PAPER

EFFECT OF THE SOIL NATURE ON SELECTED CHEMICO-PHYSICAL AND THERMAL PARAMETERS OF EXTRA VIRGIN OLIVE OILS FROM *CV* CHEMLALI

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ABSTRACT

The current study assessed the effect of different soils (clay, EVOOsC, stony, EVOOsS, brown, EVOOsB, limestone and gypsum, EVOOsLG) on the chemico-physical (free acidity, peroxide value, oxidative stability, fatty acids, pigments, colour, viscosity, heat capacity) and thermal (upon DSC) quality of four 'Chemlali' extra virgin olive oils. EVOOsC showed the lowest peroxide value, the highest amount of monounsaturated fatty acids and the narrowest crystallization range. EVOOsLG had the highest content of saturated and polyunsaturated fatty acids as well as the lowest stability to oxidation and melting enthalpy. EVOOsS and EVOOsB revealed the highest viscosity, while the heat capacity measurement didn't show any difference among the oils. EVOOsB exhibited the highest b^* colour parameter in relation to the highest carotenoids content. These preliminary findings have showed that the nature of the soil has an effect on the final quality of EVOO, as also revealed by the PCA analysis.

Keywords: composition, DSC, extra virgin olive oil, physical properties, soil

1. INTRODUCTION

EVOO is a traditional product of the Mediterranean countries. Among these the olive culture represents one of the main economic and agricultural strategic sectors in Tunisia. About 60 million olive trees are distributed and spread on 1.6 million hectares extended from the northern to the southern regions of the country. Tunisia contributes to more than 4% of the world olive oil production the 'Chemlali' being the most abundant olive variety (ISSAOUI *et al*., 2010). The chemical characterization of 'Chemlali' olive oils, as influenced by different either natural or human factors, was the object of several scientific studies (BEN HASSINE *et al*., 2014; HANNACHI *et al*., 2007; BEN HASSINE *et al*., 2015). Significant variability on fatty acids (FA) composition other than minor components such as polyphenols and/or pigments and oxidative stability was found.

Closely related to chemical composition, but less debated in literature, is the physical and thermal characterization of the oils, which could be considered of great interest for consumers and industries. The use of differential scanning calorimetry (DSC) has been mainly suggested as an alternative interesting approach for the characterization of the oils from different vegetable sources (TAN and CHE MAN, 2002) or for the evaluation of different oxidative stability (CHIAVARO *et al*., 2009; CHIAVARO *et al*., 2011; CHIAVARO *et al*., 2012) by means of the phase transition behaviour. Recently, DSC has also been applied for the characterization of Tunisian olive oils on the basis of the cultivar–environment interaction showing good correlations between thermal and chemical properties(KOTTI *et al*., 2009).

DSC can be also used for the determination of the heat capacity (Cp). SANTOS *et al*. (2005) reported an increase in the Cp of vegetable oils, measured by DSC, as a function of the fatty acids saturation. The measurement of viscosity along with the heat capacity provides useful information to optimize the equipment design (settling and storage tanks, centrifuges, pumps, etc.) thanks to the determination of their behaviour during different technological processes. The correlation between viscosity and chemical composition of vegetable oils was slightly mentioned only when, after prolonged heating, an increasing of the viscosity was observed in relation with the increased saturation of the oil constituents and the generation of different polymer compound classes (KALOGIANNI *et al*., 2011).

The colour is a basic criterion affecting the consumer preference although the European Union regulations do not require its measurement for an assessment of the virgin olive oil quality. Olive fruits contain two main classes of pigments that are transferred to the virgin olive oil during the extraction process: the green chlorophylls and the yellow and orange carotenoids. They can be used as indicator of the olives genetic make-up other than on the habitat where the olive trees are grown(CERRETANI *et al*., 2008).

It is well-known that the unique nutritional and organoleptic properties of the olive oil are closely related to olives genotype other than agronomic, environmental and technological factors (GARCÍA-GONZÁLEZ *et al*., 2010). Among these factors, the effect of the soil nature on the chemical composition of the oils is barely debated and often just regarded to as a combined effect with other factors (BEDBABIS *et al*., 2015; PAPADIA *et al*., 2011; ROMERO *et al*., 2015). Moreover, to the authors' best knowledge, no study on the impact of the soil nature on physical and thermal properties of olive oils has been reported in literature so far. This literature gap has led us to perform a complete characterization, in terms of composition (fatty acids, quality parameters, main pigments), oxidative stability, physical (viscosity, colour, heat capacity) and thermal properties, of four monovarietal 'Chemlali' EVOOs, differing only in the nature of the cultivation soils being cultivated in the same area under the same agricultural conditions and harvested in the same season at the same ripening index.

2. MATERIALS AND METHODS

2.1. Oil samples preparations

EVOO samples were obtained from fruits of the main Tunisian olive cultivar, 'Chemlali', which were picked by hand at the same stage of maturity, according to the IOOC (2004) classification, from three trees, during the crop season 2012/ 2013 (October), placed in 4 locations with different soil nature: clay (EVOOsC), stony (EVOOsS), brown (EVOOsB), limestone and gypsum (EVOOsLG), located in Sousse center of Tunisia (35°.49′ N, 10°.30′ E). Olive trees were subjected to identical fertilisation regime and to all common olive cultivation practices. The same laboratory mill was used to prepare the olive oil samples. Only healthy fruits without any kind of infection or physical damage were processed. After harvesting, fresh olives (1.5-2.0 kg) were washed and defoliated, smashed with a hammer crusher and then paste mixed at 25°C for 30 min, centrifuged without addition of warm water (oil produced from each extraction was 200-250 mL/kg) and then transferred into dark glass bottles and stored in the dark at 4°C until analysis. Three samples of each oil were analysed and triplicate analyses were carried out for each sample.

2.2. Chemical analysis

Free acidity, expressed in oleic acid (18:1) percentage, and peroxide value (POV), given as milli-equivalents of active oxygen per kilogram of oil ($meqO_2/kg$) were determined according to the analytical methods described in the European Union Commission Regulations (EEC/2568/91; EEC/1429/92).
Oxidative stability (OSI) was evaluated by the Rancimat method (GUTIÉRREZ ROSALES *et al*., 1989). Stability was expressed as the oxidation induction time (h), measured with the Rancimat 743 apparatus (Metrohm, Herisau Switzerland), using an oil sample of 3.6 g. The oil temperature was 101.6°C and the air flow was 10 L/h.
Chlorophyll and carotenoid contents were determined colorimetrically, as previously described (MINGUEZ-MOSQUERA *et al*., 1991). Briefly, the method consists in quantitatively assessing the chlorophyll fraction by measuring the absorbance of the olive oils at 670 nm and the carotenoid fraction at 470 nm through the use of appropriate molar absorption coefficients. The results were expressed in g/kg.
The fatty acids (FA) were converted to fatty acid methyl esters before analysis by shaking a solution of 0.2 g oil and 3 mL of hexane with 0.4 mL of 2-N methanolic potassium hydroxide, and analyzed using a Hewlett-Packard (HP 4890D; Hewlett-Packar Company, Wilmington, DE) chromatograph equipped with a capillary column (Supelcowax: 30 m × 0.53 mm; 0.25 mm), a split/splitless injector and a flame ionization detection (FID) detector. The carrier gas was nitrogen at a flow rate of 1 mL/min. The temperatures of the injector, the detector and the oven were held at 220, 250 and 210°C, respectively. The injection volume was 1 μL. The results are expressed as relative area percentage of total FAs.

2.3. Thermal analysis

EVOO samples (8-10 mg) were weighed into aluminium pans, covers were sealed into place and the whole analyzed with a DSC Q100 (TA Instruments, New Castle, DE). Indium (melting temperature 156.6°C, ΔH_f= 28.45 J/g) and *n*-dodecane (melting temperature -9.65°C, ΔH_f= 216.73 J/g) were used to calibrate the instrument and an empty pan was used as reference. Oil samples were equilibrated at 30°C for 8 min and then cooled at -80°C at the rate of 2°C/min, equilibrated at -80°C for 8 min and then heated

from -80 to 30°C at 2°C/min. Dry nitrogen was purged in the DSC cell at 50 cm^3/min. DSC curves were analysed with Universal Analysis Software (Version 3.9A, TA Instruments) to obtain enthalpy change for transition (ΔH, J/g), onset temperature of transition (Ton,°C), offset temperature of transition (Toff,°C) and peak temperature at the maximum (Tp) for the two main events of cooling and heating transitions (Tp1 and Tp2,°C). Range of transition was calculated as the temperature difference between Ton and Toff. Heat flow (W/g) of the main peak (p1) of both cooling and heating was also calculated.
Heat capacity (Cp, J/g°C) measurements were taken with a DSC Q100 (TA Instruments, New Castle, DE). Each determination was performed in triplicate according to the combination of two methods (ASTM E1269-05; HEIDENREICH *et al*., 2007). According to the method (Minguez-Mosquera *et al*., 1991) three individual DSC experiments referred to as: baseline, reference, and sample were performed using the same procedure: equilibration at 5°C; isothermal for 15 min; ramp at 5°C/min up to 300°C; isothermal for 20 min. For determination of the heat capacity calibration constant E(T), the heat capacity of the sapphire ($c_{p,s}$), the mass of the sapphire (m_s) and the temperature-dependent heat flow of sapphire (Q_s) and of the empty pan (Q_E) were considered in Eq.1.

$$E(T) = \frac{c_{p,s}\dot{q}m_s}{60[\dot{Q}_S(T) - \dot{Q}_E(T)]} \qquad \text{Eq. 1}$$

The constant E(T) was then used to calculate the heat capacity of the sample by using the appropriate heat flow and mass (Q_c and m_c, respectively).

$$c_p = \frac{60E(T)[\dot{Q}_c(T) - \dot{Q}_E(T)]}{\dot{q}m_c} \qquad \text{Eq. 2}$$

2.4. Viscosity measurements

Measurements were made by means of a concentric cylinder Brookfield® DV-I Prime rotational viscosimeter (Brookfield, Middleboro, Massachusetts, USA). Data capture was obtained connecting the viscosimeter to a computer, monitoring rotation per minutes (RPM) and the apparent viscosity (Centipoise) at 1s intervals. Control of temperature was obtained connecting the jacket of the measuring cell to a water bath whose temperature was checked at 25°C±0.5. Two cylindrical spindles were used for the rheological analyses. The ULA spindle (Brookfield, Middleboro, MA, USA) (viscosity range from 0.06 to 2000 mPa s) and the SC4-18/13R spindle (Brookfield, Middleboro, MA, USA)(viscosity range from 0.3 to 9998 mPa s). Rheological behaviour was described in terms of viscosity (mPa s) at various shear rates (dv/dy). Since the apparatus measures the viscosity as a function of spindle RPM, shear rate values (1/s) were obtained multiplying RPM by a specific constant for every spindle (e.g.: 1.224 for Ultra Low Adapter, and 1.32 for the spindle SC4-18 of the Small Sample Adapter). The viscosity (μ) value was obtained from the Newton's law

$$\sigma = \mu\dot{\gamma} \qquad \text{Eq. 3}$$

where σ is shear stress (mPa), $\dot{\gamma}$ is the shear rate (1/s) and μ is viscosity (mPa s).

2.5. Instrumental colour

The software ImageJ, v.1.38x, fitted with the plugin Color Inspector 3D v. 2.3, was used to assess the oil colour applying the CIELAB colorimetric system. Each time 20 ml of samples

were put into a glass Petri dish. The images of each Petri dish were acquired with a scanner (Hewlett Packard, Palo Alto, CA, USA) at 600 dots per inch (dpi). The colour brightness coordinate L^* measures the whiteness value of a colour and ranges from black at 0 to white at 100. The chromaticity coordinate a^* measures red when positive and green when negative, and chromaticity coordinate b^* measures yellow when positive and blue when negative (MOYANO *et al*., 2008).

2.6. Statistical analysis

Means and standard deviations were calculated with SPSS (version 22.0 SPSS Inc., Chicago, IL, USA) statistical software. SPSS was used to perform one-way analysis of variance (ANOVA) and Tukey's honest significant difference test (HSD) at a 95% confidence level ($p<0.05$) to identify differences among samples. Pearson correlation coefficients were calculated among the variables at a 95% and 99% confidence levels ($p<0.05$ and $p<0.01$). Principal component analysis (PCA) was also performed by means of Statistica software (version 8.0, Stat-Soft, Tulsa, OK, USA). PCA has been used as descriptive statistical technique plotting the selected vectors (independent variables) versus all cases (samples) with the aim to find relationships among the variables, able to describe differences among the four cases.

3. RESULTS AND DISCUSSION

3.1. Chemical analysis

3.1.1 Quality parameters, oxidative stability and main pigment content

Chemical quality parameters and oxidative stability of the four samples are shown in Table 1.

Table 1. Chemical quality parameters of the EVOO samples.

	EVOOsC	EVOOsS	EVOOsB	EVOOsLG
Free acidity (%)	0.70±1.00^a	0.75±0.05^a	0.55± 0.15^a	0.70±0.01^a
POV ($meqO_2$/Kg)	2.5±0.50^c	4.5±0.51^b	4.5±0.50^b	10.5±0.49^a
OSI (h)	15.58±0.41^b	14.84±0.15^c	18.01±0.16^a	3.34±0.27^d
Chlorophylls (mg/kg)	3.48±0.09^b	3.01±0.22^b	4.32±0.10^a	4.68±0.44^a
Carotenoids (mg/kg)	1.27±0.00^b	1.20±0.11^b	1.83±0.02^a	1.60±0.12^a

Data are expressed as mean±standard deviation of three determinations ($n = 3$, $p<0.05$). Different letters in the same row are statistically different ($p<0.05$). EVOOsC, extra virgin olive oil from soil clay, EVOOsS extra virgin olive oil from soil stony, EVOOsB, extra virgin olive oil from soil brown, EVOOsLG extra virgin olive oil from soil limestone and gypsum.

The free acidity value (5.5-7.5 g/kg) was lower than the legal limit for the commercial category of extra virgin olive oil in all the samples (ECC/61/2011) and it does not seem to be affected by the nature of soil. Similarly other authors didn't find any difference in the free acidity on 'Chemlali' olive oils obtained from olives cultivated on soils irrigated with

different quality waters (BEDBABIS *et al*., 2015) or from different geographical origins (BEN HASSINE *et al*., 2014). The peroxide value (POV), on the other hand, showed (Table 1) significant differences among the four 'Chemlali' EVOO according to the nature of the soil. Among all the samples, EVOOsC showed the lowest POV value (2.5 meqO_2/kg), while EVOOsLG had the highest one (10.5 meqO_2/kg) (Table 1); however they were all below the legal limit of 20 meqO_2/kg of oil for extra virgin olive oil (EEC/2568/91). Contradictory results were previously reported in literature according to this quality parameter in EVOO. BEDABIS *et al*. (2015) didn't find any difference among 'Chemlali' EVOOs obtained from olives irrigated with different types of water. Other authors (ISSAOUI *et al*., 2010; BEN HASSINE *et al*., 2014) found differences comparing 'Chemlali' olive oils belonging to different geographical sites in Tunisia. The oxidative stability (OSI) of the 'Chemlali' samples turned out to be influenced by pedologic conditions as well significant differences among the four oils (Table 1) have been observed and EVOOsB being the most stable (18 h). In accordance with the highest POV values EVOOsLG reported the lowest oxidative stability (3h). In general, the oxidative status and the stability to the oxidation of an olive oil depend on the oil composition, both in terms of lipids profile and micro-components (BEN MANSOUR *et al*., 2015). The oil composition is demonstrated to be itself affected by several environmental factors (ISSAOUI *et al*., 2010; BEN HASSINE *et al*., 2014; HANNACHI *et al*., 2007), including the soil nature (PAPADIA *et al*., 2011).
As shown in Table 1, the oils pigments content seems to be influenced by the nature of the soils, showing significant differences among the samples. The chlorophyll content was in the range 3.0-4.7 mg/kg with EVOOsLG and EVOOsS as the richest and the poorest samples, respectively. The same trend was observed for the carotenoids, being however in the concentration range of 1.2-1.8 mg/kg (Table 1). Several authors reported different pigments concentration in 'Chemlali' olive oils as affected by the growing area in Tunisia (ISSAOUI *et al*., 2010; BEN HASSINE *et al*., 2014; BEN MANSOUR *et al*., 2015) being however in the same order of magnitude of this study. BEDBABIS *et al*. (2015) reported different pigments contents in 'Chemlali' EVOOs obtained from olives irrigated with different types of water. These authors outlined that the pigments content of the olive oils is influenced by the olives ripening, being the latter itself influenced by the salinity of the soil.

3.1.2 Fatty acid composition

The fatty acids (FA) composition of the four 'Chemlali' EVOO is shown in Table 2.
Oleic, linoleic and palmitic acids were the main FA present in the samples being oleic the main abundant compound. The FA distribution was within the range expected for high quality olive oils ECC/61/2011. The FA composition found in this study traced the ones found for extravirgin 'Chemlali' oils from other authors (ISSAOUI *et al*., 2010; BEN HASSINE *et al*., 2015; BEN MANSOUR *et al*., 2015), with differences attributable to the environmental factors. As summarized in Table 2, EVOOsLG exhibited the lowest C18:1 content (471.6 g/kg) and the highest amount of C16:0 and C18:2 (206.6 and 256.4 g/kg, respectively). On the contrary, EVOOsC showed significantly highest content of C18:1(610.5 g/kg) and lowest of C16:0 and C18:2 (180.6, 158 g/kg, respectively). Stearic acid content did not show a significant difference among samples, showing concentration around 20 g/kg. EVOOsS and EVOOsB exhibited a very similar composition and intermediate between the ones of the other two oils. While EVOOsLG exhibited the highest amount of SFA and PUFA other than the lowest amount of MUFA, EVOOsC had the highest content of this fatty acid category, among all. In particular, MUFA correlated positively with OSI ($p \leq 0.01$, $R=0.738$) and negatively with POV ($p \leq 0.01$, $R= -0.922$),

inversely PUFA correlated positively with POV ($p \leq 0.01$, $R=0.948$) and negatively with OSI ($p \leq 0.01$, $R= -0.780$). Similarly, BEN HASSINE *et al*. (2015) associated the oxidative stability of an olive oil to the value of the oleic/ linoleic acids ratio. Interestingly, PAPADIA *et al*., (2011) found significant correlations between the amount of oleic and linoleic acids in mono-varietal extra virgin olive oils and the concentration of B, Cr, Mn and Zn in the cultivation soil.

Table 2. Main fatty acid composition (%) of the EVOO samples.

	EVOOsC	EVOOsS	EVOOsB	EVOOsLG
C16:0	18.06±0.52^{c}	20.40±0.30^{ab}	19.77±0.02^{b}	21.07±0.43^{a}
C16:1	2.27±0.07^{c}	3.13±0.11^{ab}	2.88±0.01^{b}	3.40±0.16^{a}
C18:0	1.99±0.06^{a}	2.00±0.07^{a}	1.99±0.02^{a}	1.91±0.05^{a}
C18:1	61.05±0.53^{a}	53.66±0.30^{b}	53.65±0.08^{b}	47.16±0.75^{c}
C18:2	15.80±0.05^{d}	20.14±0.07^{c}	20.66±0.08^{b}	25.64±0.20^{a}
C18:3	0.82±0.02^{a}	0.67±0.02^{b}	0.85±0.03^{a}	0.82±0.01^{a}
SFA	20.05±0.52^{c}	22.40±0.24^{ab}	21.95±0.02^{b}	22.98±0.38^{a}
MUFA	63.32±0.46^{a}	56.79±0.19^{b}	56.53±0.08^{b}	50.57±0.59^{c}
PUFA	16.63±0.07^{d}	20.80±0.08^{c}	21.51±0.11^{b}	26.45±0.21^{a}

Data are expressed as mean ±standard deviation of three determinations ($n = 3$, $p<0.05$). EVOOsC, extra virgin olive oil from soil clay, EVOOsS extra virgin olive oil from soil stony, EVOOsB, extra virgin olive oil from soil brown, EVOOsLG extra virgin olive oil from soil limestone and gypsum. FA, fatty acids; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturatedfatty acids. (C16:0) palmitic, (C16:1) palmitoleic, (C18:0) stearic, (C18:1) oleic, (C18:2) linoleic, (C18:3) linolenic.

3.2. Thermal analysis

3.2.1 Cooling curves

The DSC cooling curves of the four 'Chemlali' EVOO (Fig. 1a) were similar to that previously reported for extra virgin olive oils (CHIAVARO *et al*., 2010) and those of 'Chemlali' samples in particular (KOTTI *et al*., 2009).
The DSC cooling curves of the olive oils are well known to be simply influenced by the chemical composition of the oils (TAN and CHE MAN, 2000). All the cooling profiles (Fig. 1a) showed two well distinguishable exothermic events. In all the samples, the major event (Peak 1 of Fig. 1a), at lower temperature (~ -45°C), showed a symmetrical line shape, indicating a highly cooperative and more ordered transition due to the homogeneity of the crystallizing molecules; the minor exothermic transition (Peak 2 of Fig. 1a), (~ -10°C) showed an asymmetrical line shape, suggesting a more heterogeneous crystallization process (TAN and CHE MAN, 2000). The major exothermic event was previously attributed to the crystallization of TAG rich in oleic acid, while the other was attributed to the crystallization of the more saturated TAG (TAN and CHE MAN, 2000; TAN and CHE MAN, 2002; CHIAVARO *et al*., 2010). Besides, a less defined exothermic event was also quite evident appearing as a shoulder of the major peak.

The thermal parameters extrapolated from the cooling thermograms (Table 3) exhibited differences among the four studied EVOOs, probably attributable to the nature of the soil on which they were grown.

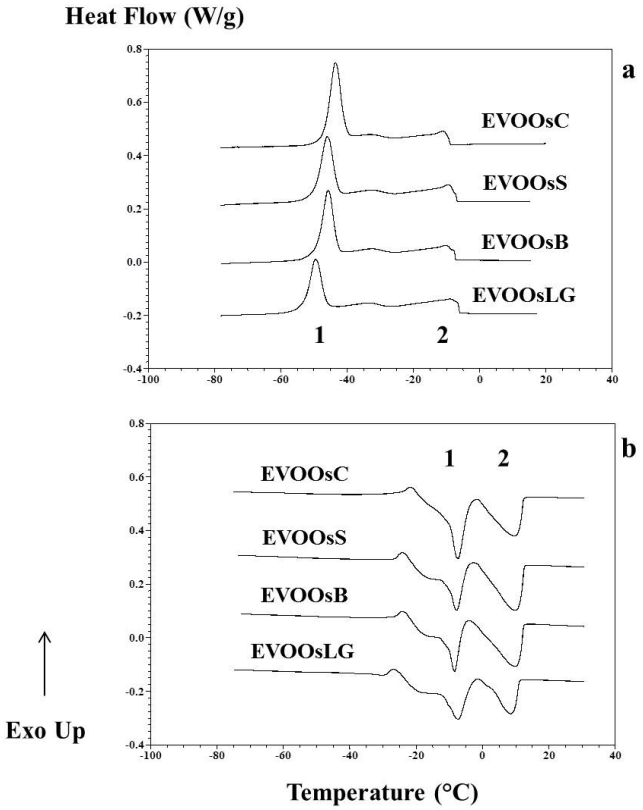

Figure 1. Cooling a) and heating b) curves of the EVOO samples. The main transitions are indicated with number 1 and 2. EVOOsC, extra virgin olive oil from soil clay; EVOOsS extra virgin olive oil from soil stony; EVOOsB, extra virgin olive oil from soil brown; EVOOsLG, extra virgin olive oil from soil limestone and gypsum.

Very different values were found for EVOOsC and EVOOsLG, while similar parameters were registered for EVOOsB and EVOOsS, intermediate from the other two. For EVOOsC, the major exothermal peak (Fig. 1a-Peak 1) resulted as the tallest among all the samples, as visible from the significant highest heat flow (Table 3). This observation could be related to the highest content of MUFA (CHIAVARO *et al*., 2010), in particular oleic acid, of this olive oil (Table 2). Moreover, it is reported that olive oils rich in oleic acid and MUFA, crystallized at lower temperature than samples rich in palmitic acid and SFA (CHIAVARO *et al*., 2010), as confirmed from our results (Tables 2-3). The minor exothermal event (Fig. 1a-peak 2) of EVOOsLG was peaked at higher temperatures (Table 3) than the other oils. This may be related to the significantly higher degree of lipid saturation (SFA content) of EVOOsLG (Table 2), as already reported for olive oil (CHIAVARO *et al*., 2010; KOTTI *et al*., 2009). EVOOsLG and EVOOsC exhibited respectively the broadest and the narrowest range of transition (Table3). Narrow range of transition has been associated to high oleic virgin olive oil (JIMENEZ MARQUEZ *et al*., 2003). About this, a high statistical correlation was found between the oleic acid content and the range of crystallization ($p \leq 0.01$; $R = -0.964$). Moreover high statistical correlations were also found between the range of crystallization and oxidative status of the oils by means of peroxide values ($p \leq 0.01$; $R = -0.912$) and oxidative stability ($p \leq 0.01$; $R = 0.797$). VITTADINI *et al*. (2003) reported that the

broadening of the crystallization phenomena is one of the changes related to the formation of lipid oxidation products that could form mixed and disordered triglyceride crystals. These crystals require lower energy to crystallize, and retard the occurrence of phase transition. The crystallization enthalpy on the other hand was not able to discriminate among the four EVOO according to the nature of the soil (Tab.3), as already observed for different 'Chemlali' EVOO from different geographical origin (KOTTI *et al*., 2009).

Table 3. DSC data obtained from the cooling and heating thermograms of the different samples.

	Enthalpy (J/g)	T_{on} (C°)	T_{off} (C°)	Range (C°)	Heat Flow (W/g)	T_{p1} (°C)	T_{p2} (°C)
Cooling							
EVOOsC	73.46±0.34 a	-8.83±0.32 c	-52.95±0.67 a	44.12±0.83 c	0.37±0.00 a	-43.84±0.45 a	-11.36±0.07 c
EVOOsS	74.72±2.38 a	-6.79±0.14 b	-56.79±0.58 b	50.00±0.67 b	0.32±0.02 bc	-46.38±0.45 b	-9.60±0.51 b
EVOOsB	77.52±1.81 a	-7.21±0.00 b	-55.70±0.29 b	48.49±0.29 b	0.36±0.02 ab	-45.51±0.34 b	-10.12±0.16 b
EVOOsLG	75.30±4.30 a	-5.88±0.08 a	-60.77±1.00 c	54.89±1.00 a	0.28±0.01 c	-49.67±0.29 c	-8.79±0.09 a
Heating							
EVOOsC	83.78±1.56 a	-24.75±0.22 a	13.31±0.08 a	38.06±0.17 c	0.30±0.01 c	-7.11±0.63 a	9.75±0.07 a
EVOOsS	76.20±3.82 ab	-27.31±0.22 b	13.07±0.16 a	40.38±0.25 b	0.25±0.01 ab	-7.32±0.79 ab	9.81±0.09 a
EVOOsB	79.54±2.08 a	-27.26±0.14 b	12.74±0.08 b	40.00±0.08 b	0.28±0.01 bc	-8.49±0.14 b	9.90±0.08 a
EVOOsLG	70.76±4.23 c	-30.25±0.14 c	11.79±0.16 c	42.04±0.21 a	0.24±0.01 a	-7.11±0.25 a	8.52±0.08 b

Data are expressed as mean±standard deviation of three determinations (n = 3, $p<0.05$). Same letters within each column do not significantly differ. Range(C°): Temperature difference between T_{on} and T_{off}; Heat Flow (W/g): value measured on the peak 1. EVOOsC, extra virgin olive oil from soil clay, EVOOsS extra virgin olive oil from soil stony, EVOOsB, extra virgin olive oil from soil brown, EVOOsLG extra virgin olive oil from soil limestone and gypsum.

3.2.2 Heating curves

The heating curves of the four EVOO (Fig. 1b) showed shapes similar to those reported in literature for 'Chemlali' samples (KOTTI *et al*., 2009). The thermal properties during heating were previously found to be largely influenced by difference in macro-components (FA) (CHIAVARO *et al*., 2009), despite those curves appeared to be more complex than cooling ones, due to the polymorphism of oils and fats (TAN and CHE MAN, 2000). Multiple exo-endothermic events were observed for all the studied samples (Fig. 1b). A first exothermic peak, visible around -25°C could be attributed to the crystallization of the minor components that did not solidify under the cooling regime used in this study and/or to a solid–solid polymorphic transformation resulting from the rearrangement of a portion of the crystals formed during cooling (BARBA *et al*., 2013). The following increase of temperature caused two major endothermic events: the melting of the unsaturated components (PUFA, MUFA) (Fig. 1b-peak1), at lower temperatures (~ -7.5°C), followed by that of the SFA ones (Fig. 1b-peak2), at higher temperatures (~ 9.5°C), as previously reported (JIMENEZ MARQUEZ *et al*., 2013). Peak 1 exhibited a quite different profile between the samples (Fig. 1b). It appeared more symmetric for EVOOsC,

probably in relation to the highest content of oleic acid and thus, to a more cooperative transition. The presence of a small endothermic event, as a shoulder of the major endothermic peak, was also observed in all the samples (~ -20°C), and with a lower intensity for EVOosC. It is generically attributed to the melting of lowest stables polymorphic forms of TAG (e.g. α) (BARBA *et al*., 2013).
As shown in Table 3, the thermal parameters of heating thermograms showed significant difference among the studied 'Chemlali' EVOO, according to the different nature of the soils. Heating enthalpy values were significantly different among samples; EVOOsC exhibited the highest value and EVOOsLG showed the lowest one. Positive correlations between heating enthalpy and oleic acid were found ($p \leq 0.01$; $R = 0.829$) in this study, as also previously observed on EVOO (ILYASOGLU *et al*., 2011). EVOOsC exhibited the highest and EVOOsLG the lowest T_{on} of heating. EVOOsLG moreover had a significant lowest T_{off} than the other oils, in relation to the high instauration of its FA profile, as previously suggested (KOTTI *et al*., 2009). Finally, EVOOsB and EVOOsS presented similar values for mostly of the heating thermal properties, probably in relation with the similarity shown in their chemical composition.

3.2.3 Heat capacity

The variation of the heat capacity as a function of temperature, in the range 26.85 – 296.85°C, is reported in Fig. 2 for all the samples.

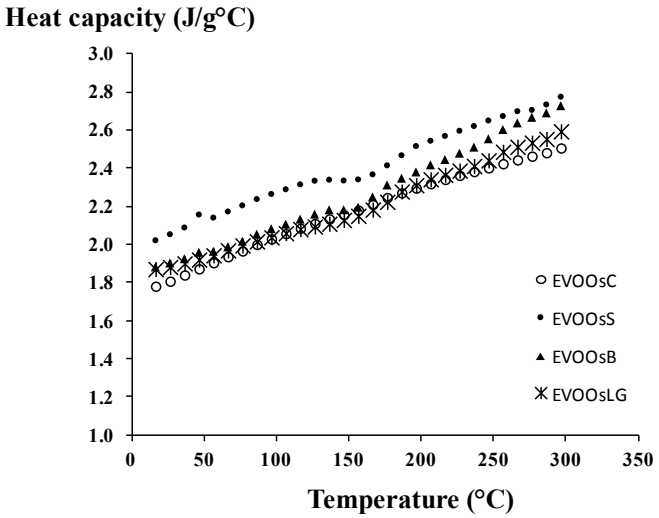

Figure 2. Heat capacity as a function of temperature (16.85 – 296.85°C) for the four EVOO samples. EVOOsC, extra virgin olive oil from soil clay; EVOOsS extra virgin olive oil from soil stony; EVOOsB, extra virgin olive oil from soil brown; EVOOsLG, extra virgin olive oil from soil limestone and gypsum.

The values obtained at 50°C were in accordance with those reported in literature for olive oil and calculated with the same method (1.99 ± 0.02)(HEIDENREICH *et al*., 2007). A linear increase of the heat capacity was observed with temperature, showing coefficient of linearity (R^2) of 0.9943, 0.9876, 0.9894 and 0.9905 for EVOOsC, EVOOsS, EVOOsB and EVOOsLG respectively.
The values obtained, by means of interpolation, at room temperature (25°C) are summarized in Table 4 ranging from 2.1 to 1.8 (J/gC°) for EVOOsS and EVOOsC

respectively, without any significant differences. Thus, the measurement of the heat capacity seems to not discriminate among the EVOO according to the nature of the soil.

3.3. Physical analysis

3.3.1 Viscosity

Edible oils are almost all Newtonian liquids (KALOGIANNI *et al*., 2011). The linear relationship of shear stress to shear rate found in this study (data not shown) indicates that all the vegetable oil samples in the tested shear rate conditions exhibited a Newtonian behaviour. The viscosity value at room temperature (25°C) was therefore obtained from the fitting of the slope of experimental shear stress-shear rate data to the Newton's law of viscosity equation (Eq. 1)(FASINA *et al*., 2008) and reported in Table 4.

Table 4. Physical quality parameters of EVOO samples.

	EVOOsC	EVOOsS	EVOOsB	EVOOsLG
Viscosity (mPa s)	59.21±0.12^{b}	61.58±0.24^{a}	61.79±0.19^{a}	59.24±0.12^{b}
Heat capacity (J/gC°)	1.81±0.07^{a}	2.06±0.18^{a}	1.89±0.07^{a}	1.88±0.09^{a}
Colour				
*L**	57.67±1.15^{a}	57.67±0.58^{a}	56.00±0.00^{a}	57.67±0.58 ^{a}
*a**	-3.67±0.58^{a}	-5.00±0.00^{b}	-5.33±0.61^{b}	-3.33±0.59^{a}
*b**	14.33±1.53^{c}	21.00±0.00^{b}	41.0±2.65^{a}	14.0±1.73^{c}

Data are expressed as mean standard deviation of three determinations. Different letters in the same row are statistically different ($p<0.05$). EVOOsC, extra virgin olive oil from soil clay, EVOOsS extra virgin olive oil from soil stony, EVOOsB, extra virgin olive oil from soil brown, EVOOsLG extra virgin olive oil from soil limestone and gypsum.

The values of viscosity found in this study are in accordance to that reported from other authors (GILA *et al*., 2015; BONNET *et al*., 2011) on virgin olive oils at room temperature. EVOOsB and EVOOsS showed values of viscosity (61.6 and 61.8 mPas respectively) significantly higher than that of EVOOsC and EVOOsLG (59.2 mPas for both). Some authors reported slight but significant correlation between viscosity and olive oil composition (GILA *et al*. 2015). GILA *et al*. (2015) observed an increase of viscosity increasing the oleic acid content of virgin olive oils from different varieties. BONNET *et al*. (2011) affirmed however that the intravarietal variance of the fatty acid and TAG compositions is much smaller than the intervarietal variance, therefore viscosity differences should not be visible. Thus, the differences found in this study may be ascribable to other factors influencing the final composition, such as the oxidative status, as effect of the soil nature. In support of this hypothesis, a correlation was found in this study between viscosity measurement and the oxidative stability of the oils ($p\leq0.05$ $R=0.617$). It is reported that lipid oxidation products may weak and/or hinder intermolecular bonding between TAG molecules leading to the increase of the oil viscosity (VITTADINI *et al*., 2003).

3.3.2 Instrumental colour

The colour of the oils showed significant differences related to the nature of the soil, except for *L** (Table 4), which was found to be not statistically different among the samples, even if the lowest value was registered for the EVOOsB, the sample richest in carotenoids. In general, *L** is reported to increase with the reduction of the pigment content in the oils, as pigments would capture part of the light, instead of transmitting it (CERRETANI *et al*., 2008). The values of *a**, ranging from -3.3 to -5.3, described a green colour for all the samples. It resulted significantly higher for EVOOsC and EVOOsLG than for the other two oils. The values of *b**, representing the yellow colour, ranged from 41 to 14. EVOOsB resulted the most yellow among all the samples in relation with the highest content of carotenoids, as already reported (MOYANO *et al*., 2008). Two Pearson's correlations were also found between the chlorophylls/carotenoids ratio and the chromatic coordinates *a** ($p<0.01$, $R=0.841$) and *b** ($p<0.01$, $R= -0.828$), confirming the function of the pigments on the color of the studied olive oils, as affected by the soil nature.

3.4. PCA analysis

Based on previous works (CAPONIO *et al*., 2013; CHIAVARO *et al*., 2013), in which PCA analysis was successfully performed to discriminate among olive oils according to different refined steps or oxidative status by means of the correlation among thermal and chemical properties, it was again proposed in this study to tentatively discriminate among the four EVOO according to the nature of the soils. Twenty one variables were selected after factor extraction using as selection criteria loading values higher than 0.7, with PC1 and PC2 being the first two principal components that explained about 85% of the total variance. In Fig. 3a, the projection of the variables on the factor plane is reported.
All the DSC thermal properties, except the cooling enthalpy, were represented on the plane and better described by the PC1. Most of the DSC thermal properties showed positive factor loadings on PC1, being instead negative only for onset temperature of cooling (T_{on} cooling) and peak 2 cooling temperature at the maximum (T_{p2} cooling). As far as the chemical properties are concerned, positive factor loadings on PC1 were observed for MUFA and OSI, being instead negative for SFA, PUFA and POV. Few variables were represented on PC2, having this component a low contribution on the total results, as revealed from the high distance of the vectors from the boarders if compared to the ones on PC1. Positive factor loading on PC2 were showed by viscosity, carotenoids and colour parameter *b**, being instead negatives for *L**, *a** and peak 1 heating temperature at the maximum (T_{p1} heating). The heat capacity was not able to discriminate among the four EVOOs, thus it was excluded from the factor analysis.
The score plot obtained for the samples was shown in Fig. 3b. PC1 clearly divided EVOOsLG and EVOOsC in different clusters, having the first negative scores and the second positive ones. EVOOsS showed an intermediate behavior among all the samples, being instead EVOOsB better described by PC2 with positive scores. By comparing the score with loading plots it is evident how EVOOsLG and EVOOsC were well discriminate by the thermal parameters, being themselves influenced by the fatty acids composition and the oxidative stability. In particular, EVOOsC, the EVOO richest in MUFA, resulted well discriminated by the thermal properties of the cooling and heating major peak, confirming previous correlation found among this peaks with this class of fatty acids (CHIAVARO *et al*., 2007).

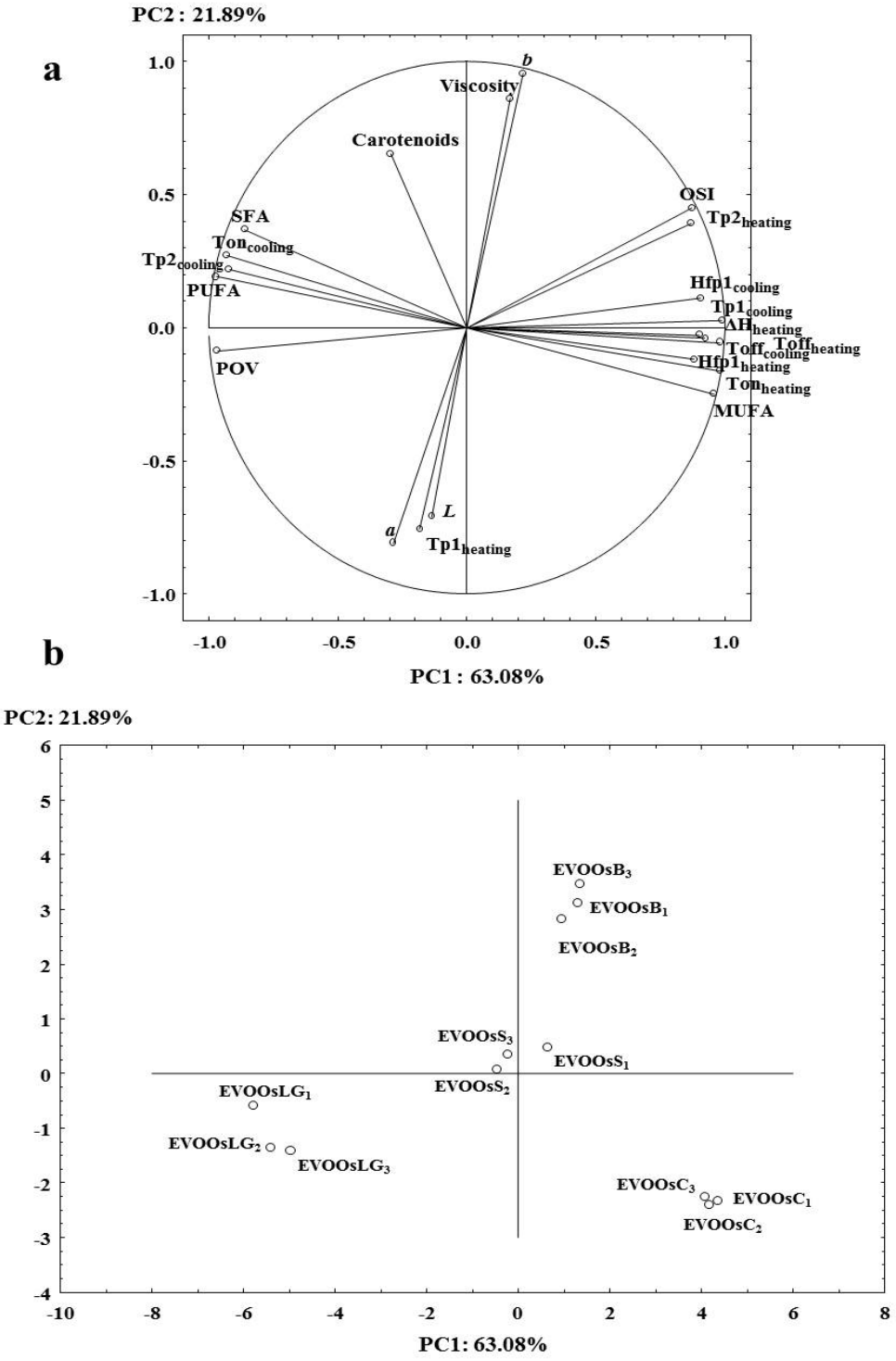

Figure 3. Principal Component Analysis (PCA) results obtained for the two principal components, showing a) the projection of the cases on the factor plane, and b) the projection of the variables on the factor plane. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; OSI, oxidative stability; POV, peroxide value; *T*on, onset temperature of transition; *T*off, offset temperature of transition; Hfp, peak heat flow at the maximum; *T*p, peak temperature at the maximum; Δ*H*, enthalpy change of transition; EVOOsC, extra virgin olive oil from soil clay; EVOOsS extra virgin olive oil from soil stony; EVOOsB, extra virgin olive oil from soil brown; EVOOsLG extra virgin olive oil from soil limestone and gypsum.

EVOOsLG on the other hand, with its high content of SFA and PUFA and the related POV value, was well described by the cooling parameters of the thermal event peaking at higher temperatures: T_{p2} and T_{on} of cooling, having also an inverse relation with the heating enthalpy. EVOOsS and EVOOsB had an intermediate composition in comparison to the other two oils, thus they showed intermediate values on PC1. EVOOsB was better discriminated on PC2 particularly from the viscosity value and from the b^* colour parameter, being the latter related to the carotenoid content, as observed in this study and already reported in literature (MOYANO *et al*., 2008).

4. CONCLUSIONS

The current study has investigated for the first time how the soil may impact on different quality parameters of four 'Chemlali' extra virgin olive oils considering physical and thermal variables other than the traditional chemical parameters. The PCA analysis helped in identifying the variables able to discriminate among the four studied EVOOs. EVOOsC and EVOOsLG resulted better discriminated by their chemical composition and thus by the related thermal properties. In particular, EVOOsC resulted as the sample with the highest MUFA content, exhibiting the highest heat flow of the first cooling peak and the narrowest cooling range in relation with lowest POV. EVOOsLG was the richest in SFA and PUFA showing the lowest heating enthalpy and the highest cooling and heating ranges together with the lowest OSI value. EVOOsB was distinguished on the base of its colour, being more yellow than the others, as revealed from the b^* parameter in relation to the highest carotenoid content, and having also the highest OSI value. EVOOsS showed intermediate properties to the other samples.
These first encouraging results have revealed the effective influence of the soil on chemico-physical and thermal properties of 'Chemlali' EVOOs which need further confirmation through the analysis of a larger set of samples to sort out the best pedologic conditions for the olive cultivation. Their relation with the soil composition should be also considered in the future.

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