fatty acids	Oleic/linoleic acid
Cel_SouthSS	0.20^{bce}	11.5^{hi}	125.6^{c}	42.4^{f}	7.18^{d}	16.11^{o}	1.91^{f}	68.96^{b}	9.12^{cd}	0.85^{h}	4.45^{abc}	7.56^{de}
Cel_SouthLS	0.22^{cde}	9.0^{f}	221.5^{g}	56.3^{m}	9.73^{h}	13.91^{fg}	1.75^{de}	73.30^{g}	8.34^{c}	0.65^{abc}	5.27^{efg}	8.79^{f}
Ogl_SouthSS	0.22^{cdef}	12.7^{i}	133.7^{d}	35.8^{e}	6.60^{c}	14.09^{g}	1.30^{a}	69.88^{c}	10.19^{ef}	0.64^{ab}	5.37^{fg}	6.86^{b}
Ogl_SouthLS	0.19^{ab}	8.8^{ef}	309.7^{m}	72.5^{p}	10.20^{j}	15.62^{n}	1.82^{e}	68.87^{b}	10.22^{ef}	0.64^{ab}	4.63^{bcd}	6.74^{b}
Crn_SouthSS	0.18^{a}	7.0^{bcd}	152.3^{e}	47.5^{h}	10.59^{l}	9.35^{a}	2.46^{m}	79.71^{m}	6.32^{b}	0.62^{a}	7.16^{i}	12.62^{i}
Bam_SouthSS	0.30^{gh}	11.6^{hi}	291.5^{l}	50.0^{i}	9.32^{g}	17.71^{q}	1.70^{d}	65.29^{a}	11.27^{g}	0.74^{ef}	4.08^{a}	5.80^{a}
Usc_SouthSS	0.23^{df}	9.3^{fg}	59.7^{a}	7.4^{a}	5.89^{b}	12.63^{d}	3.81^{o}	72.34^{f}	8.58^{c}	0.79^{fg}	4.81^{cde}	8.43^{f}
Olr_SouthSS	0.42^{m}	9.0^{ef}	119.8^{c}	25.8^{d}	7.73^{e}	14.43^{i}	1.57^{c}	71.35^{e}	9.09^{cd}	1.07^{i}	5.13^{efg}	7.85^{e}
Lec_SouthSS	0.23^{def}	7.5^{cd}	338.0^{n}	68.58^{o}	12.65^{n}	17.28^{p}	1.77^{de}	71.05^{l}	6.94^{b}	0.60^{a}	4.18^{ab}	10.24^{g}
Fra_SouthSS	0.32^{hi}	12.0^{i}	125.0^{c}	23.35^{c}	4.89^{a}	14.86^{l}	1.73^{d}	70.56^{d}	10.04^{ef}	0.74^{ef}	4.90^{cdef}	7.03^{bc}
Fs17_SouthSS	0.34^{i}	10.4^{gh}	84.6^{b}	11.7^{b}	4.56^{a}	14.92^{l}	1.44^{b}	70.18^{cd}	10.79^{fg}	0.85^{h}	5.00^{defg}	6.51^{b}
Noc_SouthSS	0.32^{hi}	6.2^{abc}	330.1^{n}	40.5^{f}	7.88^{e}	13.74^{f}	2.15^{i}	71.56^{e}	9.54^{de}	0.73^{de}	5.16^{efg}	7.50^{de}
Ogb_Centre_SS	0.25^{f}	7.3^{bcd}	240.5^{h}	45.2^{g}	6.20^{b}	14.13^{gh}	2.07^{gh}	74.50^{h}	6.98^{b}	0.64^{abc}	5.05^{defg}	10.68^{h}
Ogb_NorthSS	0.37^{l}	7.0^{bcd}	434.3^{o}	64.3^{n}	11.20^{m}	15.36^{m}	2.64^{n}	69.90^{c}	9.52^{de}	0.81^{gh}	4.44^{abc}	7.35^{cd}
Cor_CentreSS	0.24^{df}	5.0^{a}	316.2^{m}	44.6^{g}	12.53^{n}	10.37^{b}	2.37^{l}	79.85^{m}	5.37^{a}	0.70^{cde}	6.58^{h}	14.90^{l}
Cor_CentreLS	0.20^{abc}	6.0^{ab}	516.8^{p}	111.8^{r}	18.28^{p}	11.15^{c}	2.13^{hi}	78.62^{l}	6.26^{b}	0.65^{abc}	6.33^{h}	12.60^{i}
Kor_SouthLS	0.28^{g}	7.7^{de}	211.6^{f}	52.2^{l}	14.34^{o}	13.07^{e}	2.06^{gh}	77.52^{j}	4.94^{a}	0.65^{abc}	5.45^{g}	15.70^{m}
Per_CentreLS	0.39^{l}	14.0^{l}	270.3^{i}	79.02^{q}	8.40^{f}	14.34^{hi}	2.02^{g}	71.38^{e}	10.00^{def}	0.68^{bcd}	4.99^{cde}	7.14^{bcd}

Samples with same letter within each column are not significantly different ($n = 2$, $p < 0.05$).

2.5. Analysis of fatty acids

The fatty acid composition of oil samples was determined in agreement with the EC Regulation No. 2568/91. Fatty acids were converted to fatty acid methyl esters (FAME) and then analyzed by using a Varian CP-3800 (Burladingen, Germany) chromatograph equipped with a Supelco-wax capillary column (30 m lenght, 0.25 mm ID and 0.25 μm thickness) and a flame ionization detector (FID). Helium was used as carrier at a flow rate of 0.8 ml/min. The temperatures of the injector and detector were set at 240 °C and 260 °C, respectively. The oven temperature was initially set at 140 °C and kept constant for 5 min. An increase of 4 °C min^{-1} raised the temperature to 230 °C and was kept constant for 10 min. The identification of FAME used a standard FAME reference mixture (C8-C24 Sigma Aldrich). FAME peaks were identified by comparing retention times to a standard mixture.

2.6. Calorimetric test

The calorimetric analysis was performed by using a modulated adiabatic scanning calorimeter (MASC) constructed by the Institute for Physical Chemical Processes, IPCF-Pisa, CNR, Italy (Salvetti, Ferrari, Papucci, & Tombari, 1998). In the present work, MASC was used to study oil phase transitions in a temperature scanning mode by using a tailor-made time–temperature protocol.

Olive oil samples (0.4 g) were transferred to a glass capillary tube that was fame-sealed and introduced into the calorimeter cell at −30 °C. The sample was kept at constant temperature (−30 °C) for 6 min to reach the apparent complete crystallization, with the overall heat flow equal to zero at the end of isotherm. Then, temperature was increased at 2 °C/min rate up to 15 °C in order to obtain complete sample melting. The protocol adopted allowed for a supercooled liquid phase at the beginning, and polymorphic crystals in the solid sample at the end of the isotherm (−30 °C). The thermograms were recorded by a PC connected to MASC and the registered data were processed by using a dedicated software. The crystallization curve (Peak 1) was analyzed by measuring the following features: peak area, namely crystallization enthalpy (J/g); induction time (s), defined as the time interval from the beginning of the isotherm and the minimum of the peak; and peak width (s) that was measured at half peak height (Fig. 2a). Similarly, melting peaks (Peak 2 and Peak 3), were characterized by measuring: total peak area (J/g), width at half peak height (°C) and temperature value at maximum peak height (°C) (Fig. 2b).

2.7. Statistical analysis

Means and standard deviations were calculated with SPSS (Version 13.0, SPSS Inc., Chicago, IL) statistical software. The SPSS software was used to perform one-way analysis of variance (ANOVA) and the Student–Newman–Keuls test at a 95% confidence level ($p < 0.05$) to identify differences among groups. Pearson correlation coefficients (r) were calculated between thermogram features and chemical parameters of oil samples, at confidence levels of 95% ($p < 0.05$), and of 99% ($p < 0.01$) and 99.9% ($p < 0.001$) to reduce the probability of false positive identification.

3. Results and discussion

3.1. Chemical composition of EVOO samples

General information and abbreviations used for EVOO samples are shown in Fig. 1. The considered monovarietal EVOOs were produced from the foremost widespread olive cultivars in the Apulia region (Italy). Twelve samples were obtained from olive cultivars grown in the experimental field of CNR at Carpignano, south Apulia (Cimato, De Rinaldis, Calogiuri, & Sani, 2001). Four samples came from the

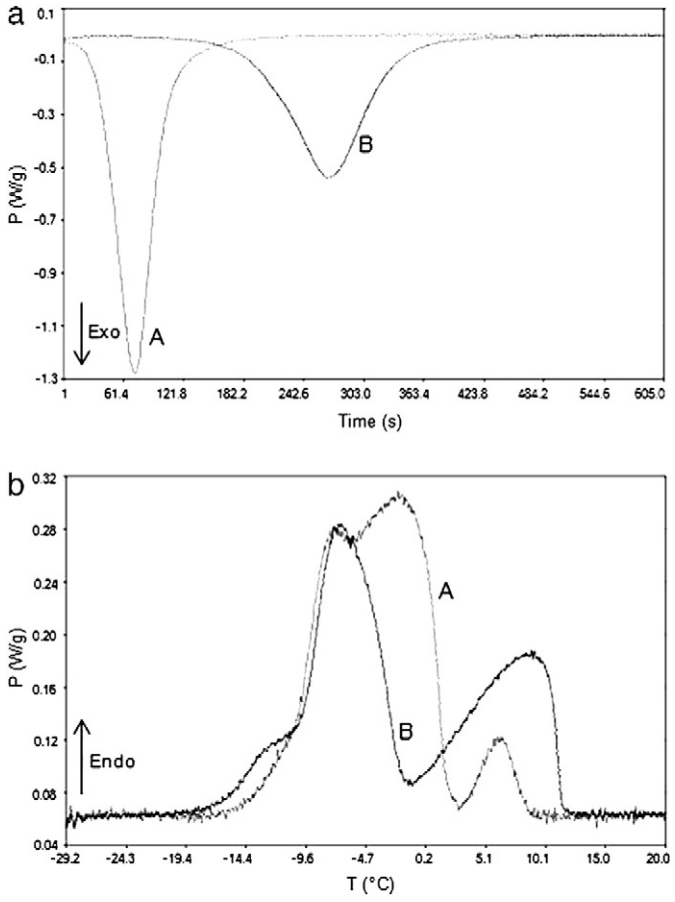

Fig. 3. Thermograms of two monovarietal EVOOs: A) cv. Cornulara (Crn_SouthSS); B) cv. Cellina di Nardò (Cel_SouthSS). (a): crystallization curve; (b): melting curve.

same farm in Bitonto (center Apulia) and two samples from Lecce (south Apulia) and Foggia (north Apulia), respectively (Fig. 1).

All samples exhibited free acidity, POVs and ΔK values within the limits set by the EC Regulation No. 1513/2001 (free acidity <0.8%, POV < 20 meq O2/kg, ΔK ≤ 0.010). EVOOs produced by using the SS system had lower total phenol content compared to the same monovarietal EVOOs produced with the LS apparatus (Table 1). In fact, the milling process required more water to extract the oil compared to the LS apparatus, determining greater loss of hydrosoluble phenolic compounds. EVOO samples produced with the LS apparatus showed lower POVs than the same monovarietal oils produced with the SS system. The extraction process influenced also the content of *o*-diphenols and the oil oxidation status. EVOO samples produced by the LS system exhibited a higher content of *o*-diphenols that positively influenced OSI time and POV value. Indeed, *o*-diphenols were identified as the main source of the overall antioxidant activity in olive oil (Rotondi et al., 2004).

The fatty acid analysis revealed significant variations among the analyzed EVOOs (Table 1). Samples from Coratina showed the highest level of oleic acid and phenolic content. Among EVOOs, linoleic acid was the most abundant polyunsatured fatty acid, varying from 4.94% in Kor_SouthLS and 11.27% in Bam_SouthSS. Linolenic and palmitic acids also showed significant variation among samples, from 0.60 (Lec_SouthSS) to 1.07% (Olr_SouthSS) and 9.35% (Bam_SouthSS) to 17.71% a (Lec_SouthSS), respectively. Significant differences were observed for unsaturated/saturated fatty acid and oleic/linoleic acid ratio, with best values in Cornulara and Coratina oils (Table 1).

3.2. Calorimetric analysis

Thermal properties of the assayed monovarietal EVOOs were evaluated by measuring the amount of heat exchanged during the liquid↔solid phase transition. The heat exchanged during phase transitions was expressed as a function of time (crystallization curves) and of temperature (melting curves).

3.2.1. Crystallization process

The crystallization process of EVOO samples was analyzed isothermally and resulted in one distinguishable exothermic event, named Peak 1 (Figs. 3a and 4a), thus differing from DSC that in temperature scanning mode, typically shows two exothermic peaks at ~−10 °C and ~−33.5 °C (Barba, Arrighetti, & Calligaris, 2013).

Peak 1 features of EVOOs were characterized by measuring the relative area, width and induction time. Results revealed that the induction time significantly differed among all the assayed EVOO samples, being particularly low in Crn_SouthSS (88 s) and Cor_CentreSS (73 s) or high in Bam_SouthSS (348 s) and Ogl_SouthLS (271 s).

The width of Peak 1 provided information about the crystallization rate and also allowed a good discrimination of EVOOs (Table 2). Some samples required more time to complete the crystallization process (e.g., Bam_SouthSS, 101 s) compared to others (e.g., Cor_CentreLS, 48.7 s). Peak 1 area (enthalpy) also contributed to differentiate samples and showed a variation range from a maximum of 64.8 J/g (Cor_CentreLS) to a minimum of 48.9 J/g (Bam_SouthSS).

Relative standard deviations (RSD) in a range of 0.02–1.42 indicated the good reproducibility of the method (Table 2 and Fig. 5). Although multivariate statistical analysis of a larger set of samples will be

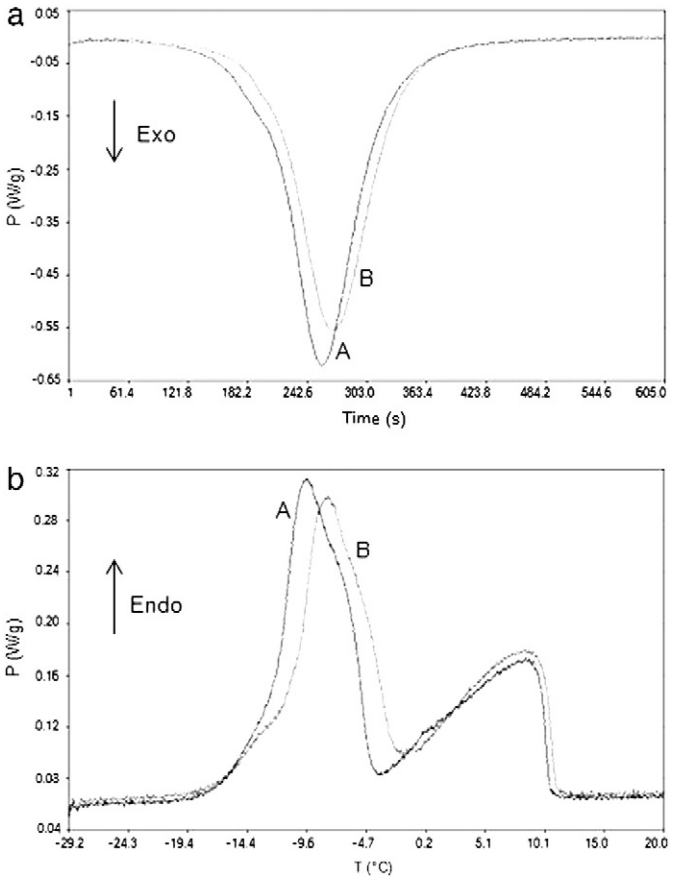

Fig. 4. Thermograms of two monovarietal EVOOs (Ogl_SouthLS and Ogl_SouthSS) obtained by using two different production systems: A) small scale and B) large scale. (a): crystallization curve; (b): melting curve.

Table 2
Crystallization peak (Peak 1) of 18 EVOOs as described by its area, width and induction time.

EVOO sample	Peak 1								
	Area			Width			Induction time		
	(J/g)	±sd	RSD (%)	(s)	±sd	RSD (%)	(°C)	±sd	RSD (%)
Cel_SouthSS	53.1^{il}	0.06	0.11	81.5^{il}	0.50	0.61	268^{l}	0.56	0.21
Cel_SouthLS	58.4^{de}	0.02	0.03	54.3^{de}	0.58	1.07	188^{f}	0.47	0.25
Ogl_SouthSS	57.8^{ef}	0.16	0.28	68.3^{g}	0.53	0.77	258^{i}	0.60	0.23
Ogl_SouthLS	54.3^{h}	0.12	0.22	77.0^{hi}	0.60	0.78	271^{l}	0.12	0.04
Crn_SouthSS	64.8^{a}	0.19	0.29	41.0^{b}	0.58	1.42	88^{b}	0.10	0.11
Bam_SouthSS	48.9^{m}	0.15	0.31	101.0^{m}	0.65	0.64	348^{m}	0.36	0.10
Usc_SouthSS	54.0^{hi}	0.07	0.13	54.7^{de}	0.25	0.46	168^{e}	0.44	0.26
Olr_SouthSS	58.5^{de}	0.10	0.17	73.0^{gh}	0.58	0.79	239^{h}	0.52	0.22
Lec_SouthSS	52.8^{l}	0.04	0.07	77.7^{hi}	0.72	0.93	191^{f}	0.60	0.31
Fra_SouthSS	55.3^{g}	0.05	0.09	84.0^{l}	0.49	0.58	246^{h}	0.40	0.16
Fs17_SouthSS	57.8^{ef}	0.05	0.09	56.0^{def}	0.70	1.25	258^{i}	0.40	0.15
Noc_SouthSS	57.3^{f}	0.06	0.10	52.0^{cd}	0.53	1.02	217^{g}	0.36	0.16
Ogb_CentreSS	59.2^{d}	0.05	0.08	57.7^{ef}	0.58	1.01	141^{d}	0.29	0.20
Ogb_NorthSS	53.7^{hil}	0.02	0.04	70.0^{g}	0.36	0.51	220^{g}	0.23	0.10
Cor_CentreSS	61.9^{d}	0.08	0.13	48.7^{c}	0.25	0.51	73^{a}	0.38	0.52
Cor_CentreLS	64.3^{a}	0.04	0.06	30.0^{a}	0.19	0.63	95^{bc}	0.23	0.24
Kor_SouthLS	60.3^{c}	0.05	0.08	51.2^{cd}	0.21	0.41	102^{c}	0.29	0.28
Per_CentreLS	57.5^{ef}	0.07	0.12	60.0^{f}	0.37	0.62	238^{h}	0.42	0.18

Values followed by the same letter within each column are not significantly different ($n = 3$, $p < 0.05$).

required (Chiavaro, Cerretani, et al., 2011), the crystallization curve obtained by MASC was able to differentiate among different monovarietal EVOOs. In particular, EVOOs of different cultivar origin and produced with the same milling system (Fig. 1), showed statistically significant variations in Peak 1 features (Table 2). Moreover, Peak 1 of monovarietal EVOOs from the same cultivar and field (e.g. Cel_SouthSS and Cel_SouthLS, from Cellina di Nardò; Ogl_SouthSS and Ogl_SouthLS, from Ogliarola salentina; and Cor_CentreSS and Cor_Centre LS, from Coratina), but obtained by using different milling processes also varied significantly (Table 2). The observed variations depended on differences in the chemical composition of EVOO samples, as it will be discussed in the correlation analysis session.

3.2.2. Melting process

The melting process was generated by raising the sample temperature at a rate of 2 °C/min, from −30 °C to +15 °C. The solidified samples underwent transitions from unstable polymorphic forms into more stable forms; these transitions may depend not only on sample temperature, but also on the time, as supported by the well known dependence of thermograms on the temperature scanning rate (Che Man & Tan, 2002).

Representative melting curves of the assayed EVOOs are shown in Figs. 3b and 4b. The transition from unstable polymorphic crystalline forms into more stable forms occurred from −10 °C to −1.5 °C in the majority of EVOOs. In this temperature interval, a reorganization of the polymorphic crystal structures is known to occur, giving rise to a first melting peak, here named Peak 2 (Sato, 2001). A second melting peak was observed in the temperature range 0–10 °C (Peak 3, Figs. 3b and 4b) as a result of the subsequent solid–liquid transition of β-type polymorphic crystals (Wesdorp et al., 2005). The ANOVA test reveled that Peak 3 was more effective to differentiate EVOO samples of different cultivar origin (e.g. EVOOs from Carpignano) compared to Peak 2 (Table 3). Low relative standard deviation (RSD) values, in a range of 0.0–3.0, showed the good repeatability of the adopted scan rate protocol (Table 3 and Fig. 5).

As a preliminary result on a low number of samples, the overall thermogram (crystallization and melting curves) obtained by MASC was able to differentiate among different monovarietal EVOOs.

Thermograms shown in Fig. 3 enlighten differences between samples obtained from different cultivars (Cornulara and Cellina di Nardò), but identical growing area (Carpignano) and extraction system (SS). Moreover, crystallization and melting profiles of EVOOs from the same cultivar (Ogliarola salentina) and growing area (Carpignano), but milled by SS and LS system, clearly differed (Fig. 4).

Differences in thermograms depended on the chemical composition of EVOOs (Table 1) confirming previous findings obtained by DSC. In particular, lipid oxidation products and free fatty acids were shown to

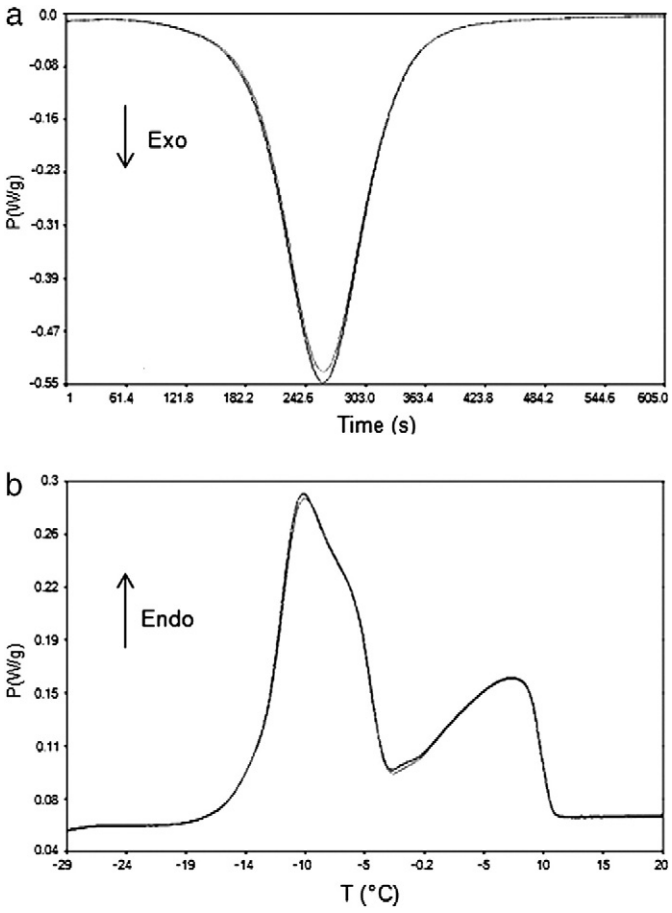

Fig. 5. Thermograms of two replicates of the monovarietal EVOO sample FS17_South_SS indicating the reproducibility of thermal analysis. (a): crystallization curve; (b): melting curve.

Table 3
Melting peaks (Peak 2 and Peak 3) of 18 EVOOs as described by area, width and temperature at maximum height.

EVOO sample	Peak 2									Peak 3								
	Area			Width			Tmax			Area			Width			Tmax		
	(J/g)	±sd	RSD (%)	(°C)	±sd	RSD (%)	(°C)	±sd	RSD (%)	(J/g)	±sd	RSD (%)	(°C)	±sd	RSD (%)	(°C)	±sd	RSD (%)
Cel_SouthSS	47.5^{b}	0.10	0.21	5.9^{b}	0.00	0.00	-6.7^{f}	0.01	0.15	30.3^{p}	0.10	0.33	8.3^{i}	0.02	0.24	8.9^{q}	0.04	0.45
Cel_SouthLS	56.8^{g}	0.81	1.43	7.8^{e}	0.10	1.28	-7.2^{ef}	0.01	0.14	22.5^{i}	0.18	0.80	7.6^{h}	0.10	1.31	6.8^{l}	0.01	0.15
Ogl_SouthSS	49.3^{c}	0.12	0.24	6.1^{b}	0.12	1.97	-9.6^{a}	0.01	0.10	29.2^{o}	0.15	0.51	9.2^{m}	0.19	2.06	8.4^{n}	0.01	0.12
Ogl_SouthLS	49.8^{c}	0.26	0.52	6.1^{b}	0.10	1.64	-7.9^{cd}	0.01	0.13	28.7^{no}	0.10	0.35	8.8^{l}	0.15	1.70	8.6^{o}	0.01	0.12
Crn_SouthSS	82.4^{p}	0.31	0.38	11.3^{m}	0.20	1.77	-2.1^{i}	0.01	0.48	0.6^{a}	0.06	10.0	2.0^{a}	0.06	3.00	5.2^{a}	0.01	0.19
Bam_SouthSS	44.8^{a}	0.29	0.65	4.8^{a}	0.06	1.25	-8.8^{b}	0.01	0.11	33.0^{r}	0.12	0.36	9.1^{m}	0.12	1.32	8.8^{p}	0.03	0.34
Usc_SouthSS	74.8^{m}	0.38	0.51	11.0^{l}	0.15	1.36	-7.1^{f}	0.01	0.14	3.8^{b}	0.10	2.63	3.6^{c}	0.03	0.83	5.9^{b}	0.01	0.17
Olr_SouthSS	55.1^{f}	0.10	0.18	7.9^{ef}	0.06	0.76	-8.6^{bc}	0.01	0.12	24.3^{l}	0.11	0.45	8.4^{i}	0.05	0.59	7.2^{h}	0.04	0.55
Lec_SouthSS	44.4^{a}	0.26	0.58	5.9^{b}	0.06	1.02	-5.9^{g}	0.01	0.17	32.2^{q}	0.12	0.37	8.3^{i}	0.03	0.36	9.9^{r}	0.01	0.10
Fra_SouthSS	51.2^{d}	0.25	0.49	6.4^{c}	0.06	0.93	-8.9^{ab}	0.01	0.11	28.2^{n}	0.10	0.35	8.9^{l}	0.08	0.90	8.4^{n}	0.01	0.12
Fs17_SouthSS	53.5^{e}	0.35	0.65	7.2^{d}	0.06	0.83	-8.6^{b}	0.04	0.46	26.6^{m}	0.14	0.53	9.3^{m}	0.02	0.21	7.4^{l}	0.01	0.13
Noc_SouthSS	59.0^{h}	0.15	0.25	8.1^{f}	0.00	0.00	-7.8^{de}	0.01	0.13	20.0^{h}	0.19	0.95	7.0^{f}	0.02	0.28	6.9^{f}	0.01	0.14
Ogb_CentreSS	62.2^{i}	0.17	0.27	8.8^{g}	0.06	0.68	-7.0^{f}	0.01	0.14	18.5^{f}	0.15	0.81	6.5^{e}	0.03	0.46	7.1^{g}	0.00	0.00
Ogb_NorthSS	53.8^{e}	0.37	0.69	5.9^{b}	0.17	2.88	-6.9^{f}	0.02	0.29	24.5^{l}	0.14	0.57	7.6^{h}	0.09	1.18	8.4^{m}	0.01	0.12
Cor_CentreSS	77.1^{n}	0.52	0.67	10.3^{i}	0.10	0.97	-1.9^{i}	0.00	0.00	5.8^{d}	0.08	1.38	3.0^{b}	0.08	2.66	6.3^{c}	0.02	0.32
Cor_CentreLS	79.5^{o}	0.06	0.07	10.8^{i}	0.23	2.13	−1.5i	0.00	0.00	4.8^{c}	0.02	0.42	2.9^{b}	0.01	0.34	6.3^{c}	0.05	0.79
Kor_SouthLS	64.3^{l}	0.35	0.54	9.5^{h}	0.28	2.94	-3.2^{h}	0.01	0.31	17.6^{e}	0.05	0.28	5.9^{d}	0.04	0.68	7.4^{i}	0.02	0.27
Per_CentreLS	58.6^{h}	0.26	0.44	8.1^{f}	0.08	0.98	-7.3^{def}	0.06	0.82	19.1^{g}	0.02	0.10	7.3^{g}	0.03	0.41	6.7^{d}	0.01	0.15

Values followed by the same letter within each column are not significantly different ($n = 3$, $p < 0.05$).

influence the nucleation process and the polymorphic crystal growth kinetics (Chiavaro et al., 2007, 2010; Ferrari et al., 2007).

On the other end, the endothermic events determining the melting of crystallized lipids were previously found to depend on minor components (diacylglycerols, free fatty acids) and oxidative stability indices (Chiavaro, Vittadini, Rodriguez-Estrada, Cerretani, & Bendini, 2008; Jiménez & Maza, 2003).

3.3. Correlation analysis between thermal and chemical EVOO properties

Correlation analysis between thermogram parameters and chemical composition of the assayed EVOOs is shown in Table 4. Thermal properties of the cooling transition (crystallization kinetics and transition enthalpies) were significantly correlated to free fatty acids (palmitic, oleic and linoleic acids), fatty acid composition (unsaturated/saturated fatty acid ratio and oleic/linoleic acid ratio), and oxidative stability indices. Positive correlation was observed between Peak 1 area, time range (width) and induction time and palmitic and linoleic acids ($p < 0.001$ and $p < 0.01$, respectively). Similarly, positive correlation was observed between Peak 1 width and induction time and POV ($p < 0.01$ and $p < 0.001$, respectively), while inverse relationship resulted between Peak 1 descriptors and oleic acid, unsaturated/saturated fatty acid ratio and oleic/linoleic acid ratio ($p < 0.001$ and $p < 0.01$).

The present results enlightened how the crystallization curve significantly shifted towards higher times and over longer time range depending on higher peroxide index and linoleic acid content. Similar evidences came from conventional scanning mode (DSC), although in that case the crystallization process was expressed as a function of temperature (Chiavaro et al., 2007, 2010; Tan & Che Man, 2000). It was reported that the shape of the crystallization curve generated by DSC can be influenced by the composition in free fatty acids and primary and secondary oxidation products (Angiuli et al., 2007; Chiavaro et al., 2010). High temperature onset and wide crystallization range were previously observed in DSC analysis of oils characterized by a high degree of fatty acid saturation (Tan & Che Man, 2000); while narrow range of crystallization was found in virgin olive oils with high oleic acid content (Márquez & Maza, 2003). Single fatty acids were found to influence melting kinetics and enthalpies, but melting process is a complex process in which other chemical components (e.g. TAGs and DAGs) are mainly involved (Chiavaro, Vittadini, et al., 2008). However, in the present work we wanted to test the role of parameters (free acidity, fatty acid composition, phenolic content and oxidation stability) that are mainly considered to assess EVOO's quality.

The outcome data showed that both endothermic Peak 2 and Peak 3 were correlated with palmitic (C16), stearic (C18), oleic (C18:1) and linoleic (C18:2) acids, although differences in the Pearson correlation coefficient (r) sign were observed (Table 4). As a result, an increase in palmitic and linoleic acids determined a reduction of Peak 2 size in favor of Peak 3. On the contrary, oleic acid content, unsaturated/saturated

Table 4
Correlation coefficients (r) between crystallization/melting features and chemical parameters of 18 EVOOs.

	Peak 1			Peak 2			Peak 3		
Chemical parameters	Area	Width	Induction time	Area	Width	Tmax	Area	Width	Tmax
POV	ns	0.63^{**}	0.74^{***}	-0.56^{*}	-0.51^{*}	-0.66^{**}	0.53^{*}	0.61^{**}	ns
Total phenols	ns	ns	ns	ns	ns	ns	ns	ns	ns
o-phenols	ns	ns	ns	ns	ns	ns	ns	ns	ns
OSI time	-0.49^{*}	ns	-0.55^{*}	ns	ns	0.78^{***}	ns	-0.49^{*}	ns
Free acidity	ns	ns	ns	ns	ns	ns	ns	ns	ns
Palmitic acid (C16)	0.80^{***}	0.81^{***}	0.83^{***}	-0.94^{***}	-0.91^{***}	-0.72^{***}	0.92^{***}	0.88^{***}	0.88^{***}
Stearic acid (C18)	ns	ns	ns	0.61^{**}	0.58^{*}	ns	-0.68^{**}	-0.68^{**}	-0.50^{*}
Oleic acid (C18:1)	-0.85^{***}	-0.79^{***}	-0.97^{***}	0.88^{***}	0.87^{***}	0.88^{***}	-0.84^{***}	-0.86^{***}	-0.72^{***}
Linoleic acid (C18:2)	0.65^{**}	0.62^{**}	0.93^{***}	-0.67^{**}	-0.70^{**}	-0.88^{***}	0.64^{**}	0.74^{**}	ns
Linolenic acid (C 18:3)	ns	ns	ns	ns	ns	ns	ns	ns	ns
Unsatured/satured fatty acids	-0.87^{***}	-0.75^{***}	-0.76^{***}	0.84^{***}	0.79^{***}	0.73^{***}	-0.80^{***}	-0.78^{***}	-0.77^{***}
Oleic/linoleic acid	-0.69^{**}	-0.63^{**}	-0.91^{***}	0.70^{**}	0.71^{**}	0.90^{***}	-0.65^{**}	-0.74^{**}	ns

*, **, ***: significant at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively; ns: non significant.

fatty acid ratio and oleic/linoleic acid ratio showed positive correlation with Peak 2 whole size, while negative relationship was observed between the above chemical parameters and Peak 3 descriptors. No significant correlations were observed between thermal properties and free acidity, linolenic acid, polyphenol and *o*-diphenol content. These results confirm previous evidences showing the absence of a direct role of phenolic compounds on the crystallization profile of EVOOs in the presence or absence of phenols (Cerretani et al., 2012; Chiavaro, Mahesar, et al., 2011). On the contrary, lipid oxidation products were markedly correlated with EVOO crystallization process (Calligaris, Sovrano, Manzocco, & Nicoli, 2006; Chiavaro, Mahesar, et al., 2011).

4. Conclusions

Authentication and traceability of extra virgin olive oils are highly requested by the market. However, the analytical methods mostly employed for this purpose require highly specialized personnel. We have optimized and applied a simplified, fast and reliable calorimetric method for the authentication of monovarietal EVOOs based on the use of MASC. The preliminary results here presented show that MASC can provide characteristic fingerprints of different monovarietal EVOOs. Compared to DSC, the presented method was based on a tailor-made calorimetric protocol and as major advantages offered rapid measurements and friendly outcome data. The use of glass capillary flame-sealed tubes, allowed forty times larger oil sample mass to test compared with DSC, thus increasing the instrumental sensitivity. The preliminary results here presented might suggest the practical use of MASC for the characterization of EVOOs by operators working along the olive oil supply chain.

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