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Comparative investigation of minerals, chlorophylls contents, fatty acid composition and thermal profiles of olive leaves (*Olea europeae* L.) as by-product

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SUMMARY: This work presents a chemical (the minerals, chlorophyll contents and fatty acids) and thermo-physical investigation (DSC profile) of four varieties of olive leaves grown in Tunisia. The total chlorophy11 contents of olive leaves ranged from 1132.33 to 1795.93 ppm. The results showed that linolenic acid ($C_{18:3}$) is the major fatty acid in olive leaves (from 30.02 to 42.16%), followed by oleic acid ($C_{18:1}$) and palmitic acid ($C_{16:0}$). The thermal profiles of olive leaf extracts determined by their DSC melting curves revealed simple thermograms with a single peak after melting. The hexane extract of the Chemchali variety, which contained relatively high unsaturated fatty acids and low saturated fatty acid levels, exhibited the lowest peak temperature value (54.59 °C) and required the smallest amount of energy for melting (31.57 $J \cdot g^{-1}$). This study showed that olive leaves possessed physicochemical properties and a fatty acid composition that may become interesting for industrial applications.

KEYWORDS: *Chlorophylls; DSC melting curves*; *Fatty acid composition; Minerals; Olive leaves*

RESUMEN: ***Investigación comparativa de los contenidos de minerales, clorofila, composición de ácidos grasos y perfiles térmicos de hojas de olivo (*Olea europaea *L.) como subproducto***. Este trabajo presenta estudios de la composición química (minerales, contenido de clorofila y ácidos grasos) e investigaciones termofísicas (perfil DSC) de cuatro variedades de hojas de olivo cultivadas en Túnez. Los contenidos totales de clorofila de las hojas de olivo oscilaron entre 1132,33 y 1795,93 ppm. Los resultados mostraron que el ácido linolénico (C18:3) es el ácido graso principal en las hojas de olivo (30,02 a 42,16%), seguido de los ácidos oleico (C18:1) y palmítico (C16:0). Los perfiles térmicos de los extractos de hoja de olivo determinados por sus curvas de fusion, DSC mostró termogramas simples con un solo pico después de la fusión. El extracto de hexano de la variedad Chemchali, que contenía ácidos grasos insaturados relativamente mas altos y bajos niveles de ácidos grasos saturados, exhibió el pico de temperatura más baja (54,59 °C) y requiere menor energía para la fusión (31,57 $J \cdot g^{-1}$). Este estudio mostró que las hojas de olivo poseen propiedades fisicoquímicas y composición en ácidos grasos que pueden ser interesantes para aplicaciones industriales.

PALABRAS CLAVE: *Clorofilas; Composición en ácidos grasos; Curvas de fusión DSC*; *Hojas de olivo; Minerales*

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1. INTRODUCTION

The olive is one of the most extensively cultivated fruit crops in the world. The worldwide planted area dedicated to olive trees is about 9.9 million hectares with 95% of them in the Mediterranean basin (FAOSTAT, 2009). Olive leaves are one of the by-products of the farming of the olive grove and can be found in high amounts in the olive oil industries (10% of the total weight of olives) and during the pruning of olive trees (Tabera *et al*., 2004). Further, olive leaves are considered as a cheap raw material which can be a source of high-added value products (Briante *et al*., 2002). Indeed, recent research has demonstrated that olive leaves are a good source of phenolic compounds, such as secoiridoids and flavonoids which have strong protective effects against oil oxidation (Farag *et al*., 2003; Paiva-Martins *et al*., 2007) and a positive impact on human health (Japon-Lujan and Luque de Castro, 2006; Altiok *et al*., 2008). Several reports have shown that the olive leaf has the capacity to lower blood pressure (Khayyal *et al*., 2002) and increase blood flow in the coronary arteries (Zarzuelo 1991). Olive leaves are often prepared as an infusion or decoction extraction. Nowadays, many homoeopathic remedies are sold as capsules containing the powder or the extract of dried olive leaves (Bahloul *et al*., 2009). Moreover, olive leaf extracts have several applications in the pharmaceutical and food industries.

Tabera *et al*., (2004) have used the counter-current supercritical fluid extraction for the fractionation of high-added value products from a raw extract of olive leaves in hexane. Some compounds such as waxes, hydrocarbons, squalene, β-carotene and α-tocopherols were identified in olive leaves. Other important compounds may be found in olive leaves essentially fatty acids, minerals and chlorophylls. Fatty acids are phytochemicals which can be used as nutraceuticals. The polyunsaturated fatty acids, including the omega-3 and omega-6 families, constitute an important class of phytochemicals due to their beneficial health effects. Indeed, according to Tessier (1994), the fatty acids of olive leaves should be associated to the phenolic compounds to lower cholesterol levels. There is quite a lot of literature on the lipid profile of olive oil (Christopoulou *et al*., 2004; Manai *et al*., 2008; Kerem *et al*., 2011) and the phenolic composition of olive leaves but a dearth of information on the fatty acid composition of olive leaves.

The thermal stability of esters depends on their chemical structure and fatty acid composition. In this respect, differential scanning calorimetry (DSC) gives valuable information on the thermal properties of fats and their suitability for a particular application. Indeed, DSC measures the temperatures and heat flows associated with transitions in materials as a function of time and temperature in a controlled atmosphere. Any endothermic or exothermic event is registered as a peak in the chart, and its area is proportional to the enthalpy gained or lost, respectively (Gloria and Aguilera, 1998). Differential Scanning Calorimetry (DSC) measures the change in the difference in the heat flow rate to the material and to a reference material while they are subjected to a controlled temperature program. The result of DSC is a curve of heat flux versus time or temperature and is therefore used also for the determination of the enthalpy, the melting temperature and the specific heat (Klancnik *et al*., 2010).

In this work, we aimed to investigate the mineral composition of olive leaves, to establish the fatty acid composition and to study the thermal behaviour of olive leaf extracts by differential scanning calorimetry in an effort to achieve efficient uses of such by-products for industrial applications.

2. MATERIAL AND METHODS

2.1. Chemicals

All chemicals and reagents were of analytical reagent grade. Potassium hydroxide, Hydrochloric acid and Nitric acid were purchased from Panreac (Spain). Acetone, methanol, ether and hexane were supplied from Carlo Erba (France).

2.2. Plant material

Olive leaves were harvested from the Olive Tree Institute farm of Sfax, Tunisia (34°43N, 10°41E) in April 2011. Nineteen-year-old olive trees (*Olea europaea* L.) were used. Trees were spaced 4 m×6 m and subjected to the same olive cultivation practices. The sandy soil had an organic matter content of 1.3%; 12.3% $CaCO_3$; 1.2% N and a pH of 7.8. Four varieties were selected for this study: Chemlali (CL), Chemchali (CH), Chetoui (CT) and Zarrazi (ZR). Samples of olive leaves (120 g), characterized as being from the present year (aged 3 months), were collected from each variety. All foreign matter was removed from the material, especially adhering soil or sand. Since the moisture contents of the leaves were about 50%, the samples were oven dried at 37 °C for 48 h. Olive leaves from each variety were separately milled in a blender to pass a 1 mm screen and stored in light protected glass bottles for further use.

2.3. Mineral content determination

Samples of each olive leaf powder (1 g) were ashed at 550 °C in a muffle furnace for 4 hours. The ashes were then cooled in a desiccator and used for the determination of calcium, potassium, magnesium, sodium, iron, zinc and copper contents (AOAC, 1984). Therefore, a known amount of ash was dissolved in 4 mL of concentrated HNO_3 in

a porcelain crucible. The solution was stirred and evaporated to dryness on a hot plate set at 120 °C. After that, the crucible was returned to the furnace at 550 °C for 1 hour. The ash was dissolved in 10 mL of concentrated HCL and then filtered into a 50 mL volumetric flask. The ash residue was rinsed three times and made up to volume with deionized water. The mineral constituents present in the olive leaves were analyzed separately using an atomic absorption spectrophotometer (Hitachi Z6100, Japan). A calibration curve for each of the mineral to be determined was prepared using the standard solutions before the readings were obtained.

2.4. Chlorophyll content determination

Total chlorophyll, chlorophyll a and b contents were determined by the spectrophotometric method according to AOAC (1984). Briefly, fresh olive leaf samples (5g) were ground into a Waring Blendor and then 25 mL of 85% acetone solution were added. The samples were homogenized for 3 min. The homogenate was filtered through filter paper Whatman no. 1, with a Büchner funnel under vacuum. The filter cake residues were then washed with an 85% acetone solution. The extraction procedure was repeated until the tissue was devoid of any green and washings were colorless, and then the filtrate was brought to a final volume of 100 mL. An aliquot of 25 mL of acetone extract was transferred into a separator containing 50 mL of ether. After that, distilled water was added until it was apparent that all fat solution pigments had entered the ether layer. Washings of ether solutions were continued with distilled water until all the acetone was removed. Then the ether solution was transferred to a 100 mL volumetric flask, diluted to volume and mixed. The absorbance readings of this solution were recorded at wavelength 660 and 642.5 nm using a UV/Vis spectrophotometer (Shimadzu, Japan). The concentrations of total chlorophyll, chlorophylls a and b (ppm) were calculated as:

$$\text{Total chlorophyll} = 7.12\ A_{660} + 16.8\ A_{642.5} \quad \text{(Eq. 1)}$$

$$\text{Chlorophyll a} = 9.93\ A_{660} - 0.777\ A_{642.5} \quad \text{(Eq. 2)}$$

$$\text{Chlorophyll b} = 17.6\ A_{642.5} - 2.81\ A_{660} \quad \text{(Eq. 3)}$$

2.5. Hexane extraction

The olive leaf powder (50 g) was placed in a dark flask and homogenized with 250 mL of hexane (1:5, w/v). After mixing for 4 h in a shaker (Selecta, Spain), the mixture was centrifuged for 15 min at 1000 g. The supernatant was then filtered through a filter paper (Whatman no. 2). The extraction procedure was repeated twice. The solvent was removed by a vacuum rotary evaporation at 40 °C. The extract was pooled, drained under a stream of nitrogen and then stored in a freezer until analysis.

2.6. Fatty acid composition

The hexane extracts of olive leaves were filtered with a millipore membrane filter (0.45 μm) prior to gas liquid chromatography analysis. Samples (1 mg) were converted into their corresponding fatty acid methyl esters using a methanolic solution of potassium hydroxide (2 M). The mixture was maintained at 100 °C for 1 h. The reaction was stopped with 0.5 mL of distilled water. Then, the extracted fatty acid methyl esters were dissolved in heptane. GC analyses were performed on a GC-17-A SHIMADZU (Japan), equipped with a hydrogen flame ionization detector and a capillary column: Carbowax (15 m, 0.25 mm).The column temperature was fixed at 180 °C and the injector and detector temperatures were set at 230 °C and 250 °C, respectively. Nitrogen was the carrier gas. Fatty acid methyl esters were identified by comparison of their retention times with respect to pure standards purchased from Sigma and analyzed under the same conditions. Fatty acid methyl esters were quantified according to their percentage area, obtained by the integration of the peaks. The results were expressed as a percentage of individual fatty acids in the extract.

2.7. Differential scanning calorimetry (DSC)

The thermal properties of olive leaf extracts in hexane were determined using a differential scanning calorimeter (NETZSCH-Gerätebau GmbH Thermal analysis DSC 204, Germany). The hexane extract (8±0.1 mg) was weighed in a DSC-aluminium pan. An empty DSC-pan was used as an inert reference. The sample and the reference pans were then placed inside the calorimeter and were quickly cooled to −60 °C at a speed of 5 °C·min^{-1}, held at this temperature for 15 min, and heated to 100 °C with a heating speed of 5 °C·min^{-1} and the DSC thermographs were recorded during the melting transition. The peak temperature is the temperature maximum of a thermal transition. The onset temperature is the temperature where the extrapolated leading edge of the endotherm intersects with the baseline.

2.8. Statistical analysis

Analyses were carried out in triplicate. The values of different parameters were expressed as the mean ± standard deviation. The results were statistically analyzed by one-way analysis of variance (ANOVA) and Tukey tests using SPSS (Version 11, SPSS Inc., Chicago, USA). Statistical significance was accepted at a level of $p<0.05$.

3. RESULTS AND DISCUSSION

3.1. Mineral composition

The mineral compositions of the olive leaf varieties namely Chemlali (CL), Chemchali (CH), Chetoui (CT) and Zarrazi (ZR) are shown in Table 1. It can be seen that the ash contents of olive leaves ranged from 6.60 to 9.82%. The values were in the same range of those reported by Delgado-Pertinez *et al*., (2000) for olive leaves (6.5 to 9.6%). The ash amounts of olive leaves are considered high in comparison with other vegetal products such as pumpkin and watermelon seed kernels (3.21% and 3.6%, respectively) (El-Adawy and Taha 2001).

The mineral composition of olive leaves showed that calcium [9.25 $mg \cdot g^{-1}$ d.m. (CT)–10.39 $mg \cdot g^{-1}$ d.m. (CL)] was the predominant mineral. Similar values (9.296 $mg \cdot g^{-1}$ d.m.) were reported by Lee *et al*., (2005). Calcium develops and maintains strong bones and teeth and enhances the use of other nutrients. Therefore, calcium supplements may be used to prevent and to treat calcium deficiencies.

Potassium amounts in the olive leaves ranged from 4.47 to 9.14 $mg \cdot g^{-1}$ d.m. Potassium is a micronutrient that plays an important role in the regulation of the heartbeat. Besides, it maintains fluid balances and helps muscles contract. An adequate intake of calcium and potassium contributes to preventing cardiovascular diseases (McCarron and Reusser, 2001). Thus, the presence of calcium and potassium in olive leaf powder extracts could enhance the beneficial effects on health of other phytochemicals such as phenolic compounds and tocopherols.

The potassium concentrations of CL and CT olive leaf varieties were significantly ($p<0.05$) different from those of CH and ZR. The mineral contents of olive leaves could be influenced by the differences in the nutrient status of the soils in which the olive tree grows and by the environmental effects around the tree. Higueras *et al*., (2012) studied the uptake of metallic trace elements from soils for olive-tree growing in the Almadén mercury mining district. The authors found good correlations between soil and olive leaf contents for major elements from the soils (Fe, Al, Mg and Ca), indicating a direct and important uptake of these elements from the soil. However, some element contents in leaves do not show any relationship with the element contents in the soil (Ce, Cr, Hg, Nd, Pb, Zn). These variations have been explained by the different availability of the elements in the soil. Several methods for estimating nutrient availability and their limitations were reviewed by Marschner and Rengel (2012).

The effects of soil chemistry on plants were also studied by Lynch *et al*., (2012). In fact, the soil nutrient availability, pH, aeration and low molecular weight organic solutes were among the soil chemical factors affecting the plant root growth and development.

The magnesium contents varied from 1.5 to 3 $mg \cdot g^{-1}$ in olive leaves. The magnesium average value measured for Spanish olive leaves was 1.5 $mg \cdot g^{-1}$ (Higueras *et al*., 2012). Sodium was present in relatively low concentrations in the olive leaves.

TABLE 1. Mineral composition of olive leaves ($mg \cdot g^{-1}$ dry matter)

	Variety			
Minerals	**Chemlali**	**Chemchali**	**Chetoui**	**Zarrazi**
Ash*	$8.47^{a}\pm0.14$	$9.82^{b}\pm0.09$	$8.05^{a}\pm0.36$	$6.60^{c}\pm0.23$
Calcium	$10.39^{a}\pm0.32$	$9.66^{a,b}\pm0.33$	$9.25^{b}\pm0.36$	$9.37^{b}\pm0.29$
Potassium	$7.87^{a}\pm0.23$	$4.47^{b}\pm0.45$	$9.14^{a}\pm0.35$	$5.33^{b}\pm1.61$
Magnesium	$1.50^{a}\pm0.25$	$3.00^{b}\pm0.13$	$1.55^{a}\pm0.10$	$2.75^{b}\pm0.00$
Sodium	$0.82^{a}\pm0.03$	$1.51^{b}\pm0.10$	$0.35^{c}\pm0.03$	$0.34^{c}\pm0.02$
Copper	traces	traces	traces	traces

*(% dry matter).

Different letters in the same row indicate that means are significantly different ($p<0.05$).

3.2. Total chlorophyll, chlorophyll a and chlorophyll b

Chlorophylls are very common pigments, which give color to plants and several algae. The color of olive leaves is mainly related to their chlorophyll content, as this compound is the main pigment of green vegetables and masks the bright color of carotenoids.

The total chlorophyll, chlorophyll a and chlorophyll b contents of olive leaves are presented in Figure 1. The total chlorophy1l contents of olive leaves ranged from 1132.33 to 1795.93 ppm. These values were in the same range obtained for watercress leaves (1400 ppm) (Gonçalves *et al*., 2009). The chlorophyll content of olive leaves seems to vary with variety. Indeed, the amounts of chlorophyll a were significantly ($p<0.05$) different among varieties [281.69–854.59 ppm] being higher for the CL variety which also had the highest contents of total chlorophyll. The chlorophyll b content of olive leaves ranged from 851.83 to 1114.23 ppm. The influence of genetic factors on chlorophyll content was confirmed by Bojovic and Markovic (2009) who investigated the variability of the chlorophyll content of wheat leaf with cultivar. Furthermore, chlorophyll content is usually affected by various environmental factors such as temperature, photoperiod, nitrogen, water and degree of maturity (Chen *et al*., 2011; Lee *et al*., 2011).

Chlorophylls are used as additives to food products due to their color and physico-chemical properties. In fact, chlorophyll molecules are extracted and used as natural pigments in processed foods (Gutiérrez-Rosales *et al*., 1992). They can be chemically modified before being incorporated into

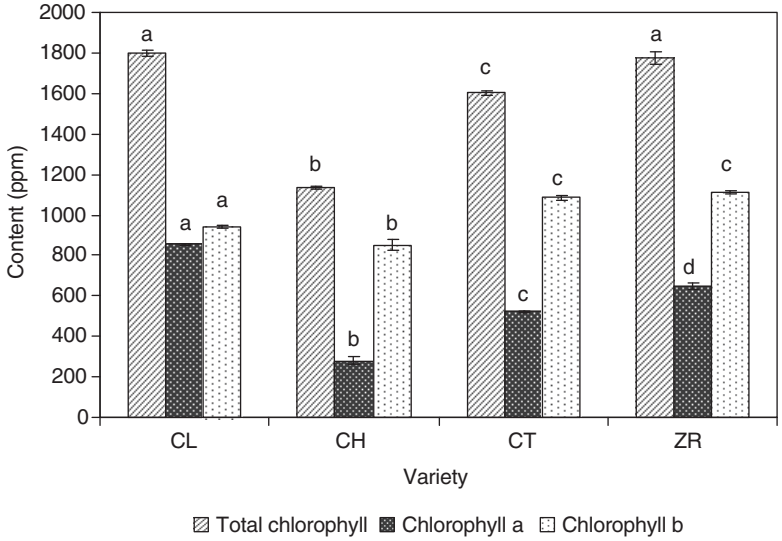

FIGURE 1. Total chlorophyll, chlorophyll a and chlorophyll b contents of olive leaves (CL): Chemlali, (CH): Chemchali, (CT): Chetoui and (ZR): Zarrazi. Bars with the same letter are not significantly different ($p>0.05$).

food products (e.g. replacement of Mg^{2+} by Cu^{2+} in chlorophyll). Further, chlorophyll precursors and chlorophyll derivatives are used in medicine for photodynamic treatments (Schoefs, 2002).

3.3. Hexane extracts

The hexane extract yields of olive leaves are shown in Table 2. The percentages varied from 3.11 to 3.88 %. The extraction yields of the olive leaf varieties were statistically similar ($p>0.05$). This can be explained by the fact that the olive trees were grown in the same region with similar rainfall. Additionally, the olive leaves were harvested during the same season. Martin-Garcia and Molina-Alcaide (2008) found higher yields (7.81–9.76%) for Spanish olive leaves using the soxhlet technique for the extraction. This difference can be attributed to the effect of heat on fat extraction and to the progressive depletion by the successive and multiple washing cycles by the soxhlet extraction. Based on the fact that the temperature of extraction can affect the initial quality of the fat, cold extraction was selected in this study.

3.4. Fatty acid composition

The fatty acid composition of olive leaf extracts in hexane is shown in Table 2. The major fatty acids found in the olive leaf samples were linolenic acid ($C_{18:3}$) [30.02–42.16%], oleic acid ($C_{18:1}$) [18.28–26.36%] and palmitic acid ($C_{16:0}$) [18.22–22.42%]. The fatty acid composition of olive leaves seems to vary slightly with variety. For instance, the fatty acid composition of the CL variety is statistically similar to that of ZR, except for the amounts of linoleic acid.

Linolenic acid is a polyunsaturated omega-3 fatty acid which is metabolized to eicosapentaenoic acid, a precursor of eicosanoids with anti-inflammatory and antithrombotic activity (Ruiz *et al.*, 2002). Many researchers have found evidence that linolenic acid is related to a lower risk of cardiovascular diseases (William, 2000; Simopoulos, 2002; Schacky and Harris 2007).

The percentage of polyunsaturated fatty acids [44.50–58.29%] in olive leaves was higher than that of saturated fatty acids [23.30–28.99%]. The olive leaves exhibited higher levels of saturated fatty acids than olive oil (21.22%) (chemlali variety) (Dabbou *et al.*, 2010). Besides, the olive leaves showed higher levels of polyunsaturated fatty acids in comparison with olive oil (18.11%), which contains about 17.48% of linoleic acid and 0.63% of linolenic acid. The polyinsaturated fatty acids could make olive leaves specifically prone to oxidation. However, this could be counterbalanced by the levels of antioxidants that protect the olive leaf extract.

The polyunsaturated fatty acids including the omega-3 and omega-6 families detected in the plants constitute an important class of phytochemicals due to their generalized beneficial health effects (Guimarães *et al.*, 2009).

Linoleic acid is observed at appreciable percentages in olive leaf extracts [14.48–16.52%]. This essential fatty acid has become increasingly popular because of its beneficial properties for the skin, including anti-inflammatory, acne reduction and moisture retention properties (Darmstadt *et al.*, 2002). Therefore, it is often used in making soaps, creams and emulsifiers. Thus, the olive leaf extracts containing polyunsaturated fatty acids constitute a

Table 2. Fatty acid composition of four olive leaf varieties (%)

	Variety			
Fatty acid	**Chemlali**	**Chemchali**	**Chetoui**	**Zarrazi**
Hexane extract yield	3.11[a]±0.26	3.58[a]±0.00	3.88[a]±0.53	3.21[a]±0.41
Myristic acid ($C_{14:0}$)	3.15[a]±0.35	2.49[a,b]±0.65	1.80[b]±0.32	3.27[a]±0.27
Palmitic acid ($C_{16:0}$)	22.42[a]±0.53	18.22[b]±1.27	20.39[a,b]±0.95	21.19[a]±0.71
Palmitoleic acid ($C_{16:1}$)	0.15[a]±0.00	0.13[a,b]±0.01	0.10[b]±0.02	0.17[a]±0.01
Stearic acid ($C_{18:0}$)	3.42[a]±0.41	2.59[a]±0.22	3.09[a]±0.41w	3.88[a]±0.39
Oleic acid ($C_{18:1}$)	26.36[a]±1.39	18.28[b]±3.46	25.07[a]±0.34	25.15[a]±0.44
Linoleic acid ($C_{18:2}$)	14.48[a]±1.11	16.13[b]±0.61	16.52[b]±0.56	15.84[b]±0.53
Linolenic acid ($C_{18:3}$)	30.02[a]±1.95	42.16[b]±4.66	33.03[a]±1.19	30.50[a]±1.44
Saturated fatty acids	28.99±0.42	23.30 ±1.31	25.29±0.91	28.34±0.79
Monounsaturated fatty acids	26.51±1.40	18.41±3.45	25.17±0.35	25.32±0.44
Polyunsaturated fatty acids	44.50±1.28	58.29±4.06	49.54±0.84	46.35±1.22
Unsaturated fatty acids	71.01±0.42	76.70±1.31	74.71±0.91	71.66±0.79

Different letters in the same row indicate that means are significantly different ($p<0.05$).

potential source of fatty acids which can be used to manufacture dietary supplements.

In addition, the use of olive leaves in animal feeding appears to increase the content of unsaturated fatty acids and lower the content of saturated fatty acids in animal milk (Fegeros *et al*., 1995; Moline-Alcaide and Yanez Ruiz, 2008) and therefore provides an appreciable dietary nutraceutical value. Notably, the fatty acid composition in the milk fat produced by the animals fed olive leaves had more oleic and linolenic acid and less myristic and palmitic acids. This trend is due to the fatty acid composition of olive leaves, which are rich in unsaturated fatty acids.

3.5. DSC thermal profiles

DSC is a fast and direct way to assess the quality of fats and to study their physical properties. DSC heating profiles help to explore the nature of the phase transition taking place during the melting of fats and oils (Gloria and Aguilera, 1998).

Olive leaf extracts in hexane showed the same melting profile (Figure 2). Olive leaf extracts exhibited a simple thermogram with a single peak after melting in the DSC. The melting of a fat results in volume expansion and a negative heat effect (Tan and Che Man, 2002). This showed the endothermic transition upon heating from a crystalline solid to the liquid state. The thermal parameters from the DSC melting curves of olive leaf extracts are shown in Table 3. The onset temperatures of the olive leaf extracts were significantly different ($p<0.05$). The values ranged from 26.50 to 30.10 °C.

The enthalpy of melting is the heat energy required for melting. This is calculated by integrating the area of the DSC peak on a time basis. Significant differences ($p<0.05$) were also encountered for the melting enthalpy of the samples and values ranged from 31.57 to 54.97 $J \cdot g^{-1}$. The thermal parameters and the phase transitions in fats may be affected by the compositional changes between the samples such as fatty acid chain length, degree of unsaturation and nature of distribution of fatty acids in triacylglycerol species. The DSC application upon heating allowed for discriminating among oil samples from olives of different cultivars and/or harvesting periods. The thermal properties of monovarietal extra virgin olive oil samples were found to correlate well with the chemical composition i.e. triacylglycerols, diacylglycerols, total and free fatty acids and oxidation status (Chiavaro *et al*., 2008).

A mathematical model based on a simple regression procedure was also developed to correlate melting parameters to mass fractions of mono and polyunsaturated fatty acids (Fasina *et al*., 2008).

Regarding the peak temperatures of olive leaf extracts, the values ranged from 54.59 to 54.80 °C. The CH variety exhibited the lowest peak temperature value (54.59 °C) and it required a smaller amount of energy for melting (31.57 $J \cdot g^{-1}$). This can be due to the higher level of unsaturated fatty acids and lower level of saturated fatty acids in the CH olive leaf extract. Indeed, the fatty acid composition influences the melting point values of the samples. Fats containing higher amounts of saturated triacylglycerol species commonly demonstrate a higher melting point than those which are highly unsaturated. Further, the peak temperatures of CL (54.79 °C) and ZR (54.80 °C) were statistically similar ($p>0.05$). This could be attributed to the similarities observed in the fatty acid composition between the two varieties.

de Man *et al*. (1991) consider the melting point as the peak temperature calculated from a DSC

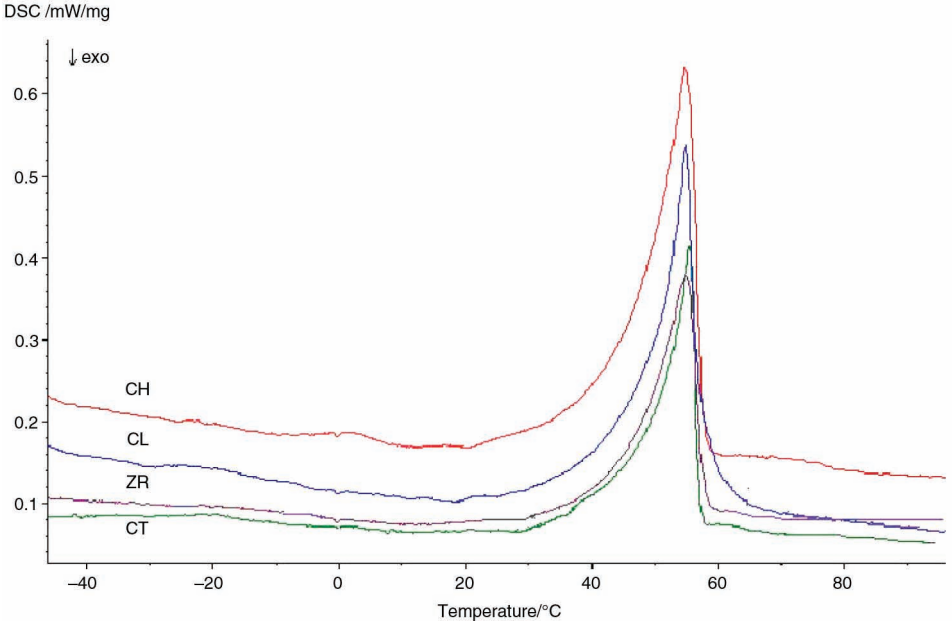

FIGURE 2. DSC melting profile of olive leaf extracts in hexane. Olive leaf varieties: CL: Chemlali, CH: Chemchali, CT: Chetoui and ZR: Zarrazi.

TABLE 3. Thermal parameters from the DSC melting curves of olive leaf extracts

	Variety			
Parameter	**Chemlali**	**Chemchali**	**Chetoui**	**Zarrazi**
Onset temperature (°C)	29.10^{a}±0.07	26.50^{b}±0.06	30.10^{c}±0.03	29.55^{d}±0.02
Peak temperature (°C)	54.79^{a}±0.04	54.59^{b}±0.10	54.70^{c}±0.07	54.80^{a}±0.07
Melting enthalpy ($J \cdot g^{-1}$)	54.97^{a}±0.19	31.57^{b}±0.11	43.24^{c}±0.01	42.12^{d}±0.03

Different letters in the same row indicate that means are significantly different ($p<0.05$).

melting curve. The melting point is used to characterize the fats and is related to their physical properties, such as hardness and thermal behaviour.

4. CONCLUSIONS

Data resulting from this work improve the fundamental knowledge about the chemical composition of olive leaves and such a determination is very important to explore varietal changes. The olive leaf is a promising source of calcium and potassium which contribute together with other phytochemicals to the health benefits of olive leaves. Total chlorophyll contents ranged from 1132.33 to 1795.93 ppm. Chlorophylls can be used as natural additives to food products.

The present study proved that the olive leaf extract in hexane constitute a source of beneficial fatty acids, namely linolenic acid, oleic acid and linoleic acid. Olive leaf extracts exhibited a simple thermogram with a single peak after melting in the DSC. The hexane extract of the CH variety exhibited the lowest peak temperature value (54.59 °C) and it required the smallest amount of energy for melting (31.57 $J \cdot g^{-1}$) in comparison with the other samples. This was due to the fatty acid composition of the CH variety which exihibited the highest unsaturated fatty acid and lowest saturated fatty acid levels.

Olive leaves are renewable resource which provide adding value to agricultural products and potentially create new rural jobs when used for industrial products.

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