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Microwave heating of different commercial categories of olive oil: Part II. Effect on thermal properties

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ABSTRACT

The effect of microwave heating of commercial categories of olive oil for human consumption (extra virgin olive oil [EVOo], olive–pomace oil [Po] and olive oil [Oo]) on DSC thermal properties was evaluated at different times of microwave treatment.

Marked changes of DSC cooling profiles were found for EVOo and Po subjected to microwaving, with the major exotherm that shifted towards lower temperature and decreased height with increasing treatment time. Thermal properties (during DSC cooling analysis) changed in all samples: crystallisation enthalpy significantly decreased and the phase transition developed over a larger temperature range, due to more heterogeneous chemical composition of all oils that resulted from triacylglycerol lysis to the formation of lipid oxidation products. Heating profiles of EVOo and Po were also modified by microwave treatment, as the minor endotherm progressively disappeared, significantly shifting offset temperature of transition towards lower temperature.

Oo did not exhibit such changes of thermal properties and phase transition profiles as described for EVOo and Po. This may be mainly related to its lower water content although the simultaneous presence of small amounts of antioxidant molecules (polyphenols) may have contributed to partially prevent thermal degradation of this oil in comparison with the others.

These preliminary results suggest that DSC can be useful, not only for monitoring modifications of chemical composition with increasing microwave treatment time, but also to discriminate amongst olive oils according to their response to microwave exposure.

1. Introduction

Microwave heating is a common and fast procedure for food preparation and manufacturing. Heating of food by microwaving results from the molecular friction of electric dipoles under an oscillating electric field of specific frequency to the presence of water, which is an electric dipole, is a determinant for the induction of modifications during and after microwaving (Mudgett, 1989). Amongst food macrocomponents, lipids are also particularly sensitive to this treatment as the specific heat for lipids is low and they are heated quickly (Vieira & Regitano-D'Arce, 1998). In particular, microwave heating of vegetable oils, which is commonly used as heat transfer medium in food processing, accelerates their oxidation, causing polymerisation and thermal-oxidative decomposition (Albi, Lanzón, Guinda, Pérez-Camino, & León, 1997a).

The application of fast, reliable and low environmental impact analytical methods for monitoring the thermal stability of vegetable oils and the oxidation degree resulting from time–temperature combination of heating treatments (microwave), are nowadays requested.

Differential scanning calorimetry (DSC) is a technique widely employed to evaluate cool and heat-related phenomena in the field of fat and vegetable oil analysis, which provides a reproducible method for their identification (Che Man & Tan, 2002; Tan & Che Man, 2002a). Its application for the assessment of the oxidative deterioration of vegetable oils is well-known and reviewed (Kowalski, 1991; Tan & Che Man, 2002b). Thermal properties obtained from cooling thermograms (such as peak enthalpy and temperature) of deep-fried vegetable oils were found to be highly correlated with changes in viscosity, colour and total polar compounds (TPC) (Gloria & Aguilera, 1998). Tan and Che Man (1999a, 1999b) also reported high correlation coefficients amongst such thermal properties and standard chemical oxidative indices after oil frying. Few studies dealt with DSC evaluation of the

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deteriorative effect of microwave heating on vegetable oils. The effect of different microwave power and heating time application on cooling thermograms of corn and soybean oils was evaluated by Tan, Che Man, Jinap, and Yusoff (2001). These authors found that the percentage area of the lowest temperature endotherm, attributed to triunsaturated triacylglycerols, decreased in both oils with increasing heating time and power set and was highly correlated with changes in standard oxidative stability indices. The lower temperature shoulder peaks present in both DSC cooling and heating thermograms of palm olein were also found to shift toward lower temperature after microwaving (Tan, Che Man, Jinap, & Yusoff, 2002). High statistical correlations were reported between DSC curve parameters and oxidative stability indices for this oil, as well.

Olive oils are largely employed in the Mediterranean area as flavouring and cooking fat ingredients of a number of formulates (such as salad dressings, sauces and ready-to-eat products) and as a filling medium for canned foodstuffs. The commercial categories of olive oils are legally classified by the European Community (EC, 2001). Extra virgin olive oil (EVOo; free acidity <0.8 g per 100 g), olive oil (Oo; free acidity <1.0 g per 100 g) and olive–pomace oil (Po; free acidity <1.0 g per 100 g) are destined for human consumption. Oo is obtained by blending virgin olive oil with refined olive oil, whereas Po is produced by blending virgin olive oil and refined pomace olive oil.

DSC evaluation of thermal properties and thermo-oxidative stability of olive oils has been the subject of few studies. DSC was found to differentiate monovarietal virgin olive oils, based, not only on crystallisation and melting profiles (Jiménez Márquez & Beltrán Maza, 2003), but also on thermal properties related to macro- and microcomponents of the oil (Chiavaro, Vittadini, Rodriguez-Estrada, Cerretani, & Bendini, 2008b; Chiavaro et al., 2007). DSC application for the discrimination of commercial categories of olive oil was reported by Jiménez Márquez (2003) and, more recently, by Chiavaro et al. (2008a). Auto- and thermo-oxidation of EVOo have been evaluated by DSC (Vittadini, Lee, Frega, Min, & Vodovotz, 2003) and modulated DSC (Kanavouras & Selke, 2004), showing good correlations between thermal parameters (crystallisation enthalpy and onset transition temperature) and the amount of oxidised volatile compounds. To the authors' best knowledge, the effect of microwave heating on thermal properties of different commercial categories of olive oil has not yet been reported.

The aim of this preliminary study was to evaluate the influence of microwave heating on DSC thermal properties (upon cooling and heating) of three commercial categories of olive oil for direct human consumption (extra virgin olive oil, pomace olive oil and olive oil) at different times of treatment. Results were also related to both chemical composition and changes of oxidative stability indices reported in Part I (Cerretani, Bendini, Rodriguez-Estrada, Vittadini, & Chiavaro, 2009).

2. Materials and methods

2.1. Sampling

EVOo was produced with hand-picked olives from the 2006 harvest in Monopoli (Apulia, Italy); olives were processed by a continuous industrial plant with a working capacity of 1 ton/h equipped with a hammer crusher, a horizontal malaxator (at a temperature of 27 °C), and a three-phase decanter. Olive oil (Oo) and pomace olive oil (Po) were donated by Coppini Arte Olearia (Parma, Italy) and obtained from olives picked in Trapani (Sicily, Italy) in 2006, as well. Oo was obtained by blending EVOo and refined olive oil, whereas pomace–olive oil (Po) was produced by blending EVOo and refined pomace–olive oil. Samples were stored in dark bottles without headspace at room temperature (23 ± 1 °C) before analysis. One sample of each type of oil was subjected to microwaving and analysed.

2.2. Microwave treatment

A domestic microwave oven was used for sample heating (Perfect Combo MW 651, DeLonghi, Treviso, Italy). Two aliquots (90 ml) of each oil were placed in opened 150 ml flasks (7.3 cm i.d.) on the rotatory turntable plate of the oven at equal distance and exposed at a frequency of 2450 Hz at medium power (720 W). The oil samples were heated for 1.5, 3, 6, 9, 12 and 15 min. Temperature of each oil was measured immediately after microwave exposure, by inserting a thermocouple (K-type; Ni/Al–Ni/Cr) connected to an acquisition system (HI 98804, Hanna Instrument, Villafranca Padovana-PD, Italy) at approximately the geometrical centre of the sample. Temperature mean values of the three oils are reported in Part I (Cerretani et al., 2009). All heated samples were allowed to cool at room temperature (23 ± 1 °C) for 60 min after thermal treatment. The two oil samples were combined after microwave treatment to obtain a homogeneous sample used for both chemical and thermal analysis (Part I; Cerretani et al., 2009).

2.3. Solvents, reagents, and standard compounds

All solvents used were of analytical or high-performance liquid chromatography (HPLC) grade (Merck, Darmstadt, Germany). Reagents and commercial standards of triacylglycerols (triolein (OOO), trilinolein (LLL)) and tridecanoic acid methyl ester were purchased from Sigma–Aldrich (St. Louis, MO, USA). The standard mixture of fatty acid methyl esters (GLC 463) was supplied by Nu-Chek (Elysian, MN, USA).

2.4. Chemical analyses

Triacylglycerols (TAG) were analysed by HPLC coupled to both diode-array (DAD) and mass spectrometer (MSD) detectors, as previously described (Chiavaro, Vittadini, Rodriguez-Estrada, Cerretani, & Bendini, 2008c). TAG was tentatively identified based on their UV–vis and mass spectra obtained by HPLC-DAD/MSD and literature data (Nagy et al., 2005). TAG are grouped according to the type of FA bonded to the glycerol structure as monosaturated triacylglycerols (MSTAG), disaturated triacylglycerols (DSTAG) and triunsaturated triacylglycerols (TUTAG). The limit of quantitation (LOQ) was 0.01 g/100 g of TAG. The following TAG were identified: LLL, trilinolein; LLP, dilinoleoyl-palmitoyl-glycerol; LLPo, dilinoleoyl-palmitoleyl-glycerol; OLL, dilinoleoyl-oleoyl-glycerol; OLLn, oleoyl-linoleoyl-linolenoyl-glycerol OLO, dioleoyl-linoleoyl-glycerol; OLnO, dioleoyl-linolenoyl-glycerol; OLP, palmitoyl-oleoyl-linoleoyl-glycerol; OLPo, palmitoleoyl-oleoyl-linoleoyl-glycerol; OOO, triolein; OOPo, dioleoyl-palmitoleoyl-glycerol; POP, dipalmitoyl-oleoyl-glycerol; POPo, palmitoyl-palmitoleoyl-oleoyl-glycerol; SLO, stearoyl-oleoyl-linoleoyl-glycerol; SOO, dioleoyl-stearoyl-glycerol; SOP, palmitoyl-stearoyl-oleoyl-glycerol.

Fatty acids (FA) were quantitatively determined by gas chromatography (GC), according to Cercaci et al. (2006). FA was expressed according to their unsaturation degree, as saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids. LOQ was 0.01 g/100 g of fatty acids.

For both determinations, three replicates were analysed per sample.

2.5. DSC

Samples of oil (8–10 mg) were weighed into aluminium pans; covers were sealed into place and the whole analysed with a DSC

Q100 (TA Instruments, New Castle, DE), as previously described (Chiavaro et al., 2008b). Thermograms were analysed with Universal Analysis Software (Version 3.9A, TA Instruments) to obtain enthalpy (ΔH, J/g), peak maximum (°C), T_{on} (°C) and T_{off} (°C) of the transitions (intersection of baseline and tangent at the transition). Range of the transitions was calculated as temperature difference between T_{on} and T_{off}. Five replicates were analysed per sample.

2.6. Statistical analysis

Means and standard deviations were calculated with SPSS (Version 14.0, SPSS Inc., Chicago, IL) 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 of evaluated thermal parameters.

3. Results and discussion

3.1. Chemical composition

TAG composition of untreated EVOo, Po and Oo is reported in Table 1. Sixteen TAG were identified in all samples; eight of them were separately quantified and the others were quantified as pairs (LLL + LLPo, OLL + OLPo, LLP + OLnO and OLP + OOPo), as previously reported (Chiavaro et al., 2008a). For all samples, more than 86% of the total TAG was represented by OLL + OLPo, OLO, OLP + OOPo, OOO and SLO triglycerides (Table 1). In general, TAG containing linoleic acid (LLL + LLPo, OLLn, and LLP + OLnO) were more represented in Po than in other samples, in accordance with the linoleic acid amount. By contrast, OOO was significantly higher in EVOo than in the other two oils. Percentage data of DSTAG, MSTAG and TUTAG are also reported in Table 1. No significant differences were observed for DSTAG. Po showed significantly higher MSTAG and lower TUTAG values than did the other samples; moreover, TUTAG were significantly higher in Oo than in the other oils. Main FA composition of the untreated samples is reported in Table 1. FA percentages were within the range indicated by the Commission Regulation for all categories (EC, 2003). EVOo showed significantly higher oleic acid and lower linoleic acid contents than did the other two oils. Moreover, all samples displayed an oleic acid to linoleic acid ratio higher than 7, which represents a good oxidation resistance index. By contrast, Po exhibited the highest amount of stearic acid. FA were grouped into different classes, according to their unsaturation degree (Table 1). EVOo showed higher MUFA than the other oils, whereas Po exhibited the highest amount of PUFA, in particular linoleic acid, and this is probably related to their more efficient extraction from seeds due to the application of organic solvents in the process of Po production.

Table 1
TAG and FA compositions of untreated EVOo, Po and Oo[A].

	EVOo	Po	Oo
TAG (%)			
LLL + LLPo	2.3 (0.1)^{c}	4.9 (0.2)^{a}	3.1 (0.1)^{b}
OLLn	0.8 (0.1)^{b}	1.1 (0.1)^{a}	0.9 (0.1)^{ab}
OLL + OLPo	13.5 (0.1)^{b}	13.3 (0.1)^{b}	15.5 (0.1)^{a}
LLP + OLnO	3.5 (0.2)^{b}	9.5 (0.2)^{a}	3.1 (0.1)^{b}
OLO	22.7 (0.5)^{a}	21.6 (0.2)^{b}	22.8 (0.4)^{a}
OLP + OOPo	12.7 (0.2)^{b}	13.6 (0.2)^{a}	12.6 (0.3)^{b}
POPo	1.7 (0.1)^{a}	1.8 (0.2)^{a}	1.6 (0.1)^{a}
OOO	24.8 (0.2)^{a}	20.8 (0.5)^{c}	23.8 (0.3)^{b}
SLO	12.5 (0.3)^{a}	10.7 (0.4)^{c}	11.4 (0.1)^{b}
POP	1.4 (0.2)^{a}	1.1 (0.1)^{a}	1.2 (0.1)^{a}
SOO	3.4 (0.3)^{a}	3.4 (0.3)^{a}	3.5 (0.5)^{a}
SOP	0.6 (0.1)^{a}	0.6 (0.1)^{a}	0.5 (0.1)^{a}
DSTAG	2.0 (0.3)^{a}	1.7 (0.1)^{a}	1.7 (0.2)^{a}
MSTAG	33.9 (0.5)^{b}	38.2 (0.4)^{a}	32.3 (0.6)^{c}
TUTAG	64.2 (0.2)^{b}	60.1 (0.5)^{c}	66.1 (0.7)^{a}
FA (g/100 g)			
Palmitic acid	11.0 (0.1)^{a}	10.8 (0.1)^{b}	10.4 (0.0)^{c}
Palmitoleic acid	0.9 (0.1)^{a}	0.9 (0.1)^{a}	1.0 (0.1)^{a}
Stearic acid	2.6 (0.1)^{c}	3.5 (0.1)^{a}	3.0 (0.1)^{b}
Oleic acid	76.0 (0.2)^{a}	71.8 (0.5)^{c}	75.2 (0.2)^{b}
Linoleic acid	7.5 (0.1)^{c}	8.8 (0.1)^{a}	8.3 (0.1)^{b}
Linolenic acid	0.8 (0.1)^{a}	0.9 (0.1)^{a}	0.8 (0.1)^{a}
SFA	14.2 (0.1)^{b}	15.6 (0.2)^{a}	14.2 (0.1)^{b}
MUFA	77.4 (0.1)^{a}	76.4 (0.3)^{b}	76.8 (0.4)^{b}
PUFA	8.4 (0.2)^{c}	9.6 (0.1)^{a}	9.1 (0.1)^{b}

[A] Same letters within each row do not significantly differ; standard deviation is given in parenthesis ($n = 1$, samples size = 3, $p < 0.05$).

3.2. Cooling thermograms

DSC cooling thermograms of unheated and microwave-heated oils are shown in Fig. 1A–C for EVOo, Po and Oo, respectively. The cooling thermogram of untreated EVOo exhibited two well distinguishable exothermic events, with the minor exotherm peaking at about −15 °C and the major at ∼−37 °C, as already reported (Chiavaro et al., 2007, 2008b). The major peak was previously associated with the crystallisation of highly unsaturated TAG, whilst the minor was attributed to the crystallisation of more saturated TAG fractions of EVOo (Chiavaro et al., 2007; Tan & Che Man, 2000).

Cooling profiles of the oils were largely altered by microwave treatment. In particular, the major exothermic event, whose maximum was skewed toward higher temperature in the untreated oil, became more symmetrical from 3 min of microwave treatment onwards. In addition, this peak decreased in height, starting from 12 min of treatment, spanning a greater temperature range at the highest time of heating treatment (15 min). A shift of ∼2−3 °C towards lower temperature was also observed for the major exotherm as time of microwave heating increased, becoming dramatically evident in samples microwaved for 15 min, where this event peaked at ∼−47 °C. Both peak height decrease and shift towards lower temperature were previously observed in cooling thermograms of EVOo (Vittadini et al., 2003), as a consequence of thermo-oxidation. Changes were less evident in the minor event (peaking at −15 °C), which slightly shifted towards higher temperature from 9 min of treatment onwards. This shift became evident in 15 min-treated sample, where crystallisation also developed over the largest temperature range, and it is probably related to the presence of TAG lysis products, such as diacylglycerides (DAG) and saturated free fatty acids (FFA) (Vittadini et al., 2003).

Cooling profiles of untreated Po exhibited two well distinct exothermic events with the major event shifted toward lower (∼−45.3 °C) and the minor toward higher (∼−12 °C) temperatures as compared to EVOo. This could be related to the different unsaturation degree of lipid composition between the two oils, as Po exhibited both higher amounts of unsaturated lipids (i.e. linoleic acid, PUFA, LLL + LLPo, OLLn, LLP + OLnO) and SFA than did EVOo (Table 1). Temperature regions of crystallisation were commonly found to be influenced by the degree of lipid saturation and/or unsaturation in vegetable oils (Che Man & Tan, 2002). Microwave heating treatments appeared to slightly shift the major exotherm of Po cooling thermograms towards lower temperature (∼−46.4 °C), starting from 12 min of treatment (Fig. 1B). Peak height also decreased when thermal treatment reached 9 min. The minor exothermic event slightly changed its profile, becoming less evident and shifting toward higher temperature (∼−10.1 °C) with increasing treatment time.

Cooling profiles of untreated Oo (Fig. 1C) were more similar to EVOo than Po, with the major event peaking at a lower temperature than did EVOo (∼−39.2 °C), probably as a consequence of the higher TUTAG and PUFA amounts (Table 1); the minor

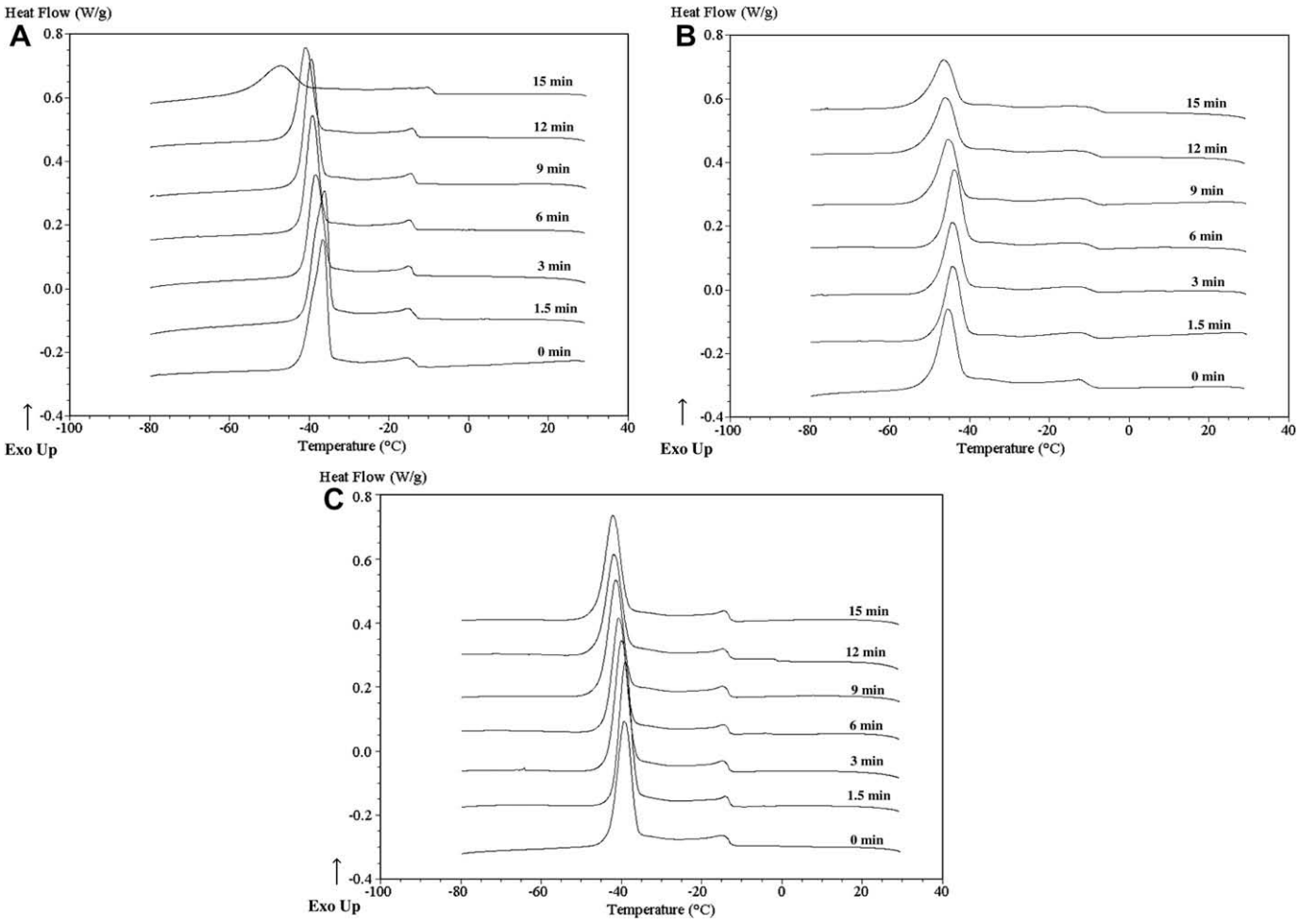

Fig. 1. DSC cooling thermograms of EVOo (A), Po (B) and Oo (C) at different microwave heating times.

exotherm peaked at – 15 °C, as did EVOo. Microwave treatment did not induce significant changes in the thermal profile of this olive oil category, except for a slight shift of major peak toward lower temperature (~3 °C), starting from 6 min of heating treatment.

Table 2 shows the thermal properties of untreated and microwave-heated oils. Crystallisation enthalpy of EVOo significantly decreased from 6 min of microwave treatment onwards. Reduction of crystallisation enthalpy was previously reported for thermo-oxidised samples of EVOo (Vittadini et al., 2003) and other vegetable oils (Gloria & Aguilera, 1998; Tan & Che Man, 1999a); this seemed to be related to the partial lysis of TAG and the formation of their degradation products (monoglycerides (MAG), DAG, FFA, oxidised TAG), which did not crystallize in the scanning temperature range of crystallisation (Gloria & Aguilera, 1998; Tan & Che Man, 1999a). FFA and secondary lipid oxidation products (PAV), which were found in relevant amounts at 6 min of treatment in EVOo (Cerretani et al., 2009), were also reported to weaken and/or hinder intermolecular bonding between TAG molecules, leading to the formation of more irregular crystals that required lower energy to undergo phase transition (Che Man & Swe, 1995; Vittadini et al., 2003). T_{on} of EVOo crystallisation was shifted toward lower temperature up to 9 min of treatment (Table 2). The formation of lipid oxidation products that altered TAG crystallisation was previously associated with temperature shift of crystallisation in EVOo (Vittadini et al., 2003) and other vegetable oils (Tan & Che Man, 1999a, 1999b). By contrast, a significant shift toward higher temperature was found in samples after 12 min of heating (Table 2). Tan et al. (2001) attributed the decrease of peak area of the highly unsaturated TAG fraction (by DSC cooling thermograms) to the formation of more saturated TAG for microwave-treated vegetable oils. An increase of saturated (16:0) and a decrease of unsaturated (18:1 and 18:2) FA profiles was also observed after prolonged microwave heating of vegetable oils by Farag, Hewedi, Abu-Raiia, and El-Baroty (1992). Lipids with high saturation degrees were reported to have higher crystallisation onset temperatures (Che Man & Tan, 2002). However, Cossignani, Simonetti, Neri, and Damiani (1998) did not find significant changes in FA composition of TAG fractions of microwave-heated EVOo samples. Increases of DAG and MAG contents were found in the same samples, though. DAG was previously found to shift crystallisation onset temperature toward a higher temperature in EVOo samples (Chiavaro et al., 2007). Prolonged microwave treatment (15 min) might have favoured TAG lysis in EVOo, due to the water content of untreated oil; this is evinced by a significant increase of FFA (Part I) (Cerretani et al., 2009), which shifted crystallisation T_{on} toward higher temperature. DAG, FFA and lipid secondary oxidation products may also have contributed to shift T_{off} of crystallisation toward lower temperature, at prolonged times of treatment, significantly enlarging the range of transition (Table 2). In particular, DAG was previously reported to influence TAG crystallisation by either accelerating or delaying crystallisation of vegetable oils and fats (Siew, 2002; Wright & Marangoni, 2002).

Enthalpy of lipid crystallisation was significantly lowered in the Po sample, already at 1.5 min of microwave treatment, being more

Table 2
DSC data obtained from the cooling thermograms of microwave-heated oils at different treatment times[A].

Time (min)	ΔH (J/g)	T_{on} (°C)	T_{off} (°C)	Range[B] (°C)
EVOo				
0	71.0 (1.7)^{a}	−12.2 (0.2)^{b}	−45.8 (0.1)^{b}	33.6 (0.2)^{cd}
1.5	70.2 (0.5)^{a}	−12.5 (0.2)^{b}	−45.5 (0.3)^{ab}	33.1 (0.4)^{d}
3	67.5 (1.9)^{ab}	−12.9 (0.2)^{c}	−45.9 (0.3)^{b}	33.1 (0.3)^{d}
6	59.9 (2.3)^{b}	−13.1 (0.2)^{c}	−46.6 (0.3)^{c}	33.3 (0.7)^{cd}
9	61.9 (2.0)^{b}	−12.9 (0.2)^{c}	−47.2 (0.4)^{c}	34.3 (0.3)^{c}
12	57.2 (2.0)^{b}	−12.3 (0.3)^{b}	−49.9 (0.5)^{d}	37.2 (0.4)^{b}
15	45.6 (0.8)^{c}	−8.3 (0.3)^{a}	−61.9 (0.3)^{e}	53.6 (0.2)^{a}
Po				
0	63.9 (1.8)^{a}	−7.6 (0.3)^{cd}	−54.3 (0.8)^{a}	46.5 (1.0)^{c}
1.5	51.1 (3.1)^{b}	−7.9 (0.2)^{d}	−54.2 (0.3)^{a}	46.3 (0.1)^{c}
3	52.4 (0.7)^{b}	−7.8 (0.1)^{d}	−54.5 (0.6)^{a}	46.7 (0.6)^{c}
6	52.3 (1.8)^{b}	−7.7 (0.1)^{d}	−54.3 (0.1)^{a}	46.5 (0.3)^{c}
9	54.2 (1.6)^{b}	−7.2 (0.0)^{bc}	−58.0 (0.5)^{b}	50.8 (0.5)^{b}
12	52.6 (1.8)^{b}	−6.8 (0.2)^{b}	−57.9 (0.5)^{b}	51.2 (0.5)^{b}
15	50.2 (2.2)^{b}	−6.2 (0.1)^{a}	−59.1 (0.4)^{c}	52.9 (0.4)^{a}
Oo				
0	70.3 (0.9)^{a}	−12.0 (0.1)^{a}	−48.0 (0.7)^{a}	36.0 (0.8)^{c}
1.5	64.9 (1.9)^{bc}	−12.2 (0.3)^{a}	−47.2 (0.1)^{a}	35.0 (0.4)^{c}
3	65.1 (1.6)^{bc}	−12.1 (0.2)^{a}	−47.8 (0.1)^{a}	35.7 (0.2)^{c}
6	63.0 (2.0)^{c}	−12.3 (0.2)^{a}	−48.0 (0.6)^{a}	35.7 (0.5)^{c}
9	65.6 (1.4)^{bc}	−12.1 (0.2)^{a}	−48.5 (0.6)^{ab}	36.4 (0.4)^{bc}
12	62.1 (2.5)^{c}	−12.0 (0.1)^{a}	−50.2 (0.6)^{b}	38.2 (0.6)^{ab}
15	64.9 (0.9)^{bc}	−12.0 (0.1)^{a}	−51.2 (1.4)^{c}	39.2 (1.3)^{a}

[A] Same letters within each column for each oil do not significantly differ; standard deviation is given in parenthesis ($n = 1$, samples size = 5, $p < 0.05$).
[B] Temperature difference between T_{on} and T_{off}.

marked than in EVOo. However, thermal properties were significantly changed only by the application of prolonged microwave treatment, as lipid crystallisation significantly started at higher T_{on} values and finished at lower T_{off} occurring over a larger temperature range. Po showed higher content of linoleic acid, PUFA and TAG esterified with this fatty acid (LLL + LLPo, OLLn, LLP + OLnO, OLP + OOPo) than EVOo (Table 1). Vegetable oils with high degrees of unsaturated fatty acids were found to be more sensitive to the effect of microwave energy (Farag, Hewedi, Abu-Raiia, & El-Baroty, 1992; Yoshida, 1993). Albi, Lanzón, Guinda, Pérez-Camino, and León (1997b) observed that thermal degradations of TAG were more abundant than were oxidative ones, especially after microwave heating, and this effect was more evident for highly unsaturated vegetable oils. It may be hypothesised that those molecular species deriving from thermal degradations of TAG (e.g. dimers, oligomers) were formed already at short microwaving times in Po, leading to the formation of more irregular crystals that required lower energy to crystallize. In addition, less formation of both secondary oxidation products (PAV) and chemical species from TAG lysis (FFA), as compared to EVOo (Cerretani et al., 2009), did not significantly change onset, offset and range of the transition, except for prolonged microwave treatment (from 9 to 15 min).

Crystallisation enthalpy of Oo significantly decreased after 1.5 min of treatment, but it was less pronounced than in Po. T_{on} remained substantially unvaried, whilst T_{off} shifted toward lower temperature, leading to the enlargement of the transition range only at the longer treatment times (12 and 15 min). The contents of unsaturated lipid molecules (linoleic acid, TUTAG, PUFA) in Oo

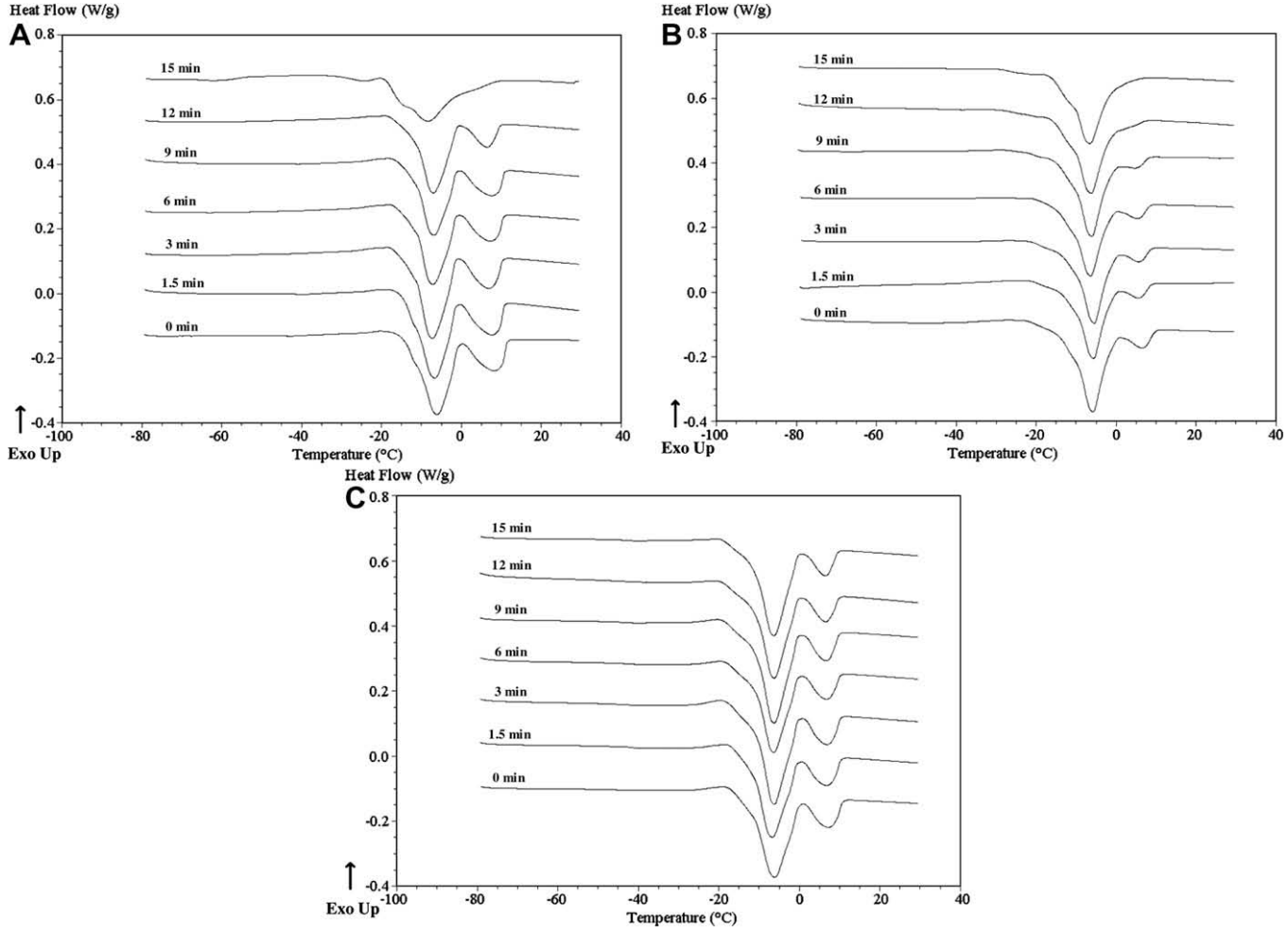

Fig. 2. DSC heating thermograms of EVOo (A), Po (B) and Oo (C) at different microwave heating times.

Table 3
DSC data obtained from the heating thermograms of microwave-heated oils at different treatment times[A].

Time (min)	ΔH (J/g)	T_{on} (°C)	T_{off} (°C)	Range[B] (°C)
EVOo				
0	77.8 (0.8)^{a}	−28.3 (1.0)^{a}	12.1 (0.2)^{a}	40.4 (0.9)^{c}
1.5	76.0 (1.7)^{a}	−27.1 (0.3)^{a}	11.4 (0.1)^{b}	38.5 (0.3)^{d}
3	74.2 (1.8)^{a}	−26.9 (0.6)^{a}	11.4 (0.1)^{b}	38.3 (0.6)^{d}
6	67.6 (2.4)^{b}	−27.1 (0.7)^{a}	11.0 (0.2)^{bc}	38.2 (0.6)^{d}
9	70.8 (1.5)^{b}	−33.9 (1.5)^{b}	10.8 (0.2)^{cd}	44.6 (1.4)^{c}
12	56.0 (1.8)^{c}	−38.2 (0.5)^{c}	10.3 (0.2)^{d}	48.5 (0.4)^{b}
15	37.3 (4.8)^{d}	−60.3 (1.1)^{d}	10.2 (0.3)^{d}	70.5 (1.2)^{a}
Po				
0	63.5 (1.8)^{ab}	−38.6 (0.2)^{cd}	10.5 (0.0)^{a}	49.1 (0.2)^{a}
1.5	62.0 (1.0)^{b}	−39.8 (0.9)^{d}	9.4 (0.3)^{b}	49.4 (1.3)^{a}
3	61.0 (2.1)^{b}	−40.1 (1.0)^{d}	9.2 (0.1)^{b}	49.1 (0.9)^{a}
6	61.8 (1.5)^{b}	−39.7 (0.5)^{d}	9.2 (0.2)^{b}	49.4 (0.7)^{a}
9	66.9 (1.8)^{a}	−28.6 (0.2)^{a}	8.4 (0.2)^{c}	36.9 (0.1)^{b}
12	65.7 (2.0)^{ab}	−29.8 (0.1)^{ab}	7.5 (0.2)^{d}	37.3 (0.1)^{b}
15	63.2 (2.3)^{ab}	−30.6 (0.2)^{b}	6.7 (0.1)^{e}	37.3 (0.4)^{b}
Oo				
0	75.6 (2.1)^{a}	−27.1 (0.2)^{a}	11.3 (0.2)^{ab}	38.3 (0.2)^{a}
1.5	73.6 (2.2)^{a}	−26.1 (1.0)^{a}	10.9 (0.2)^{bc}	37.0 (0.9)^{a}
3	71.7 (1.1)^{a}	−27.6 (0.7)^{a}	10.9 (0.2)^{bc}	38.5 (0.7)^{a}
6	71.7 (1.4)^{a}	−26.4 (0.8)^{a}	10.7 (0.1)^{c}	37.0 (0.8)^{a}
9	73.0 (1.2)^{a}	−27.4 (0.3)^{a}	10.7 (0.1)^{c}	38.1 (0.4)^{a}
12	71.6 (2.1)^{a}	−27.5 (0.4)^{a}	10.5 (0.2)^{cd}	38.0 (0.3)^{a}
15	73.1 (1.2)^{a}	−27.4 (0.3)^{a}	10.3 (0.1)^{d}	37.7 (0.4)^{a}

[A] Same letters within each column for each oil do not significantly differ; standard deviation is given in parenthesis ($n = 1$, samples size = 5, $p < 0.05$).
[B] Temperature difference between T_{on} and T_{off}.

were similar and higher than in Po and EVOo, respectively; however, Oo also displayed higher polyphenol and lower water contents than did Po (Cerretani et al., 2009), which may have partially protected TAG from microwave heating effects. Polyphenols, which have well-known antioxidant properties, were found to partially prevent thermo-oxidation of EVOo and Oo when subjected to different thermal treatments, e.g. microwave (Albi et al., 1997b). A less marked TAG lipolysis may also be hypothesised for this type of oil, as FFA did not significantly increase after microwave heating (Cerretani et al., submitted for publication). This may be related to the lower FFA and water contents of the untreated oil, as compared to those of EVOo. Other researchers found that DAG values did not significantly increase in Oo subjected to prolonged microwave treatment (Caponio, Pasqualone, & Gomes, 2002). The limited formation of molecules found to be able to hinder crystallisation of TAG (FFA and probably DAG and MAG) (Okiy, 1978; Riiner, 1971) may not have substantially changed thermal properties, even though the pattern of secondary lipid oxidation product (PAV) increase was similar to that of EVOo (Cerretani et al., 2009).

3.3. Heating thermograms

DSC heating thermograms of untreated and microwave-heated oils are shown in Fig. 2A–C for EVOo, Po and Oo, respectively. The effects of thermo-oxidation on DSC heating curves of vegetable oils have rarely been reported, probably because of the very complex lineshapes of DSC thermograms originating from the melting of several crystal polymorphic forms that are partially overlapped.

Unheated EVOo showed a melting profile similar to those previously described (Chiavaro et al., 2008a, 2008b). Three well noticeable events were found: a first exothermic event, occurring over the −30 to −15 °C temperature range and related to the transition/rearrangement of TAG polymorphic crystals into more stable forms in other vegetable oils (Tan & Che Man, 2002a), and two endotherms with maxima at −6.0 °C (A) and 8.5 °C (B), previously attributed to the melting of TUTAG and MSTAG, respectively (Chiavaro et al., 2008a). An additional endothermic event (at ∼−14 °C) was observed as a shoulder in the major peak. Microwaving induced some changes in all events of the EVOo heating thermogram (Fig. 2A). The lower temperature exothermic event spread over a larger temperature range, starting from 9 min of microwave treatment. Both endotherms were shifted toward lower temperature, even at short heating times. In particular, temperature shift was more evident (∼2 °C) for the minor endotherm at the higher temperature, which also became smaller and shorter at 12 min of treatment. The EVOo heating profile dramatically changed after 15 min of microwave treatment (Fig. 2A). Multiple exo–endothermic transitions appeared at the lower temperature region (from −60 to −20 °C) of the thermogram. In addition, the major endotherm broadened, exhibiting a decrease of peak height, whilst the minor endothermic event disappeared as it was probably embedded into the major one. It may be hypothesised that the increase of secondary lipid oxidation products (PAV), FFA and products of TAG lysis (i.e. DAG, MAG) might have led to the formation of different and less stable polymorphic crystals than in pure oil (melting at lower temperature) (Che Man & Swe, 1995; Chiavaro et al., 2008a). These molecules also made the transition/rearrangement of TAG polymorphic crystals more difficult, as previously reported (Che Man & Swe, 1995), changing the phase transition profile at the lower temperature region of the thermogram.

The Po heating profile was similar to that of EVOo (Fig. 2B), with some differences: the initial exothermic event spread over a larger temperature range (from −40 to −15 °C) and the less evident minor endothermic event peaked at a lower temperature (∼6.5 °C). A shoulder endothermic event (at ∼−11 °C) was also observed in the major endotherm with increasing microwaving time; in addition, the initial exothermic became less evident and an endothermic event also appeared at ∼−23 °C. The minor endotherm became less evident with increasing time of microwave treatment, from 9 min onwards, and it disappeared at 15 min of heating when the major event broadened, as observed for EVOo. Modifications of Po heating profile with microwave treatment time may be related to the increase of secondary lipid oxidation products (PAV), FFA and products of TAG lysis (DAG, MAG), as for EVOo. However, change of the minor endotherm appeared at a lower time of microwave treatment than with EVOo. This may be related to a higher content of such MSTAG (e.g. LLP, Table 1), which may be more sensitive to microwave heating effects, due to the presence of several double bounds in their chemical structure (Yoshida & Kajimoto, 1986).

Oo heating profiles were more similar to those of EVOo than Po (Fig. 2C), with the major and the minor endothermic events peaking at −6.0 and −7.2 °C, respectively. Heating profiles of this oil did not change significantly with increasing time of microwave exposure, except for a slight shift toward lower temperature and a sharpening of the minor endothermic peak starting from 9 min of the thermal process.

Table 3 lists the thermal parameters obtained from the DSC melting curves of all oil samples. The overall enthalpy of EVOo phase transition significantly decreased, starting from 6 min of microwave exposure. Phase transition also significantly shifted toward lower temperature and occurred over a larger temperature range, starting from 9 min of treatment. The increase of lipid oxidation products (PAV), FFA (Cerretani et al., 2009) and probably DAG, generated by TAG lysis (reported to be adsorbed into TAG crystal lattices) (Okiy, 1978; Siew & Ng, 1996), led to the formation of mixed crystals that melted at lower temperature and over a narrower temperature range than did pure TAG crystal; this results in an heterogeneous structure, that is more easily disrupted upon heating, than pure TAG, as previously observed (Che Man & Swe, 1995; Chiavaro et al., 2008a).

Enthalpy of heating transition did not significantly vary for microwave-treated Po as compared to the unheated oil (Table 3), whilst T_{on} significantly shifted toward higher temperature. This behaviour was related to change in the exothermic event profile. In this study, the enthalpy value of the total heating transition was calculated by subtracting the exothermic enthalpy from the endothermic peak enthalpies, as phase transition was considered to be from the onset of the exothermal to the offset of the endothermic events. T_{off} shifted to lower temperature and range of transition significantly narrowed, as the minor endothermic peak slowly disappeared with increasing time of treatment.

Thermal properties of heating thermograms did not significantly change in Oo (Table 3), except for a slight shift towards lower temperature of T_{off} with increasing time of treatment, as a consequence of the shift of the minor endotherm toward lower temperature; the latter might be related to the formation of lipid oxidation products. The analysis of phase transition profile and thermal properties under heating confirmed that Oo seemed more resistant to microwave effects than were the other two oils, even though lipid oxidation (PAV) appeared to be comparable (Cerretani et al., 2009).

4. Conclusions

The results of this study showed that the evaluation of cooling thermograms is very promising in estimating the oxidation and the degradation state induced by the microwaving of olive oils, as significant changes were found in both phase transition profiles and derived thermal properties at different microwave exposure times. However, heating profiles seemed to be less informative, due to their complexity, as changes at lower times of treatment were clearly evinced only in the minor endotherm peaking at the highest temperature region of thermograms.

DSC may also potentially discriminate amongst olive oils that exhibit different resistances to thermo-oxidative effects of microwave heating. This is possibly related, not only to major (e.g. TAG, fatty acids) but also to minor (e.g. water, phenols, lipid oxidation products) chemical components and is clearly shown by the evaluation of both cooling and heating thermograms. This could be very important for DSC evaluation of thermo-degradative effects of microwaves on complex food systems prepared with different olive oils. These findings must be confirmed by the analysis of a larger number of samples, taking into account the incidence of different amounts of such minor chemical components (e.g. phenolic compounds, water) of olive oils that play an important role in microwave resistance and which are ascribable to olive cultivar, geographical origin, harvesting period, and agronomical and processing practices (e.g. refining).

Further and more detailed information on alterations of crystal forms of olive oils induced by microwave heating could be obtained by coupling DSC with X-ray diffraction.

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