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Microwave heating of different commercial categories of olive oil: Part I. Effect on chemical oxidative stability indices and phenolic compounds

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ABSTRACT

The effect of microwave heating of extra virgin olive oil (EVOo), olive oil (Oo) and pomace olive oil (Po) in domestic appliances, was investigated in terms of chemical oxidative indices (peroxide, *p*-anisidine and Totox values), free acidity, water content, total phenol content and different classes of phenolic compounds.

Water content of unheated EVOo was higher as compared to the other two oils and it was found to decrease with increasing treatment time in all oils, especially in EVOo. Lipolysis was noticeable in EVOo only at highest treatment times. *p*-Anisidine values showed a sinusoidal trend in EVOo and Oo with a maximum at 6 and 15 min, respectively, while they gradually increased in Po.

Significant amounts of phenols were detected only in unheated EVOo, even though about 30% were lost after 6 min. Among the different classes of phenolic compounds, *o*-diphenols and lignans displayed the highest microwave heating resistance in the whole time range of microwave application, which is of great importance from a nutritional standpoint. The utilisation of Po may be encouraged especially at short microwave treatment times for both domestic and food catering applications.

1. Introduction

The use of the microwave has been largely applied in many food processes, such as cooking, thawing, blanching, dehydration, pasteurisation, sterilization and tempering (Rosenberg & Bogl, 1987). Microwaves are commonly found in both domestic and food catering operations and are very popular for high speed and convenience, as compared to conventional heating treatments (Burfoot et al., 1990). Their effects on macro- and microcomponents, as well as on flavour formation and colour changes in several foods, have been extensively evaluated (Vikram, Ramesh, & Prapulla, 2005). The influence of microwave heating on the thermo-oxidative stability of common oils and fats in household use, was also investigated. Different vegetable oils subjected to various heating times and microwave powers exhibited the formation of reactive free radicals that rapidly reacted with the atmospheric oxygen to produce hydroperoxides and secondary oxidation products (Albi, Lanzón, Guinda, León, & Pérez-Camino, 1997a; Farag, Hewedi, Abu-Raiia, & El-Baroty, 1992; Hassanein, El-Shami, & El-Mallah, 2003; Lie Ken Jie & Yan-kit, 1988). Other chemical effects were observed: free fatty acids moderately increased in microwaved vegetable oils as a consequence of fat hydrolysis (Albi et al., 1997a; Farag et al., 1992; Hassanein et al., 2003), tocopherols decreased (Albi et al., 1997a; Hassanein et al., 2003; Yoshida, Hirooka, & Kajimoto, 1990), and fatty acid isomerization (*trans* formation) was contradictory (Mai, Tsai, Armbruster, Chu, & Kinsella, 1980). Density and viscosity of oils were reported to increase and the higher viscosity was directly related to the formation of triacylglycerol and fatty acid dimers and polymers, whereas the density raise was ascribable to oxygen incorporation into oxidation compounds (oxidised triacylglycerols) (Albi, Lanzón, Guinda, Pérez-Camino, & León, 1997b). The development of oil rancidity and oxidative alterations were found to be faster during microwave treatment than in conventional oven heating and deep fat frying (Albi et al., 1997a; Farag et al., 1992). This was referred to a combination of temperature and energy effects induced by microwaves, which could have strengthened the effect of heating as a consequence of the internal friction of the molecules leading to zonal overheating in the oils (Albi et al., 1997a).

Oils produced from olive fruit represent the main fatty substance in the Mediterranean diet and their consumption represents more than 50% of all edible oils in this area. Among different commercial categories of olive oils, four of them are intended for direct human consumption: extra virgin olive oil, virgin olive oil, olive oil and pomace olive oil. Extra virgin or virgin olive oil were recently defined by the European Community Regulation as "obtained from

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the fruit of the olive tree solely by mechanical or other physical means under conditions that do not lead to alteration in the oil." (EC, 2001). In particular, extra virgin olive oil (EVOo) (free acidity <0.8 g/100 g) is recognised by the EC Regulation as the product with the highest quality among different commercial categories of olive oils (EC, 2003). Olive oil (Oo) (free acidity <1.0 g/100 g) is obtained by blending virgin olive oil with refined olive oil. Pomace olive oil (Po) (free acidity <1.0 g/100 g) is a blend between virgin olive oil and refined pomace olive oil, which is produced by refining crude olive pomace oil, previously extracted with solvents from pomace (solid by-product). EVOo is well known for its beneficial biological activity on human health, because of the presence of phenolic compounds (Menéndez et al., 2007; Visioli & Galli 2001). The content of these compounds is related to the olive cultivar, geographical origin, seasonality, agronomical and technological conditions of oil production and storage (Gallina Toschi, Cerretani, Bendini, Bonoli-Carbognin, & Lercker, 2005). The phenolic compound content is an important parameter in evaluating olive oil quality, since phenols are strictly related to both the oil's resistance to oxidation (because of their antioxidant properties) and the typical bitter and pungent tastes of this type of product (Bendini et al., 2007). In particular, the antioxidant effect is mainly exerted by the *ortho*-diphenolic components (i.e. hydroxytyrosol and oleuropein aglycone derivatives) (Bester, Butinar, Bucar-Miklavcic, & Golob, 2008; Carrasco-Pancorbo et al., 2005).

Few reports dealt with changes in olive oil composition and phenolic compounds due to microwave heating. Cossignani, Simonetti, Neri, and Damiani (1998) evaluated the changes induced by prolonged microwave heating on traditional quality parameters (free acidity, peroxide value and ultraviolet absorbance indices), fatty acid (FA) and triglyceride (TAG) composition of EVOo, finding a significant decrease of TAG, increase of monoglyceride (MAG) and diglyceride (DAG) fractions and higher free acidity and peroxide value in microwaved oils with respect to untreated samples. More recently, Caponio, Pasqualone, and Gomes (2002) compared microwave and conventional heating in Oo used in model pizza dough, finding significantly higher polar compounds, TAG oligopolymers and oxidised TAG after microwave treatment. Brenes, García, Dobarganes, Velasco, and Romero (2002) evaluated phenolic content of EVOo from different Spanish cultivars after microwave and conventional heating, finding minor losses of phenolic compounds after 10 min microwave treatment, as well as lower oil degradation with respect to thermo-oxidation assays. A higher decrease of total phenolic content was observed by Albi et al. (1997a) in olive and virgin olive oil after prolonged microwave treatment (120 min) as compared to conventional oven heating; these modifications were actually more marked in virgin olive oil than in olive oil.

The aim of this work was to evaluate the changes related to hydrolytic and oxidative degradation (free fatty acids, peroxide and *p*-anisidine values) induced by different times of microwave heating on three commercial categories of olive oil intended for direct human consumption (EVOo, Oo and Po). In addition, modifications of water content and phenolic compounds were also considered, in particular changes related to three phenolic fractions (simple phenols, secoiridoids and lignans). These hydrolytic and oxidative chemical parameters were also related to thermal properties obtained by means of DSC on the same samples and the results are reported in Part II (Chiavaro et al., 2009).

2. Materials and methods

2.1. Samples

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. Oo and Po were donated by Coppini Arte Olearia (Parma, Italy) and obtained from olives picked in Trapani (Sicily, Italy) in 2006, as well. Samples were stored in dark bottles without headspace at room temperature before analysis. One sample for each olive oil category was subjected to microwaving and analysed.

2.2. Microwave heating treatment

A domestic microwave oven was used for sample treatment (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 subjected to microwaving for 1.5, 3, 6, 9, 12 and 15 min. Temperature of the oils 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. All heated samples were allowed to cool at room temperature (23 ± 1 °C) for 60 min after thermal treatment and before chemical analysis. The two 90 ml aliquots of each oil were combined after microwaving, in order to obtain a homogeneous sample used for both chemical and thermal analysis (Part II; Chiavaro et al., 2009).

2.3. Solvents, reagents, stock solutions and reference compounds

All solvents used were of analytical or high-performance liquid chromatography (HPLC) grade (Merck, Darmstadt, Germany). Hydranal-solvent oil, hydranal-titran 2, hydranal water standard 0.10 and 3,4-Dihydroxyphenylacetic acid (3,4-DHPAA) were purchased from Sigma Aldrich (St. Louis, MO), whereas oleuropein (oleuropein glucoside) and hydroxytyrosol were supplied by Extrasynthèse (Genay, France). The stock solution of 3,4-DHPAA was prepared in methanol:water (50:50, v/v) at a concentration of 500 μg/ml and was used to build a calibration curve for phenols quantification. Distilled water with a resistance of 18.2 MΩ was deionized by using a Milli-Q system (Millipore, Bedford, MA).

2.4. Water content

Water content was analysed with a TitroMatic 1S instrument (Crison, Barcelona, Spain) based on a Karl-Fischer titration with a bivoltametric indication (2-electrode potentiometry). A solution of chloroform:hydranal-solvent oil (a methanolic solvent) (2:1, v/v) was used to dissolve the sample, whereas hydranal-titran 2 was utilised as the titrating reagent. Hydranal-titran 2 was previously titrated using a hydranal water standard. The water content was expressed as mg/kg of oil. Loss of water was calculated as the percent content difference between the unheated and heated oil sample, with respect to the initial moisture content. Three replicates were analysed per sample.

2.5. Determination of free acidity (FA), peroxide value (POV), p-anisidine value (PAV) and Totox value (TV)

FA content (expressed as % oleic acid) and POV (expressed as meq O_2/kg lipids) were evaluated according to the official methods described in annex III of EEC Regulation 2568/91 (EEC, 1991). PAV was determined according to the IUPAC standard method 2.504, by measuring absorbance at 350 nm (IUPAC, 1987). Totox value was calculated based on POV and PAV, by using the following equation (Idris, Abdullah, & Halim, 1992):

Totox value = 2POV + PAV

For each determination, three replicates were analysed per sample.

2.6. Liquid–liquid extraction (LLE) and HPLC determination of phenolic compounds

Phenolic compounds were extracted from oil samples by LLE, using a modified version of the method suggested by Pirisi, Cabras, Falqui Cao, Migliorini, and Mugelli (2000). Briefly, 4 g of oil (± 0.001 g) were dissolved in 4 ml of *n*-hexane, and the solution was extracted successively twice with four 2 ml portions of a methanol:water (60:40, v/v) solution. The combined extracts of the hydrophilic layer were concentrated and dried by evaporative centrifuge (Mivac Duo of Genevac Inc., Valley Cottage, NY) at a temperature of 40 °C. Finally, the residue was redissolved in 0.5 ml of methanol:water (50:50, v/v) and filtered through a 0.45 μm filter (Whatman, Clifton, NJ). HPLC analyses of phenolic extracts were performed with a HP 1100 series (Agilent Technologies, Palo Alto, CA), equipped with a binary pump delivery system, a degasser, an autosampler, a diode array UV–vis detector (DAD), and a mass spectrometer detector (MSD). The HPLC column used was a 5 μm C_{18} Luna column (25 cm × 3.0 mm) (Phenomenex, Torrance, CA), with a C_{18} pre-column (Phenomenex) filter. The mobile phases were water with formic acid (0.5%) (Phase A) and acetonitrile (Phase B) and a gradient was applied according to the method by Carrasco-Pancorbo et al. (2005) at a flow rate of 0.5 ml/min. The injection volume was 10 μl. The detection was made using MS as well, and the analyses were carried out using an electrospray (ESI) interface operating in positive mode using the following conditions: drying gas flow, 9.0 l/min; nebulizer pressure, 50 psi; gas drying temperature, 350 °C; capillary voltage, 3000 V; fragmentor voltage, 60 V. Phenolic compounds were identified based on their UV–vis and mass spectra obtained by HPLC-DAD/ESI-MSD, according to Carrasco-Pancorbo et al. (2005). The phenolic compounds were quantified using the calibration curve of 3,4-DHPAA at 280 nm for all compounds, except for elenolic acid which was registered and quantified at 240 nm.

2.7. Statistical analysis

Data were analysed using SPSS (Version 14.0, SPSS Inc., Chicago, IL) statistical software. SPSS was used to perform one-way-analysis of variance (ANOVA) and Least Significant Difference test (LSD) at a 95% confidence level ($p \leqslant 0.05$) to identify significant differences among samples within the evaluated parameters.

3. Results and discussion

The different microwave treatments applied to the oils in this study induced a temperature increase in the samples that was proportional to the treatment time, as expected. Temperatures of the samples at the end of the microwave treatments are reported in Table 1.

3.1. Water content and oxidative indices

Water content of unheated oil was 786, 496 and 605 mg/kg of oil for EVOo, Oo and Po, respectively. The lower water content of Oo and Po was expected, as water content is known to be reduced by refining (Cerretani, Bendini, Barbieri, & Lercker, 2008), while difference of water content between Oo and Po may be ascribable to the EVOo amount blended. Water loss (%) in EVOo (A), Oo (B) and Po (C), are shown in Fig. 1. In all oils, water loss increased from 1.5 to 3 min of treatment, being more marked in EVOo and Po. Further water loss was observed in EVOo from 9 min onwards, reaching about 60%. Oo showed a linear increase of water loss up to 9 min of treatment, attaining about 52%, and remained practically steady thereafter. On the contrary, water loss in Po (about 55%) stayed substantially unvaried, from 3 min of treatment onwards.

Table 1
Mean temperature for oil samples at different treatment times.[a]

Treatment time (min)	Temperature (°C)
1.5	145.6 (2.2)
3	181.3 (3.2)
6	255.9 (2.1)
9	293.7 (7.4)
12	305.5 (6.9)
15	313.4 (3.7)

[a] Standard deviation is given in parenthesis ($n = 1$, sample size = 3).

Changes of FA and other chemical oxidative indices as a consequence of microwave treatment are shown in Fig. 2. FA (Fig. 2A) of untreated EVOo was higher that those of Oo and Po, as they were prepared by blending EVOo with refined olive oil and refined pomace oil, respectively. Lipolysis was significantly noticeable only at the longer treatment times (12 and 15 min), being more pronounced in EVOo as compared to the other oils. This could be related to the higher water content of this oil category, which may have favored TAG hydrolysis at temperatures ⩾300 °C (Table 1) for extended microwave heating. A moderate FA increase was generally found in vegetable oils after microwave heating at prolonged treatment time (Albi et al., 1997a; Farag et al., 1992; Vieira & Regitano-D'Arce, 1998). Cossignani et al. (1998) and Brenes et al. (2002) found that FA did not significantly increase in EVOo after 10 min of treatment, under similar microwave conditions. Albi et al. (1997a) did not find any FA increase after 120 min of microwave heating at half power (170 °C) in both EVOo and Oo.

POV values (Fig. 2B) of unheated EVOo, Oo and Po were 21.8, 20.3 and 10.6 meq O_2/kg, respectively. POV of EVOo and Oo were slightly higher than their legal limit of 20 and 15 meq O_2/kg (EC, 2003), respectively. POV values of the three commercial categories of olive oil intended for human consumption were generally higher than those of other vegetable oils, as the EVOo used to make Oo and Po, was produced without refining processes, which partially remove oxidation compounds.

POV of EVOo, Oo and Po (Fig. 2B) greatly decreased up to 6 min of heating (76%, 78% and 85%, respectively) and remained approximately constant until the end of the heating treatment (∼4–5 meq O_2/kg for all three oils). This may be related to the fast heating due to microwave exposure (Table 1), which has both decreased the oxygen availability and shifted the oxidation reaction balance towards a greater formation of secondary oxidation products.

Literature reports contradictory results about POV values of microwave treated vegetable oils. Albi et al. (1997a) found a small POV increase in EVOo after microwave heating at 170 °C for 120 min, while POV decreased in Oo under the same heating conditions. Cossignani et al. (1998) reported a POV increase in EVOo after microwave heating exposure (8 min at medium-power). Vieira and Regitano-D'Arce (1998) also found higher POV values with short periods (4–6 min) of microwave heating as compared with prolonged exposure, while Tan and co-workers observed that hydroperoxide formation was more pronounced at low-power setting than at medium or high microwave power in corn and soybean oils (Tan, Che Man, Jinap, & Yusoff, 2001), as well as in palm olein (Tan, Che Man, Jinap, & Yusoff, 2002). Exposure to microwave energy was reported to favour the formation of free radicals (Farag et al., 1992). However, peroxides usually undergo further degradation not only at high but also at low temperature

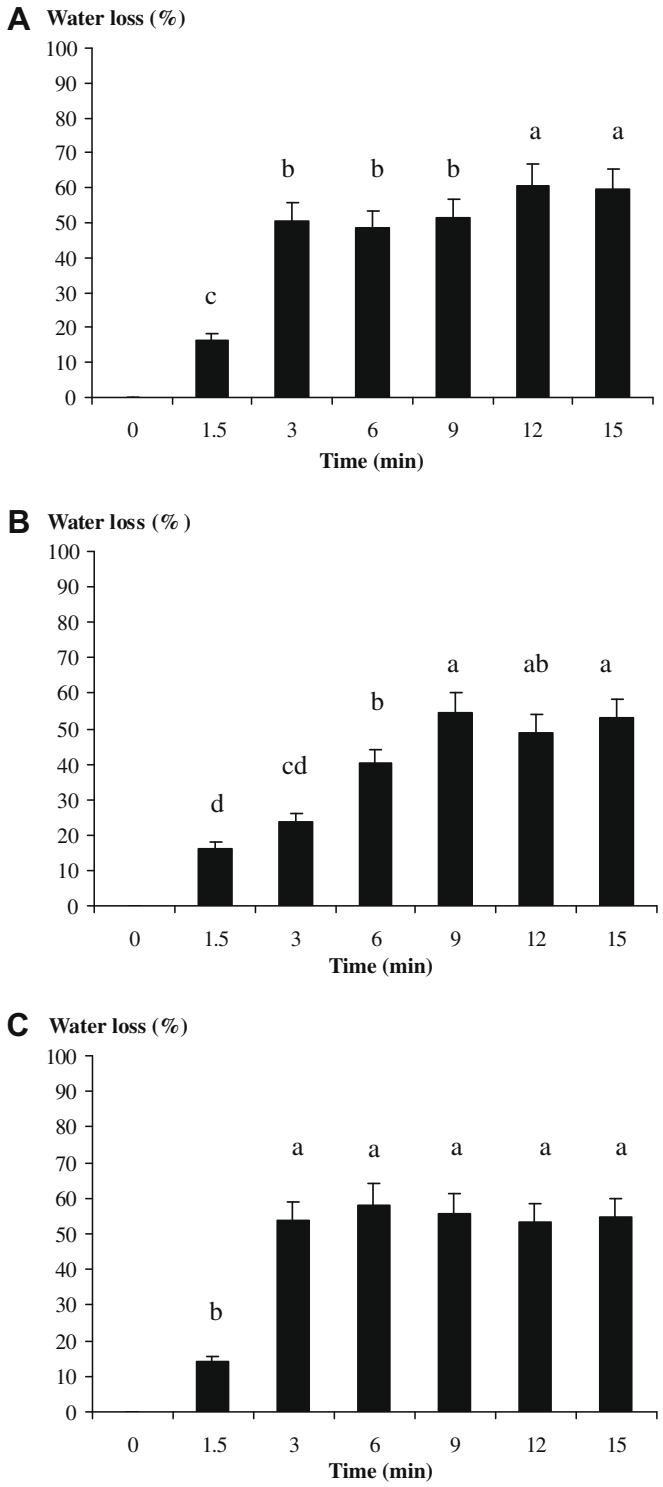

Fig. 1. Water loss (%) after microwave heating treatment in EVOo (A), Oo (B) and Po (C). Error bars represent ±10% relative standard deviation, (n = 1, samples size = 3). Bars with the same letters are not significantly different ($p \leqslant 0.05$).

(Paul & Mittal, 1997). In the present study, at the beginning of the experiments (0 min), the three oils exhibited medium–high POV values, which registered a rapid decrease during microwave treatment. It might be possible that, under these experimental conditions, the rate of the hydroperoxide demolition reaction, that leads to the formation of secondary oxidation products, exceeds that of hydroperoxides generation.

The formation of secondary oxidation products was determined by the *p*-anisidine test. PAV values (Fig. 2C) showed a sinusoidal trend for EVOo and Oo with a maximum at 6 and 15 min of treatment, respectively. However, a significant increase of PAV was found for Po, starting from 3 to 12 min of treatment, and it remained unvaried until the end of the heating treatment. It must be noted, though, that the PAV of Po at 15 min was significantly lower that those of the other two oils. Yoshida et al. (1990) found an increase of PAV values for olive and other vegetable oils, after 6 min of microwave exposure. Tan et al. (2001, 2002) also reported a PAV increase for corn oil, soybean oil and palm olein, which was more marked after 8 min of microwave heating at both medium and high power settings. The trends exhibited by both EVOo and Oo may be related to the different antioxidant properties of phenolic compounds present in these two oil categories (see below), according to chemical modifications induced by thermo-oxidation; in fact, it is well known that phenols display different antioxidant properties according to their chemical properties (Carrasco-Pancorbo et al., 2005). The oxidative status for all oils was also evaluated by the Totox index (Fig. 2D), which was obtained by considering both POV and PAV contribution in order to give a more complete picture of the EVOo and Oo oxidative trends. Totox values of unheated oils were high, since olive oils generally exhibit higher POV values than other vegetable oils. Heated EVOo and Oo displayed a similar Totox trend; the values did not increase gradually and constantly, but showed two maximums at 3 and 15 min for EVOo and 6 and 15 min for Oo. On the contrary, the Totox values of Po were found to increase after 1.5 min of treatment, even though it remained significantly lower than those of the other two oils up to 6 min of treatment. The characteristic sinusoidal Totox trend shown by EVOo and Oo may be related to a different degree of the antioxidant action exhibited by phenolic compounds that were found in detectable amounts only in EVOo and Oo but not in Po (see above). The significant loss of water at 3 and 6 min (Fig. 1A and B) in EVOo and Oo, respectively, may have destabilized hydrophilic phenols, especially hydroxytyrosol, that were found to exhibit the highest antioxidant activity (Carrasco-Pancorbo et al., 2005), and/or favoured chemical transformation of phenols leading to species with no antioxidant properties.

3.2. Phenolic compounds

Untreated and microwave heated EVOo, Oo and Po were analysed by HPLC-DAD/MSD to quantify total phenols and to evaluate change on their profiles induced by the microwaves.

To the authors' best knowledge, only one study (Brenes et al., 2002) has reported the effect of microwave heating on phenols. Total phenolic content in the pure oil samples was different: a medium–low amount of phenols was found in EVOo (93 mg/kg of oil), which is within the common range for commercial extra virgin olive oil, while Oo exhibited a lower content (13 mg/kg of oil). No detectable amount of phenols was found in Po (limit of detection = 0.1 mg/kg for each phenol type) since, as already mentioned for Oo, refining processes lead to a reduction of these compounds. HPLC chromatograms obtained for unheated EVOo, Oo and Po are reported in Fig. 3.

Phenolic compounds decreased in both EVOo and Oo with increasing microwave heating time as shown in Fig. 4A. Total phenolic content of EVOo showed a gradual reduction to reach 67 mg/kg of oil at 6 min of treatment (−28%), then a further decrease to 17 mg/kg of oil (−83%) at the highest heating time. Phenols of Oo remained substantially unaltered at the lowest treatment times (1.5 and 3 min), but they showed a marked loss at 6 min of heating (−40%, 7.8 mg/kg of oil) as compared to EVOo. A further decrease was observed thereafter, leading to undetectable levels of phenols in Oo after 15 min of microwave exposure.

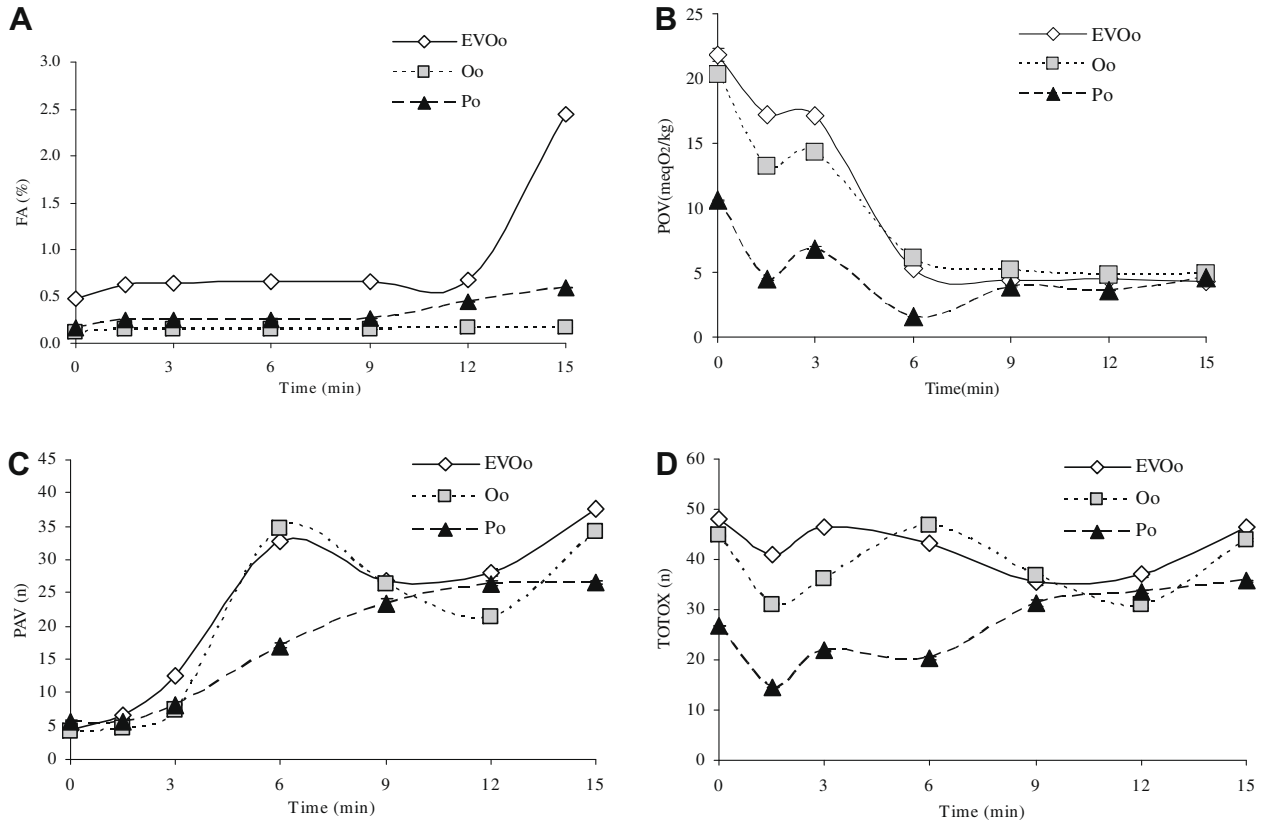

Fig. 2. Changes in oxidative indices after microwave heating treatment in EVOo, Oo and Po; free acidity (A), POV (B), PAV (C), Totox (D) (n = 1, samples size = 3).

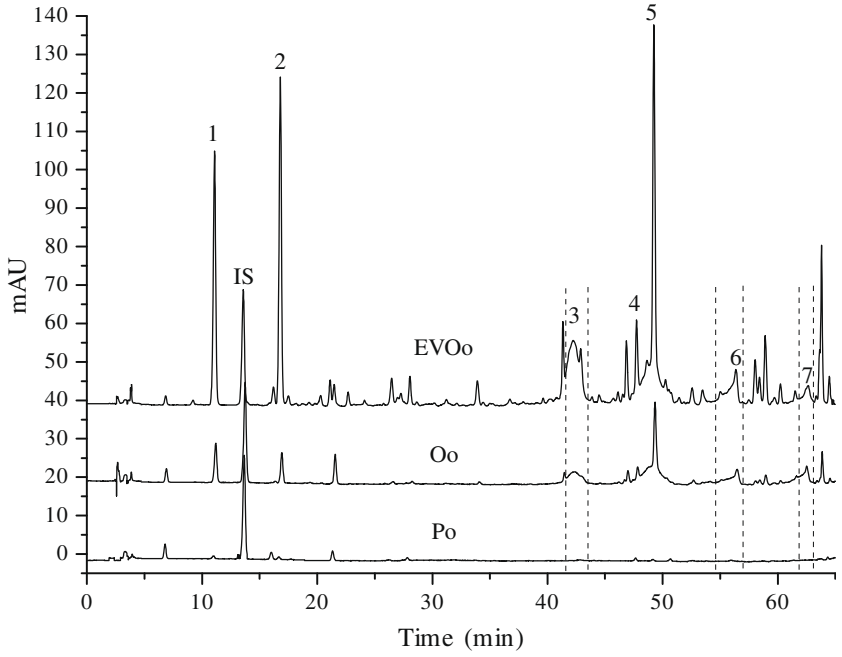

Fig. 3. HPLC chromatograms of the phenolic profile of unheated EVOo, Oo and Po. Detection was performed at 280 nm. Peak identification: HYTY, hydroxytyrosol. (1); IS, 3,4-diydroxyphenylacetic acid (internal standard); TY, tyrosol (2); DOA, isomeric forms of decarboxymethyl-oleuropein aglycon (3); Pin, (+)-pinoresinol (4); AcPin, 1-(+)-acetoxypinoresinol (5); OLA, isomeric forms of oleuropein aglycon (6); LA, ligstroside aglycon (7).

Albi et al. (1997a) also reported a great drop of total phenol content in both EVOo (about 25 times) and Oo (about 6 times), after prolonged microwave heating (120 min at 170 °C).

Phenolic compounds were also identified and classified as phenylethanol derivatives (hydroxytyrosol, HYTY, and tyrosol, TY), secoiridoids (decarboxymethyl-oleuropein aglycone, DOA,

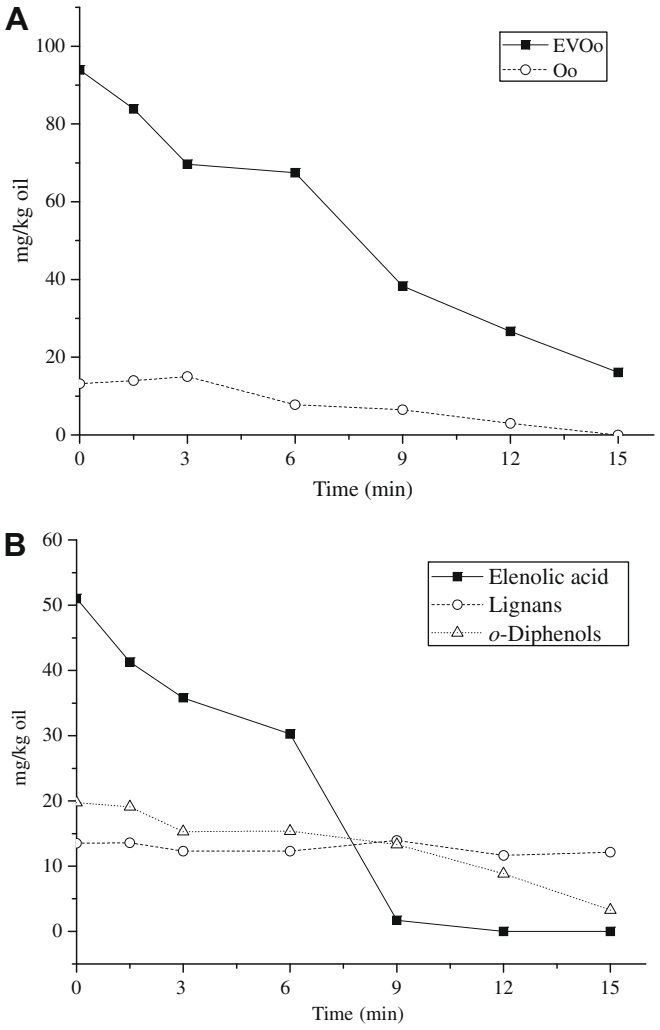

Fig. 4. (A) Total phenolic compound content (mg/kg oil) of EVOo and Oo at different times of microwave treatment. (B) Different fraction content (mg/kg oil) of phenolic compounds in EVOo, at different times of microwave treatment.

oleuropein aglycone, OA, ligstroside aglycone, LA, oleocanthal or decarboxymethyl ligstroside aglycone, OL), lignans ((+)-pinoresinol, Pin and 1-(+)-acetoxypinoresinol, AcPin) and elenolic acid (EA). Table 2 only reports data of EVOo, because it showed the highest amount of total phenols and, consequently, the loss of these compounds (Var%) was more evident than in Oo.

HYTY remained unaltered after 1.5 min of microwave heating and significantly decreased from 6.6 to 4.9 mg/kg of oil (−26.4%) after 3 min of treatment, reaching 3.6 mg/kg of oil after 9 min (−46%) and 0.4 mg/kg of oil (−94%) at the end of the treatment (15 min) (Table 2). Brenes et al. (2002) found 20 and 30% of HYTY loss in EVOo from Spanish cultivars after 5 and 10 min of microwave heating at a lower power setting, respectively. On the other hand, TY decreased from 7.7 to 7.3 mg/kg of oil (−5.2%) after 9 min of heating, to reach 2.9 mg/kg of oil (−62%) after 15 min. The same authors did not observe a reduction of TY in EVOo, after 10 min of microwave heating (Brenes et al., 2002).

OA significantly dropped from 3.4 to 1.7 mg/kg of oil (−50%) after 6 min of microwave heating, to finally disappear at the longer treatment times (12 and 15 min) (Table 2). However, Brenes et al. (2002) only observed a 20–30% decrease of OA, which might be due to the lower power setting employed by these authors that induced less chemical changes in this compound.

The time-dependent loss trend of the three different phenol classes in EVOo, is shown in Fig. 4B. Phenol compounds that were found to show greater antioxidant activity, as *o*-diphenols, HYTY, DOA and OA (Bester et al., 2008; Carrasco-Pancorbo et al., 2005), appeared to be more resistant to microwave exposure. In previous work, concentration of *o*-diphenols in EVOo decreased more quickly than other phenolic compounds, when subjected to conventional thermal treatment at 180 °C (Carrasco-Pancorbo et al., 2007). If phenol data of both studies are compared, microwave heating seems to be a less degradative treatment: the percentage variation of phenolic content after 12 min of microwave heating (∼72%) (T = 306 °C; see Table 1) was lower than that obtained in a conventional oven at 180 °C for 120 min (∼79%), while 85% of phenolic loss was found after 15 min of microwave exposure (T = 313 °C; see Table 1) and 180 min of conventional oven heating at 180 °C. Brenes et al. (2002) also reported a significant decrease of *o*-diphenols after heating at 180 °C and related this reduction to thermal destruction or oxidative degradation of these compounds. Phenol degradation compounds seem to be present at trace levels after 15 min of microwave treatment, as shown in Fig. 5. Partial identification was achieved by comparing their retention times with those of the oxidised phenols previously found after conventional oven treatment at 180 °C (Carrasco-Pancorbo

Table 2
Phenolic and their derivatives content (expressed as mg 3,4-DHPAA/kg of oil) of EVOo at different times of microwave treatment.

		0 min	1.5 min	3 min	6 min	9 min	12 min	15 min
HYTY	Mean[A]	6.6 a	6.7 a	4.9 b	4.9 b	3.6 c	2.5 d	0.4 e
	Var (%)[B]	0.0	1.1	−26.4	−25.0	−46.0	−62.7	−93.6
TY	Mean	7.7 a	7.9 a	6.3 bc	7.7 a	7.3 ab	6.0 c	2.9 d
	Var (%)	0.0	2.7	−17.9	−0.3	−5.2	−22.2	−61.8
EA	Mean	51.0 a	41.3 b	35.8 cd	30.3 d	1.7 e	n.d.	n.d.
	Var (%)	0.0	−19.0	−29.8	−40.7	−96.6	−100.0	−100.0
DOA	Mean	9.8 a	9.8 a	8.1 c	8.7 bc	9.6 ab	6.4 d	2.9 e
	Var (%)	0.0	0.0	−17.1	−10.6	−2.2	−34.7	−70.7
Pin	Mean	2.1 a	2.1 a	1.6 b	1.4 b	2.0 a	1.4 b	1.8 ab
	Var (%)	0.0	0.0	−23.4	−30.2	−2.4	−33.2	−13.2
AcPin	Mean	11.5 ab	11.5 ab	9.6 d	10.9 bc	12.0 a	10.3 cd	10.4 cd
	Var (%)	0.0	0.7	−16.1	−5.1	4.3	−10.2	−9.6
OA	Mean	3.4 a	2.7 b	2.2 cd	1.7 d	0.2 e	n.d.	n.d.
	Var (%)	0.0	−21.6	−35.8	−49.7	−93.8	−100.0	−100.0
LA	Mean	0.8 b	1.2 a	0.9 b	1.0 ab	0.8 b	0.3 c	n.d.
	Var (%)	0.0	52.5	15.0	27.5	−3.8	−68.8	−100.0

HYTY, hydroxytyrosol; TY, tyrosol; AE, elenolic acid; DOA, decarboxymethyl-oleuropein aglycon; Pin: (+)-pinoresinol; AcPin: 1-(+)-acetoxypinoresinol; OLA, oleuropein aglycone; LA, ligstroside aglycone; n.d. not detected.

[A] [a,b,c,d,e] Same letters within each row do not significantly differ ($n = 1$, sample size = 3, $p \leqslant 0.05$); RSD ⩽ 9.4.

[B] Var (%): percentage of variation respect to unheated EVOo.

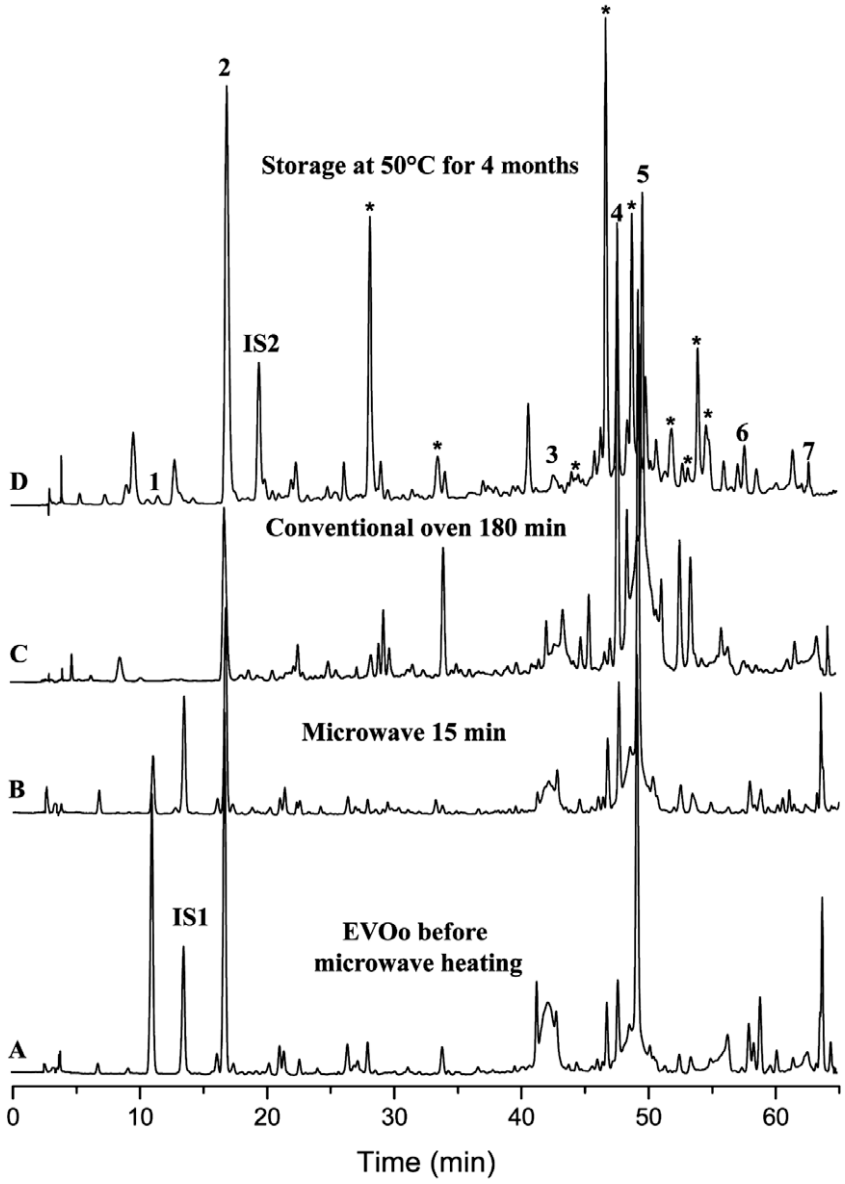

Fig. 5. HPLC analyses of four EVOo samples subjected to thermal treatment or storage conditions. (A) untreated EVOo; (B) EVOo after 15 min microwave treatment at 720 W (2450 MHz); (C) EVOo after treatment at 180 °C for 2 h in a conventional oven (from Carrasco-Pancorbo et al., 2007); (D) EVOo maintained at 50 °C for 4 months (from Armaforte et al., 2007). For peak identification see Fig. 3; IS1, 3,4-dihdroxyphenylacetic acid (internal standard 1); IS2, siringic acid (internal standard 2). The "unknown compounds" formed by phenol degradation, are marked with an asterisk.

et al., 2007), as well as after storage at 50 °C for several months (Armaforte et al., 2007). However, due to their low presence level, it was not possible to obtain neat mass spectra that confirmed their identification. It would be necessary to perform phenol extraction from a larger oil amount (60 g instead of 4 g) (Carrasco-Pancorbo et al., 2007), in order to get a higher concentration of the oxidised phenols with reduced signal-to-noise ratio that would allow their identification with the mass spectra. On the other hand, it is also possible that different heating rates lead to diverse availability of oxygen, thus affecting lipid oxidation in a different manner due to the action of antioxidants as phenolic compounds. In any case, the chemical pathway of the thermo-degradative reactions of these antioxidant compounds needs further investigation, as well as the formation, chemical structure and behaviour of oxidised phenols.

Among secoridoids, LA remained substantially unvaried (Fig. 4B) as reported by Brenes et al. (2002). The content of lignans (Pin and AcPin), that were previously found to exhibit limited antioxidant activity (Carrasco-Pancorbo et al., 2005), displayed a slight decrease (−13% for Pin and −9.6% for AcPin) after 15 min of microwave treatment. Brenes et al. (2002) did not find a significant lignan decrease after 10 min of microwave heating. A high resistance to thermal treatments was also exhibited by lignans after conventional thermal treatment at 180 °C (Brenes et al., 2002; Carrasco-Pancorbo et al., 2007). This result is of great importance as these phytoestrogens were reported to exhibit beneficial effects on human health (Schouw, Kleijn, Peeters, & Grobbee, 2000).

EA significantly decreased with increasing microwave treatment time, reaching −40% at 6 min of treatment and disappearing from 12 min onwards. A marked decrease was observed between 6 and 9 min (Fig. 4B), when the temperature exceeded 255 °C. This is probably due to its high thermo-lability, as previously observed in oven heating at 180 °C (Carrasco-Pancorbo et al., 2007).

4. Conclusions

These preliminary results show that the application of microwave heating may induce oxidative alterations, especially in EVOo and Oo. Thus, the utilisation of a cheaper olive oil, such as Po, may be encouraged especially for short microwave treatment times (1.5–6 min), that are commonly employed in domestic and food catering applications. On the other hand, the relatively high microwave stability exhibited by *o*-diphenols and lignans at the same range of treatment time, may be of great importance from a nutritional standpoint, due to the high antioxidant properties of *o*-diphenols and the beneficial effects of lignans on human health (Bendini et al., 2007). Due to their high thermal stability, lignans are particularly abundant in EVOo before and after heating treatment.

Lipolysis was significant only at the longest treatment times, especially in EVOo, and it seemed to be directly related to the water content of oils. In addition, the interaction between water content loss and stability and/or degradation of phenolic compounds into less stable and more active antioxidant forms needs further investigation, as this could be of great importance for the evaluation of the nutritional impact of microwaves on complex food systems prepared with different olive oils. The relationship between the sinusoidal trend of lipid oxidation exhibited by EVOo and Oo and the chemical change of the different phenolic classes must be also further clarified.

These findings should be confirmed by the analysis of a larger set of EVOo samples to cover the entire range of chemical composition of this oil. The effect of microwave heating treatment on Oo and Po samples refined under different conditions, as well as the application of different microwave powers on these oils, need also further investigations.

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