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Monitoring oxidative stability and phenolic compounds composition of myrtle-enriched extra virgin olive during heating treatment by flame, oven and microwave using reversed phase dispersive liquid–liquid microextraction (RP-DLLME)-HPLC-DAD-FLD method

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ABSTRACT

Lipids oxidation is one of the main ways that affect the nutritional quality of extra virgin olive oil (EVOO) by losing its phenolic substances and especially during heating treatments. This study was focused on the evaluation of the effect of *Myrtus communis* phenolic compounds-enriched extra virgin olive oil (McPC-EEVOO) on phenolic compounds composition during flame, oven at 180 °C and microwave heating, at different exposure times, evaluated by reversed phase dispersive liquid–liquid microextraction (RP-DLLME)-HPLC-DAD-FLD. The K_{232} and K_{270} were also evaluated for all heating treatments. The obtained results showed that the enrichment of EVOO by myrtle extracts significantly prevents the consumption of endogenous phenolic compound from EVOO as phenolic alcohol and flavonoids in comparison to the control (EVOO without enrichment). The most protective effect was found during flame and microwave heating. The K_{232} values were significantly reduced during flame heating compared to the control and followed by oven heating, although K_{232} values in microwave heating were similar for all the oil examines. K_{270} was not affected by this enrichment of EVOO.

1. Introduction

In recent years, the Mediterranean diet has become increasingly popular, gaining widespread attention among the nutrition and research communities (Huang and Sumpio, 2008) and their replaceable element in this dietary style is extra virgin olive oil (EVOO).

The beneficial properties of EVOO are mainly attributed to its composition, having a high percentage of mono-unsaturated fatty acids (oleic acid: C18:1, n 9) and significant amounts of minor components as phenolic compounds (phenolic acids, phenolic alcohols such as hydroxytyrosol and tyrosol, secoiridoids derivatives and flavonoids, such luteolin and apigenin) (Allouche et al., 2007; Bengana et al., 2013). EVOO phenolic compounds contribute to the antioxidant and biological properties (Tuck and Hayball, 2002), oxidation resistance, bitter and pungent taste of EVOO (Bendini et al., 2007; Bengana et al., 2013).

Virgin olive oil (VOO) may be consumed raw as an ingredient in toast, salads, and other foodstuffs, although, in addition, it is highly consumed after domestic heating, such as frying, boiling, and microwave (Carrasco-Pancorbo et al., 2007). Nevertheless, several studies have focused on possible degradation and losses of phenolic compounds present in EVOO during different heating procedures by a complex series of chemical reactions including oxidation, hydrolysis, and polymerization (Santos et al., 2013). Bešter et al. (2008) have recently found that the total phenol content decreased by 55–60% after heat treatment at 100 °C for 142 h, with an air flow of 10 L/h. Regarding single compounds, Carrasco-Pancorbo et al. (2007) reported that hydroxytyrol and its derivatives of EVOO suffered significant losses during heating at 180 °C. Likewise, Brenes et al. (2002) found between 20 and 30% of hydroxytyrol losses in EVOO from Spanish cultivars after 5 and 10 min of microwave

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heating. In the literature, a huge disparity and conflicted results were found regarding the comparison of microwave heating effect and others processes on olive oil quality (Santos et al., 2013).

Therefore, phenolic compounds present in EVOO do not remain enough to ensure a good stability during heating. For this, the enrichment of olive oil complementing the olive phenols with other kinds of phenolic compounds can be a good strategy to improve the oxidative capacity and nutritional profile of the olive oil, giving rise to the development of new food (Rubio et al., 2012). The incorporation of thyme extract in EVOO increased the oxidative stability and the antioxidant activity of EVOO (Rubio et al., 2012) and it has been also observed an enhancing effect of thyme compounds on the bioaccessibility of secoiridoids from olive oil (Rubio et al., 2014).

Regarding simulated house heating effect on enriched oil, few studies are found in the literature. The recent work of Malheiro et al. (2012) showed that the tea extracts protect olive oil from the oxidative process (until 3 min), but with higher heating periods the extracts were pro-oxidants. This means that there is a complex interaction occurring in a complex mixture of antioxidants which may be taken into account when designing a functional food. To the best of our knowledge, there is any work studying the effect of the co-occurring EVOO and plant extract on the composition of EVOO phenolic compounds during different heating processes.

Myrtle, *Myrtus communis* L., is a rich source of antioxidant compounds and possesses strong antioxidant properties (Dairi et al., 2014). The main goal of this work is to evaluate for the first time the effect of myrtle extract, obtained by two different methods, microwave-assisted extraction and conventional one, on the preservation of EVOO phenolic compounds consumption during three heating procedures. The heating in a butane-air flame heating (2, 3, 5 and 10 min) or in oven at 180 °C (1, 2 and 3 h) was assayed. The last heating treatment was applied by using for the first time scientific microwave (5, 10, 15 and 20 min). The evolution of the phenolic compounds content of EVOO was monitoring by reversed phase dispersive liquid–liquid microextraction (RP-DLLME)-HPLC-DAD-FLD method. The K_{232} and K_{270} were also determined for all heating conditions.

2. Materials and methods

2.1. Materials

EVOO samples were from Algerian "Chemlal" cultivar olives, collected in the region of Bejaia (Algeria). The oils were extracted by a three phases centrifugation system from mid-ripe olive fruit.

2.2. Chemicals and standards

For all experiments, analytical reagent grade chemicals and solvents were used. Ultrapure water was obtained from a Millipore Milli-QA10 System (Waters, Germany). Hydroxytyrosol (HYTY), luteolin (LUT) and apigenin (APIG) were obtained from Extrasynthese (Genay, France); gallic acid (GAL) tyrosol (TY), *p*-coumaric acid (*p*-CUM), ferulic acid (FER) and myricitrin analytical standards from Sigma-Aldrich Chemie GmbH (Steinheim, Germany). All solvents employed were HPLC grade; ethanol and acetonitrile were provided by Panreac (Spain), acetic acid by Romil Chemicals Ltd (England).

2.3. Plant material preparation

M. communis leaves were collected in the region of Bejaia (Algeria), and dried in an oven at 40 °C until constant weight, then crushed and sieved to have a size less than 125 μm. The samples were stored in the dark at room temperature.

2.4. Extraction procedure

2.4.1. Microwave assisted extraction (MAE) method

The extraction procedure was previously described in our previous study (Dairi et al., 2014). Briefly, domestic microwave oven (NN-S674MF, LG, Japan, 32 L, 1000 W; variable in 100 W increments, 2.45 GHz) modified in our laboratory was used for extraction of myrtle leaves phenolic compound (McPCs). The leave suspension (1 g of leaves powder per 20 mL of 50% EtOH) was irradiated by microwaves (700 W of power) for 1 min according to the following cycle: 45 s power-on, 10 s of power-off and again 15 s power-on. The sample was filtered with a sintered glass at 0.45 μm using a vacuum pump. Two others additional extractions were carried out for recovering the totality of PCs and the three fractions were combined. Then, the solvent was evaporated to obtain a dry extract.

2.4.2. Conventional extraction (CE) method

20 mL of 50%-ethanol were added to 1 g of myrtle leaves powder and let macerate during 60 min with magnetic agitation. After that, the process was the same as for MAE method (Dairi et al., 2014).

2.5. Preparation of enriched extra virgin olive oil

Weighed quantities of myrtle extracts were dissolved in an appropriate volume of ethanol (50%). Then, an appropriate volume of these (500 μL), to give 200 mg GAE kg^{-1} of oil, were added to extra virgin olive oil (500 g) by stepwise under vigorous stirring for 30 min. Oil samples were stored in the dark until use (Bouaziz et al., 2008).

2.6. Reversed phase dispersive liquid–liquid microextraction (RP-DLLME) of phenolic compounds from EVOO

The extraction of phenolic compounds from EVOO was carried out by DLLME, and their quantification was made by employing the standard addition calibration method. For this, 2 g of EVOO spiked with increasing levels of the phenolic compounds were weighed in a test tube with conical bottom and 1 mL of 1,4-dioxane (as disperser solvent) plus 150 μL of ethanol:water 60:40 (v/v) (as extraction solvent) were injected rapidly into the EVOO sample using a 2 mL syringe. The mixture was then shaken in a vortex apparatus for 1 min and centrifuged for 15 min at 4000 rpm using a Mixtasel centrifuge. The dispersed fine drops of extraction solvent were separated and settled at the conical bottom of the conical test tube. The separated phase was taken using a syringe and directly injected in the chromatographic system for its analysis in the optimized conditions (Godoy-Caballero et al., 2013).

2.7. Identification and quantification of phenolic compounds of EVOO and enriched EVOO by RP-DLLME-HPLC-FLD-DAD

The chromatographic studies were performed using an Agilent Model 1100 LC instrument (Agilent Technologies, Palo Alto, CA, USA) equipped with degasser, quaternary pump, column oven, auto sampler Agilent 1290 infinity thermostatized at 5 °C, UV–vis diode-array detector (DAD), rapid scan fluorescence detector (FLD), and the Chemstation software package to control the instrument, and for data acquisition and analyses. The analytical column employed was a 120 ODBS 5 μm, 150 mm × 4.6 mm (Teknokroma, Barcelona, Spain). The column temperature was set at 25 °C. The mobile phase components were high-purity water/0.5% acetic acid/0.1% acetonitrile (phase A) and acetonitrile/0.5% acetic acid (phase B) and were filtered before use. The gradient program was as follows: 0–20 min,

10% B; 20–30 min, 45% B; 30–45 min, 45% B; 45–50 min, 10% B; 50–65 min, 10% B. Then, the column was re-equilibrated during 15 min in the initial conditions. The flow rate was set constant at 0.5 mL min^{-1} and the injection volume was 10 μL.

Identification of individual polyphenols was carried out by using retention times and spectrometric and fluorescence data. The detection was directly performed by HPLC-DAD at the maximum absorbance wavelength for each polyphenol. Quantification of the single polyphenols was determined from standard addition calibration curve method using a four-point regression curve and analytical figures were performed by means of the ACOC program in Mat Lab code (Godoy-Caballero et al., 2012).

2.8. Measurement of specific absorbance coefficient (K_{232} and K_{270})

Coefficients of specific extinction at 232 and 270 nm (K_{232} and K_{270}) were determined according to European Union standard methods (Commission Regulation (EEC) no. 2568/91). The oil samples were diluted in isooctane placed into a 1 cm quartz cuvette, and their absorbance values were measured at the wavelengths 270 and 232 nm, against a blank of isooctane. Three replicates were prepared and analyzed per sample.

2.9. Heating procedures of un-enriched and enriched extra virgin olive oil

2.9.1. Conventional heating by flame

To simulate the conditions of heating in house cooking practices, samples (5 g) of each EVOO sample were weighed in Pyrex beakers of 42 mm diameter and placed on the center of a support and were heated by flame for different exposition times, 0, 2, 3, 5 and 10 min. Three experiments were carried out for each sample under the same conditions. After each heating period, the oil temperature was determined with a thermometer. After cooling, the samples were stored in sealed tubes at 6 °C until analysis.

2.9.2. Conventional heating in oven at 180 °C

To simulate frying conditions, nine aliquots (5 g) of each sample were weighed in Pyrex tubes and subjected to conventional heating at 180 °C in a Heraeus drying oven. The tubes were removed from the oven at fixed intervals of 60 min, obtaining samples with different heating treatments (60, 120, 180 min) to be analyzed. All heated samples were cooled at room temperature (23 ± 1 °C) and stored at 6 °C until chemical analysis.

2.9.3. Microwave heating

A Scientific microwave oven (Milestone ETHOS (800 W) with NS14100 ATC-FO NS14 stopper with quartz thermowell) was used to heating treatment of extra virgin olive oil (EVOO or EEVOO). To simulate the average quantity and conventional times used in home cooking, three aliquots (5 g) of each oil were placed in Pyrex beakers (42 mm diameter) at the oven and exposed to microwave irradiation of 2450 Hz frequency at maximum potency (1000 W) for different microwaving times, 5, 10, 15 and 20 min. The achieved temperatures of heated oils were measured immediately after microwave exposure, by inserting a thermocouple (connected to an acquisition system) at approximately the geometrical center of the sample. All heated samples were allowed to cool at room temperature for 60 min after thermal treatment and before chemical analysis. The three 5 g aliquots of each oil were combined after microwaving in order to obtain a homogeneous sample. The samples were kept under refrigeration (4 °C) until analysis.

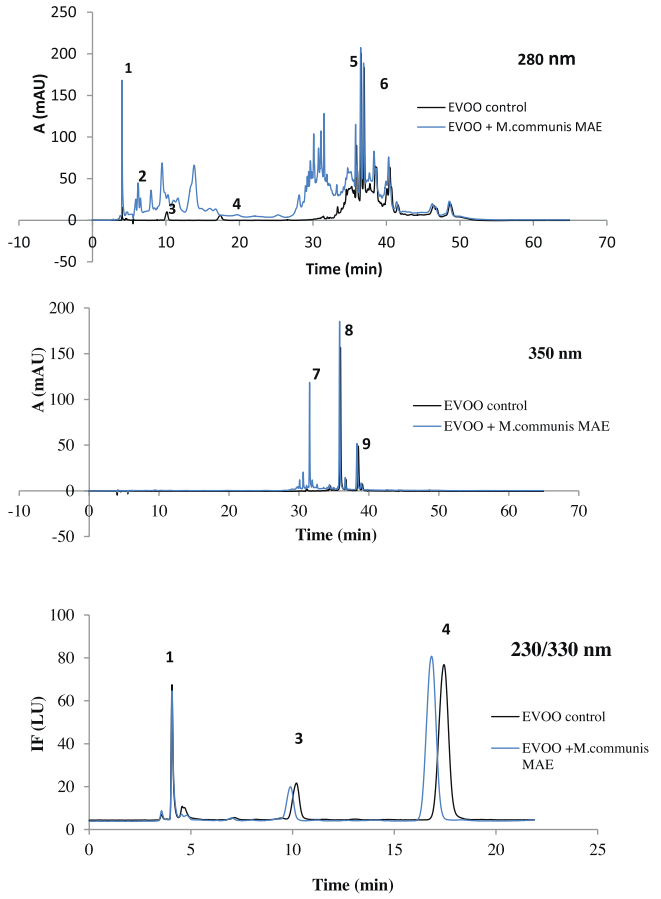

Fig. 1. DAD and FLD chromatograms of un-enriched and enriched EVOO by myrtle extract subjected to the RP-DLLME and directly injected. Galloylquinic acid (1), gallic acid (2), hydroxytyrosol (3), tyrosol (4), secoiridoid 1 (5), secoiridoid 2 (6), myricitrin (7), luteolin (8), apigenin (9). Similar chromatogram profile was obtained for myrtle CE-enriched EVOO.

3. Results and discussion

3.1. Identification and quantification of "chemlal" EVOO phenolic compounds

The extraction of EVOO phenolic compounds was carried out using a new method developed by the laboratory where this current study was done. Thus, a reversed phase dispersive liquid–liquid microextraction (RP-DLLME) procedures have been used with 1,4-dioxane as disperser solvent which is miscible with extraction solvent (ethanol/water) and with the EVOO sample. This novel extraction technique enables the increase of the contact surface area between the extraction solvent and EVOO sample, resulting in a better recovery efficiency and shorter extraction time (Godoy-Caballero et al., 2013). The main phenolic compounds identified in EVOO are shown in Fig. 1 is summarized in Table 1. The phenolic compounds detected belonging to phenolic alcohols (hydroxytyrol, tyrosol), cinnamic acid derivatives (p-coumaric acid, ferulic acid) and flavonoids (luteolin and apigenin). These compound identified were in agreement with previous works studying the same cultivar (Bengana et al., 2013; Tamendjari et al., 2014). Two another important peaks were eluted at 36.6 and 36.9 min respectively between luteolin and apigenin and showing the same spectral behavior (λ_{max} 280 nm). According to the literature (Bengana et al., 2013; Capriotti et al., 2014; Lozano-Sánchez et al., 2013) concerning Spanish and Algerian EVOO, some oleuropein and ligstroside derivatives were eluted between luteolin and apigenin. Therefore, these peaks may be tentatively attributed to secoiridoid family

Table 1
Identification of phenolic compound from Algerian extra virgin olive oil "Chemlal" cultivar by RP-DLLME-HPLC-DAD-FLD.

Phenolic compound	Retention time (Tr)	Equation	R^2	Concentration (mg kg^{-1} of oil)
Quinic acid or derivative	4.01	–	–	–
Hydroxytyrol	10.09	$Y = 72.15x + 246.1$	0.999	4.12 ± 1.57
Tyrosol	17.32	$Y = 35.98x + 222.9$	0.997	5.54 ± 1.46
Luteolin	35.92	$Y = 365.61x + 1340.8$	0.997	3.67 ± 0.77
Apigenin	38.47	$Y = 360.72x + 589.74$	0.996	1.63 ± 0.60
P-coumaric acid	31.95	$Y = 0.6695x + 32.531$	0.993	0.05 ± 0.04
Ferulic acid	32.64	$Y = 0.4119x + 13.303$	0.997	0.03 ± 0.01
Secoiridoid 1	36.65	–	–	–
Secoiridoid 2	36.94	–	–	–

(oleuropein or ligstroside derivatives) which occur in these EVOO at 63–91 mg kg^{-1} of EVOO as found in the work of Bengana et al. (2013) studying the same EVOO variety at different harvest dates. The phenolic alcohols fraction was higher than flavonoids one which is consistent with the finding of Bengana et al. (2013), whereas the work of (Tamendjari et al., 2014) studying the same Algerian cultivars shows a different result. These observations support the effect of various factors such as geographical origin, ripening degree and extraction process on chemical profiles and EVOO phenolic compounds composition (Bendini et al., 2007; Bengana et al., 2013).

The analysis of phenolic compound composition from *M. communis* leaves was carried out by HPLC-DAD-FLD in the same conditions utilized for the analysis of EVOO phenolic compounds (data not shown). The enrichment of EVOO with myrtle extract revealed the presence of new compounds in the oil sample as can be seen in the Fig. 1, and the main important compounds were resumed in Table 2. At 280 nm, the chromatogram reported the presence of an important peak at 4.1 min which was tentatively attributed to galloylquinic acid from myrtle extract (Romani et al., 2012) as mentioned above in our previous study (about 7 mg gallic acid equivalent per gram of dry myrtle extract) (unpublished data). However, this peak overlays with another small peak from EVOO both having the same elution time and spectral behavior. In the literature, the presence of a peak eluting before gallic acid has been reported in EVOO "Chemlal" variety and assigned to quinic acid present at a concentration of 3–4 mg kg^{-1} (Bengana et al., 2013). This let suppose that this interfering peak may be attributed to quinic acid or derivative. The concentration of galloylquinic acid was expressed as gallic acid equivalent (GAE) from standard addition calibration curve with removing the signal due to the interfering peak from EVOO. Gallic acid was also identified in enriched EVOO and in myrtle extracts (data not shown) according to its retention time and UV–vis data in comparison to the standard, and was present at a concentration of 1.48 ± 0.76 and 1.68 ± 0.80 mg kg^{-1} for Myrtle MAE or CE-enriched extra virgin olive oil (EEVOO) respectively. On the other hand, when the chromatogram was acquired at 350 nm, different new compounds were observed but due to the lack of standards, it was only possible to identify one flavonoid as myricitrin 3-O rhamnoside (Romani et al., 2012), one of the main compound present in myrtle extract, and was present in EEVOO at 2.5 times higher than gallic acid content. On the other hand, the EVOO samples enriched with Myrtle MAE and CE extracts gave close phenolic compounds composition which implies that the microwave extraction method may be an alternative method allowing a good recovery of antioxidant compounds from plants as reported previously in many studies (Dahmoune et al., 2013).

3.2. Heating effects on the phenolic compound composition of myrtle-enriched EVOO

To better understand the role of phenolic compounds in the EVOO oxidative stability, changes in their composition were monitored during the progress of oxidation.

3.2.1. Flame heating

Flame heating was used in order to simulate some domestic practices. The evolution of the endogenous phenolic compounds as hydroxytyrol, tyrosol, luteolin, apigenin and unknown 1 (showing a strong FL signal) during flame heating in the control (oil without enrichment) and enriched EVOO (EEVOO) was conducted by monitoring DAD or FLD signal, and the evolution of exogenous compounds such as galloylquinic acid and myricitrin was also monitored in myrtle MAE or CE-EEVOO. The result was expressed as degradation %.

With regard to phenolic alcohols, hydroxytyrol disappeared progressively during the heating treatment to achieve a content loss of 69.5% after 10 min (235 °C), while tyrosol was degraded less quickly during the first minutes of treatment. While 50% of hydroxytyrosol was degraded after 5 min (188 °C), the decrease of tyrosol content was about 7.7% in this time, diminishing later more drastically until a 58.9% after 10 min. In any case the loss of tyrosol is less than that of hydroxytyrol (Fig. 2). The phenolic compounds were degraded as a consequence of their antioxidant activity and their degradation rate was positively correlated to their antioxidant efficacy (Bouaziz et al., 2008). In fact, our result was in agreement with the tests on the antioxidative properties of the studied compounds; the antioxidant capacity decreased in the order hydroxytyrol > oleuropein aglycone > ligstroside aglycone > Tyrosol (Carrasco-Pancorbo et al., 2005). Thus, hydroxytyrosol, having two hydroxyl group, was the first compound to be oxidized in comparison to tyrosol (one OH-group and it has been claimed previously that it is the most active

Table 2
Identification of *Myrtus communis* phenolic compound in enriched extra virgin olive oil using RP-DLLME-HPLC-DAD-FLD.

	EVOO + Myrtle MAE		EVOO + Myrtle CE	
	Equation; R^2	Concentration (mg kg^{-1} of oil)	Equation; R^2	Concentration (mg kg^{-1} of oil)
Galloyl quinic acid*	–	3.44 ± 0.08^a	–	4.17 ± 0.07^b
Gallic acid	$Y = 262.35x + 389.72$; 0.99	1.48 ± 0.76^a	$262.35x + 440.59$; 0.99	1.68 ± 0.80^a
Myricitrin	$Y = 183.6x + 697.78$; 0.99	3.73 ± 1.73^a	$183.6x + 755.75$; 0.99	4.04 ± 1.81^a

The values were expressed as the mean of three measurements ($n = 3$) ± standard deviation (SD). Means with different letters for each compound are significantly different at $p \leq 0.05$.

* Galloyl quinic acid is expressed as mg gallic acid equivalent kg^{-1} of oil.

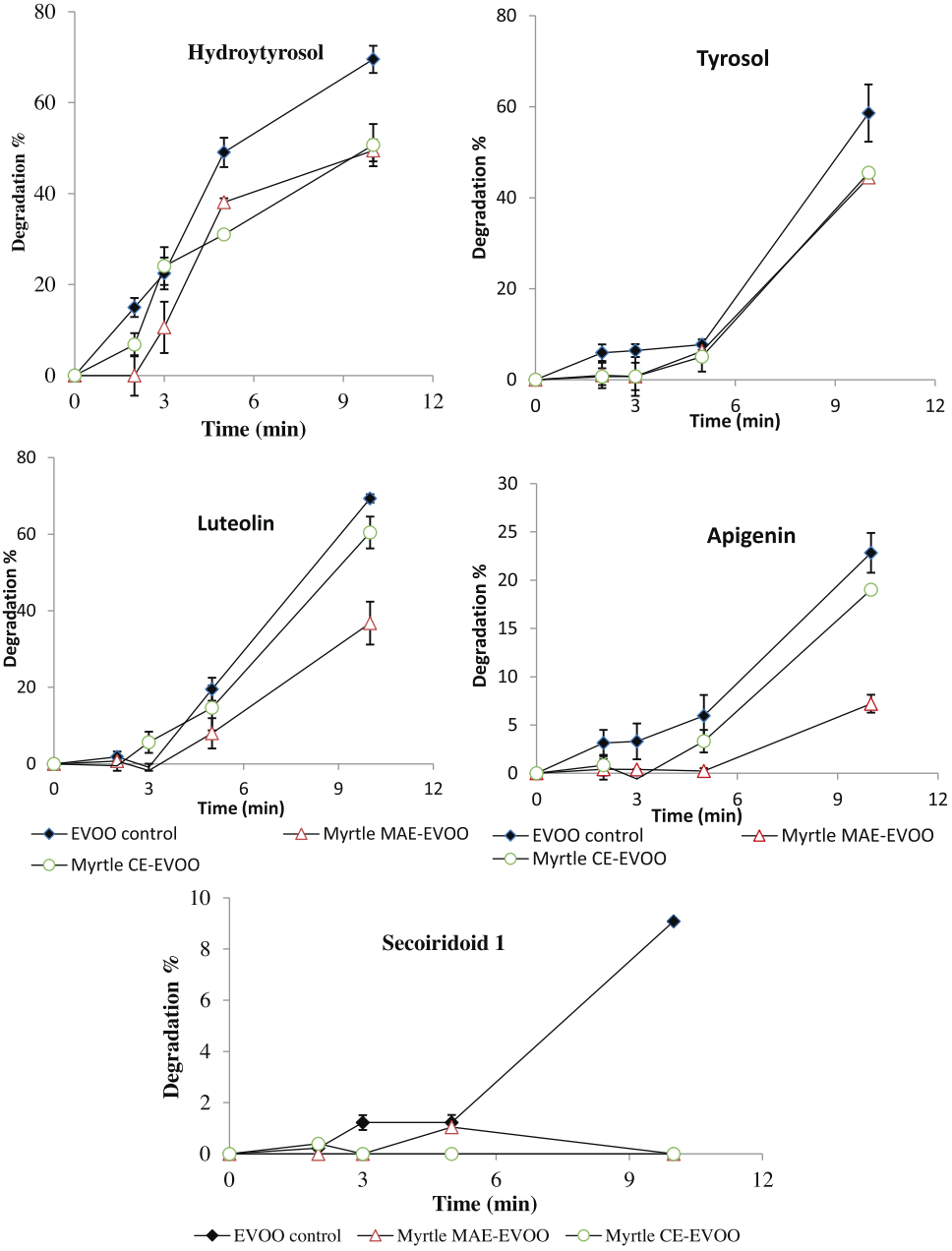

Fig. 2. Flame heating effect on EVOO or Myrtle-EEVOO phenolic compounds degradation.

antioxidant compound (Bendini et al., 2007) providing oxidative stability to the EVOO by protecting polyunsaturated fatty acids (PUFAs) (Nissiotis and Tasioula-Margari, 2002). The antioxidant activity of hydroxytyrosol is due to hydrogen donation and its ability to scavenge free radicals by forming an intra-molecular hydrogen bond between the free hydrogen of its hydroxyl group and their phenoxyl radicals (Bouaziz et al., 2008).

Luteolin (which has a catechol group) is the flavonoid most affected during flame heating and especially after 5 min (188 °C) of treatment where the degradation rate increased significantly to achieve a loss of 69.3 ± 1.1% at the highest exposition time. Regarding apigenin which has not a catechol group, it was the most stable compound and its content diminished in a 22.8 ± 2.1%. These results showed that luteolin was more efficient antioxidant than apigenin, therefore, it might play an important role in the protection of the oil against thermal oxidation process. These observations confirmed that the presence of a catechol moiety enhances the ability of the phenolic compounds to act as antioxidants by reacting with lipid radicals to form non-reactive radicals, interrupting the propagation chain (Bendini et al., 2007) and also this structure feature allows a better stabilization of the phenoxyl radical by increasing the electron delocalization.

The secoiridoid 1 (tr 36.6) showed the highest stability in comparison to the other EVOO phenolic compounds during flame heating and losing only 9.08% of its content after 10 min of heating. It is well known that secoiridoid compounds are the most important antioxidants occurring in EVOO (90%) and contribute to its oxidative stability due to their antioxidant properties and especially oleosidic form of hydroxytyrol (Nissiotis and Tasioula-Margari, 2002). According to the literature, the concentration of these complex phenols decreased during long storage time Lozano-Sánchez et al. (2013) and also during strong heating as reported previously by Bešter et al. (2008) showing that thermal treatment of EVOO for 142 h at 100 °C with an air flow 10 L/h caused a transformation

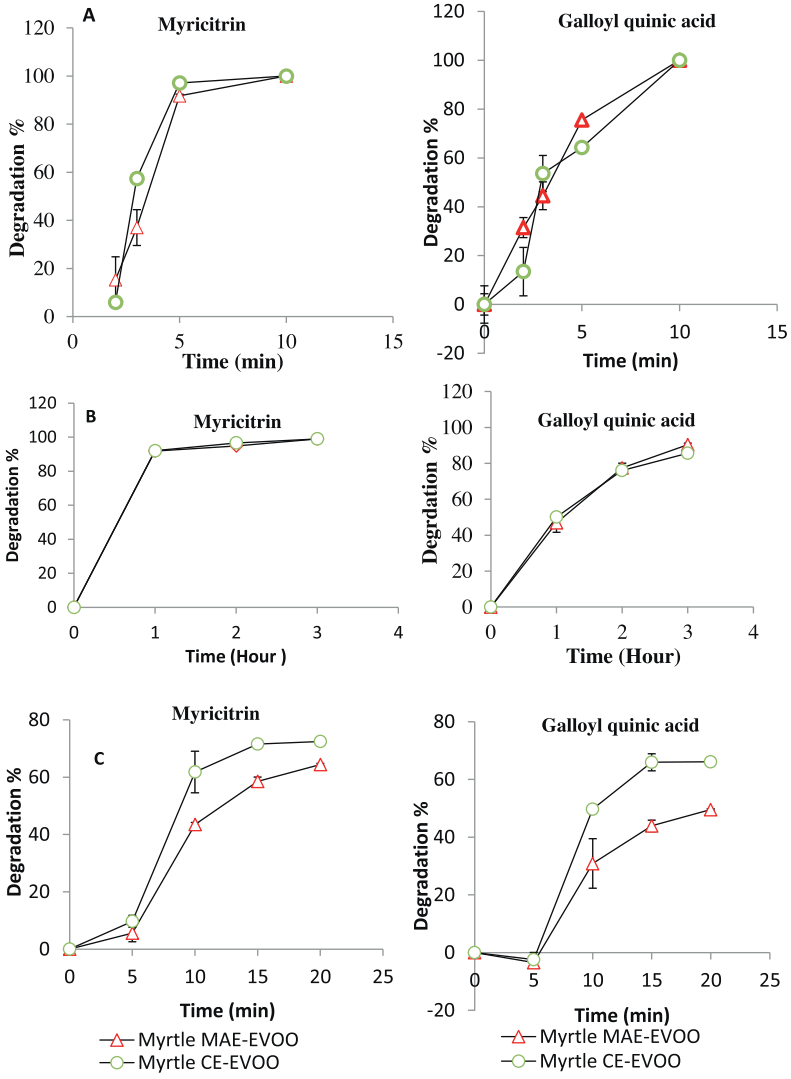

Fig. 3. The degradation of *Myrtus communis* phenolic compound during flame heating (A), oven heating at 180 °C (B) and microwave heating (C) in myrtle-EEVOO.

of secoiridoid biophenols to the simple biophenols, tyrosol and hydroxytyrosol. This supposes that our conditions were far to cause a significant losses of these compounds.

The results show also that the enrichment of EVOO by myrtle extract prevent significantly the consumption of endogenous phenolic compound from EVOO during flame heating, in comparison to the control (EVOO without enrichment) as it can be seen in Fig. 2. Myrtle MAE or CE-EEVOO decreased similarly the degradation of hydroxytyrol, 20.04 and 18.87% respectively, and of tyrosol, 14.14 and 13.10% of decrease respectively, in comparison to the control. In addition, myrtle MAE-EEVOO decreased the diminution of luteolin and apigenin, 32.55 and 15.62% respectively, and these effects were higher than the protection afforded by myrtle CE-EEVOO: only a protection of 8.90 and 3.83% for luteolin and apigenin respectively was observed. On the other hand; secoiridoid 1 was not affected during flame heating of EEVOO, in contrast to the control (EVOO without enrichment). Regarding the phenolic compounds of myrtle extracts, as can be seen in Fig. 3A, rapid degradation was observed for myricitrin and after 5 min of heat treatment the level of myricitrin decreased dramatically by 91% or 97% for myrtle MAE or CE-EEVOO respectively. With regard to galloylquinic acid which is a hydrolysable tannin, its level decreased more slowly than observed for myricitrin and achieve losses of 75.0 ± 6.7% or 64.0 ± 7.4% for myrtle MAE or CE-EEVOO respectively after 5 min of heating. The decrease of the concentrations of these both compounds could be explained by their antioxidant activities and their thermal degradation (Bouaziz et al., 2008) and the difference observed between them could be attributed to their difference in chemical structure, reactivity against lipid radicals but also their localization in the oil since galloylquinic acid is more polar that flavonol (myricitrin). Thus, myricitrin and galloylquinic acid may contribute to stabilize EVOO during heating.

3.2.2. Oven heating

The conventional heating at 180 °C simulates frying temperatures during different time intervals (1, 2 and 3 h). As can be seen in Fig. 4, the behavior of endogenous EVOO phenolic compounds during oven heating at 180 °C was different to the outcome observed during flame heating procedure.

The level of hydroxytyrosol in the control (EVOO without phenol addition) remained constant during the first hour of the treatment; after 2 and 3 h of heating at 180 °C, the hydroxytyrosol content lost by 15.45% and 53% respectively but remained less than the loss value observed during flame heating (Fig. 3). On the other hand, the level of tyrosol increased significantly by 33.5 and 49.1% compared to the unheated EVOO during the first 2 h of heating at 180 °C respectively, and after 2 h, tyrosol content started to decrease but remaining higher than the initial concentration

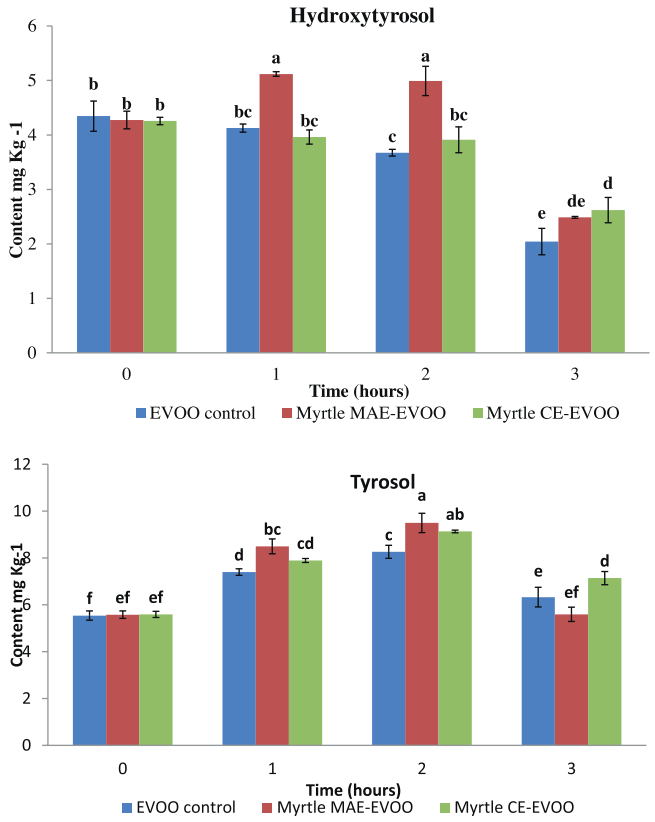

Fig. 4. Evolution content of hydroxytyrol and tyrosol during oven heating at 180 °C for EVOO and myrtle-EEVOO.

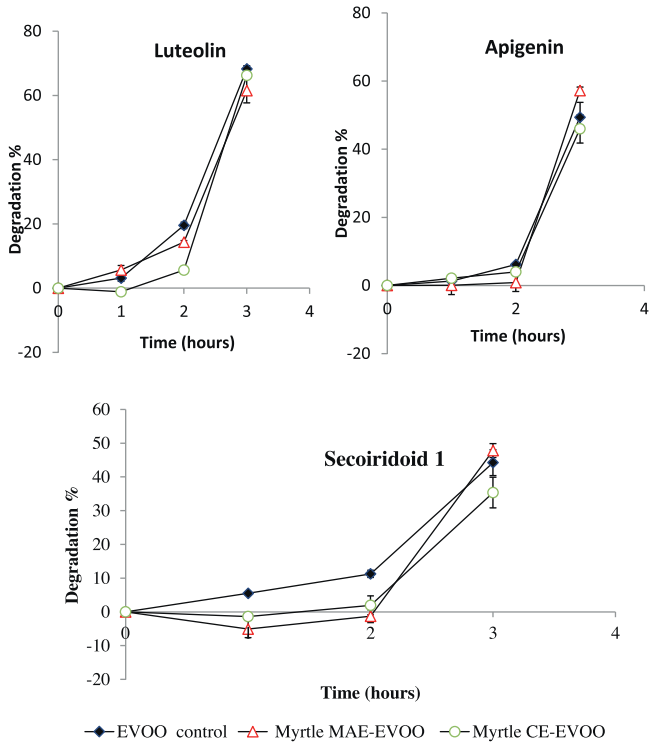

Fig. 5. Monitoring of the degradation of luteolin, apigenin and unknown 1 during oven heating at 180 °C in EVOO and myrtle-EEVOO.

before heating (+14.24% in comparison to the control). Carrasco-Pancorbo et al. (2007) who studied the deterioration of EVOO by heating at 180 °C, reported a loss of 91.5% and 57.0% for hydroxytyrosol and tyrosol respectively, which let us suppose an initial composition olive cultivar effects (Brenes et al., 2002) on antioxidant evolution during heating. In addition, Bešter et al. (2008) studied the phenolic compound changes of extra virgin olive oils after heating for 142 h at 100 °C with an air flow 10 L/h and showed an increase of tyrosol content while hydroxytyrosol and derivatives (oleuropein) and tyrosol derivatives (ligstroside) decreased. These observations supposed that the transformation of hyroxytyrosol and tyrosol derivatives may occur during heating at 180 °C releasing simple biophenols, hydroxytyrol and tyrosol respectively (Bešter et al., 2008). Therefore, the concentration of hydroxytyrol at each stage of heating would be the sum of its formation from the decomposition of its secoiridoid derivatives and its decomposition due to its role as antioxidant (Krichene et al., 2010). Since tyrosol was less effective than hydroxytyrol, it results an accumulation of this compound during their derivatives transformation and after 2 h when it started tardily acting as antioxidant, its level decreased. When EVOO was enriched by myrtle extract, hydroxytyrol level was almost unaltered during the two first step of heating (1 and 2 h) similarly to the observed in un-enriched oil, but, only for MAE-EEVOO, an increase of hydroxytyrol level was observed in comparison to the control (without enrichment). This may be attributed to the presence of interfering compound from oxidation coeluting with hydroxytyrol and thus increasing the area of this latter compound. At the end of the treatment (3 h), hydroxytyrosol was less degraded than in the control: 41.8 and 38.4% degradation for MAE and CE-enriched EVOO respectively giving an additional protection of 11.2% and 14.58% for the above oils respectively in comparison to the control. This may be explained by the active contribution of antioxidants from myrtle extract together with endogenous phenolic compound from EVOO to inhibit lipid oxidation during these heating conditions. With regard to tyrosol, the enrichment does not affect significantly the evolution of this compound during heating at 180 °C which follows the same behavior observed above for tyrosol in EVOO control. Its final concentration remained equal or superior to the initial for all oil types and this is related to the weak antioxidant activity of tyrosol and derivatives (Carrasco-Pancorbo et al., 2005).

Fig. 5 shows the degradation of luteolin during heating at 180 °C. The depletion of luteolin in EVOO increased slowly during the first steps, degradation reaching 3.1 and 19.6% in 1 and 2 h of heating, respectively. Then a further rapid decrease was observed, up to 68.3% at the end (3 h). However, apigenin showed a slower degradation and at the end of the heating about 49.4% was lost. Therefore, apigenin showed higher stability than luteolin which correspond to the result on flame heating effect. The behavior of these flavonols was in agreement with the previous work of Allouche et al. (2007) studying the heating effect at 180 °C on extra virgin olive oil. The difference observed between these compounds may be attributed to the different number of phenolic hydroxyls linked to their phenol ring. Regarding the enriched EVOO, myrtle extract did not significantly stabilize these compounds in the supplemented oil as observed for flame heating. As can be observed in Fig. 5, the depletion of luteolin and apigenin was in general the same for all oils, both un-enriched and enriched oil, although a significative decrease of the degradation of these compounds with respect to the control sample was observed after 2 h of treatment, being an average protection effect of 9.6% and 3.7% for luteolin and apigenin respectively in myrtle-EEVOO. These observations suppose also the possible contribution of flavonols to the oil stability as previously reported in the work of Artajo et al. (2006) who showed that refined oil enriched by luteolin and apigenin improve significantly its oxidative stability.

Regarding the *secoirioid 1* compound, its level decreased significantly with respect to the result of flame heating. In fact, the depletion increased progressively during the heating time to reach

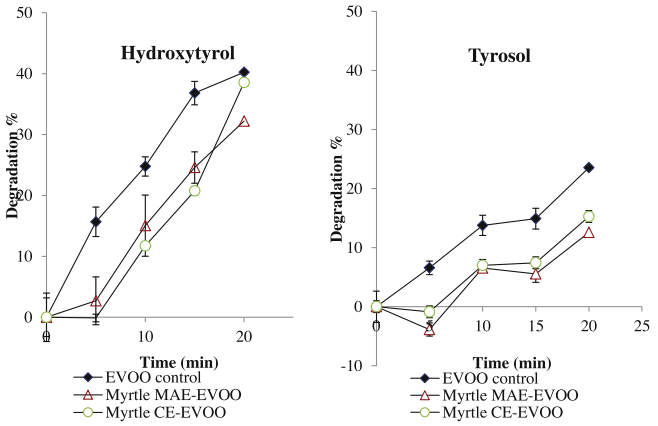

Fig. 6. Monitoring of EVOO or Myrtle-EEVOO phenolic compound during microwave heating.

significant losses of 44.2% which is higher than the value found for flame heating treatment (only 9% of degradation was observed). This confirms our hypothesis advanced from flame heating results, that this compound was degraded in drastic conditions with longer time. To better understand the impact of the depletion of this compound, we have examined the correlation between the *unknown 1* depletion and tyrosol content increase and a negative linear relationship ($R^2 = 0.90$) was found during the first 2 h of thermal treatment. This may suppose that the *secoiridoid 1* may belong to ligstroside family and its thermal hydrolysis affects the ester bound between the phenolic portion and the rest of the molecule and thus released a simple phenol as tyrosol (tyrosol as an aromatic alcoholic moiety). This is in agreement with several previous studies (Brenes et al., 2002; Carrasco-Pancorbo et al., 2007). The secoiridoid compounds are the main responsible of bitterness and pungency sensations in EVOO (Rubio et al., 2014) and according to the work of Lozano-Sánchez et al. (2013) who investigated the phenolic compounds profile of EVOO during storage (10 month) these complex phenols decreased noticeably at the end of storage suggesting that these secoiridoid compounds could be used as biomarkers of EVOO freshness. However, the *secoiridoid 1* content during the first 2 h of heating at 180 °C was not altered in myrtle MAE or CE-EEVOO which is in contrast to its behavior in control sample. But after 3 h of treatment, no significant difference was observed for all oil studied. This means that the presence of myrtle extracts in EVOO reduce the depletion of the compound *secoiridoid 1* and this may be explained by the fact that this latter compound was not only lost by thermal hydrolysis but also by a possible action as antioxidant substance reducing its consumption when myrtle extract was present. In fact, the work of Carrasco-Pancorbo et al. (2005) reported that tyrosol derivatives (ligstroside aglycon) had higher scavenging effect on DPPH radicals than tyrosol.

Regarding the antioxidants from myrtle extract (Fig. 3b), galloylquinic acid presented similar degradation behavior that observed when flame heating was applied but after 3 h of heating, galloylquinic acid was lost by 88% while during flame heating (after 10 min with 235 °C) this compound was completely lost. This may be attributed to the temperature effect since the heating time is longer in oven treatment assay. On the other hand, myricitrin decreased more abruptly during heating at 180 °C to reach a maximal loss (99%) after 1 h of treatment which is in accordance with flame heating assay and thus confirming the high reactivity of this compound and its thermolability related to its higher number of hydroxyl groups (Biesaga, 2011). In comparison to *secoiridoid 1* depletion, there is a time heating effect instead temperature heating one as found for galloylquinic acid, and thus, it can be deducted that the phenolic compound trends during thermal stress are more related to their nature of molecular structure (Brenes et al., 2002; Lozano-Sánchez et al., 2013).

Table 3
Temperatures achieved during flame and microwave heating of EVOO samples.

Flame heating		Microwave heating	
Time (min)	Temperature (°C)	Time (min)	Temperature (°C)
2	122 ± 3.1	5	93 ± 3.0
3	140 ± 3.5	10	110 ± 0.0
5	188 ± 3.0	15	112 ± 3.0
10	235 ± 5.0	20	118 ± 4.0

The values were expressed as the mean of three measurements ($n = 3$) ± standard deviation (SD).

3.2.3. Microwave heating

To the best of our knowledge, no investigations have been published on the influence of microwave irradiation on phenol composition of EVOO or EEVOO using scientific microwave. In this present study, we have working at open system during microwave heating (the temperature was not controlled) and the temperature was immediately measured after processing. The temperatures achieved were given in Table 3. An important disparity has been observed regarding the temperatures achieved by domestic microwave (170 °C vs 313 °C) reported by many authors in the literature who have studied the impact of microwave heating on chemical quality of EVOO (Santos et al., 2013).

The influence of microwave heating over the phenolic compounds of the samples was assessed and the results obtained are represented in Figs. 6 and 7. Regarding phenolic alcohols (Fig. 6), it appeared that they are more resistant to microwave exposure than observed during flame heating (Cerretani et al., 2009). The microwave heating decreased progressively the level of hydroxytyrosol by causing a loss of 40.2%, while tyrosol shows more stability and was lost only by 23.6% at the highest microwave exposure time (20 min, 118 ± 4 °C). Brenes et al. (2002) found 20 and 30% of hydroxytyrol loss in EVOO from Spanish cultivars after 5 and 10 min of microwave heating respectively while tyrosol was unaltered as found by the same authors (Brenes et al., 2002) which might be due to the lower power setting employed (500 W) by these authors that induced less chemical changes in this compound (Cerretani et al., 2009). In addition, these latter authors showed an important depletion of hydroxytyrosol and tyrosol by 93.93 and 62.33% respectively during 15 min of heating using domestic microwave (at 720 W) and the achieved temperature was 313 °C. These observations showed temperature effect on depletion of phenolic alcohols during microwave heating but also the amount of the oil analyzed and the type of microwave used must be taken in account. The enrichment had a significant protection effect on the concentration of phenolic alcohols by reducing their depletion during microwave heating and their losses started after 5 min of treatment which is in contrast with the control as can be seen in Fig. 6. With respect to hydroxytyrol, an additional protective effect was observed, about 11% and 14% for MAE- or CE-EEVOO respectively after 15 min of heating without a significant difference at $p < 0.05$ for these both oils. At the highest time of heating hydroxytyrosol content in CE-EEVOO was quite close to the control (1.9 ± 0.7% of difference). The depletion of tyrosol was reduced by 32.2 and 30.3% (after 15 min, 112 °C) in MAE- and CE-EEVOO respectively. The work of Malheiro et al. (2012) showed that about 50% of EVOO total phenolic compounds (TPC) was lost during microwave heating (1000 W, 10 min) and that tea extract enriched EVOO had not a positive effect on the TPC depletion and green tea extract presented a prooxidant effect which is not observed in our work. This means that the type of plant extract is a determinant factor in the designing of functional food since antagonist or synergic effect may occur in such a complex mixture of antioxidants.

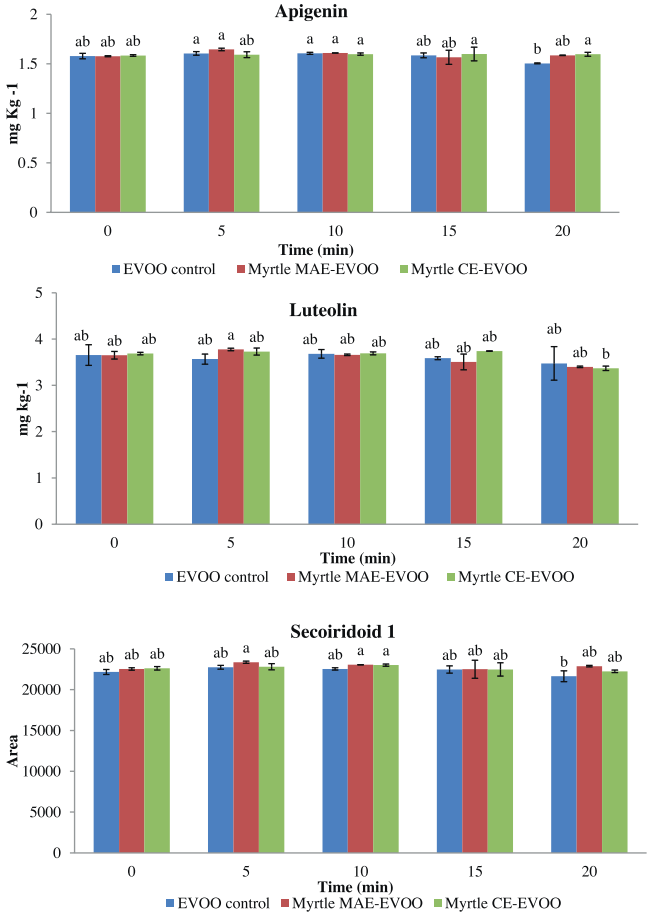

Fig. 7. Monitoring of EVOO or Myrtle-EEVOO phenolic compound during microwave heating.

Regarding flavonol fraction (Fig. 7), luteolin and apigenin contents remained unchanged during microwave heating for all exposure times assayed which is in agreement with the work of Valli et al. (2010) studying the effect of microwave heating (9 min at 750 W). The comparison of this result with flame and conventional heating, supposed that these thermal treatments led to a more important oxidative and hydrolytic degradation of flavonols than microwave heating (Valli et al., 2010) and flavonols contributed secondary on the oil stability compared to phenolic alcohols. The flavonols in the oils enriched by myrtle extracts presented also similar patterns to those observed with the control.

The *secoiridoid 1* was not changed during microwave heating for all exposure times which in contrast with the result obtained when the oils was heated in oven at 180 °C. It was already reported by Brenes et al. (2002) that the oil degradation was very low when the oils were heated by microwave, and according to the work of Cerretani et al. (2009) it was found that secoiridoids derivatives (ligstrosides) content remained constant during microwave heating until 9 min of treatment (700 W, 293 °C). These observations showed the high stability of these compounds during thermal stress which is very important to the nutritional and organoleptic quality of EVOO, since these compound contributed to the taste and pungency of the oil. The same pattern was observed for enriched EVOO by myrtle extract.

Regarding myricitrin and galloylquinic acid (Fig. 3C), microwave heating gives rise to less degradation effect compared to flame and oven heating. A depletion of 64.4 and 72.4% was observed for myricitrin in MAE and CE-EEVOO respectively during microwave heating. This may be explained by the oxidative stability and also by their hydrolytic degradation as shown in the work of Biesaga (2011) and Liazid et al. (2007) who reported the less stability of flavonoid as myricetin during exposition to microwave irradiation which is related to the greater degree of substitution (hydroxylic groups). Galloylquinic acid was lost by 49.5 and 66.1% in MAE and CE-EEVOO respectively during heating treatment which supposed the contribution of this compound in the oil stability.

The enrichment of olive oil with myrtle extract could be considered a water/oil emulsion in which small droplets of the hydro-ethanolic solution of the extract are dispersed in the oil (Suárez et al., 2011). On the other hand, the peroxidation in homogenous lipid system is predominant at the oil/air interface and thus, when the antioxidants accumulate is this interface, the antioxidant activity is greater. The fortification of EVOO with myrtle extract increased the total phenolic compounds from 250 mg GAE kg^{-1} (for the control without supplementation) at about 350 mg GAE kg^{-1} for enriched EVOO (data not shown). In addition, in our previous study, we reported that myrtle extract and EVOO phenolic compounds may act cooperatively to inhibit lipid oxidation in liposomal model, induced by iron/ascorbic acid system (unpublished data). All these observations suppose that myrtle extract through its different antioxidants being locating in oil/air interface and/or in lipid core could contribute to stabilize EVOO especially during flame heating by neutralizing lipid radicals formed and thus, decreased the consumption of endogenous phenolic compounds of EVOO.

3.3. Heating effect on specific extinction coefficients at 232 nm and 270 nm

The evaluation of specific extinction coefficients (K_{232} and K_{270}) allows checking the degree of olive oil oxidation (Malheiro et al., 2012). These specific extinction coefficients are indicative of the formation for primary (K_{232}) and secondary product (K_{270}) of oxidation respectively during thermal stress (Malheiro et al., 2013). The maximum values allowed for K_{232} and K_{270} are respectively 2.50 and 0.20 for extra virgin olive oils (EEC, 1991).

Table 4 shows the evolution of K_{232} and K_{270} during flame heating at different exposure times. The initial values were 2.07 and 0.11 respectively for K_{232} and K_{270} and thus, this oil was classified in EVOO category according to European community regulation (EEC, 1991). The addition of myrtle extracts to the olive oil did not brought significant changes on K_{232} and K_{270} values for enriched oil before heating ($t = 0$ min). During the flame heating, K_{232} showed higher values and constant increase during thermal treatment and especially after 5 min (188 °C) of heating where a drastic increase of K_{232} was observed, from 2.88 to 5.96. However, the enriched oil by myrtle extract showed less oxidative status and allowed a protective effect of about 40% at the end of the treatment (10 min) compared to the control. These observations were in agreement with the result obtained for the evolution of EVOO phenolic compound during flame treatment which indicates that myrtle extract may reduce the losses of EVOO phenolic compounds during heating and, therefore, myrtle extract may contribute to decrease the formation of primary products (hydroperoxides) by protecting the oxidation of polyunsaturated fatty acids (PUFAs) (Bouaziz et al., 2008; Malheiro et al., 2013).

Concerning K_{270}, the formation of secondary product in the control increased progressively during the flame heating but a drastic increase was observed after 3 min (140 °C) to achieve 1.07 values at the end of the treatment. The enrichment of oil by myrtle extract did not have any significant effect on K_{270} values. The comparison of the K_{232} and K_{270} obtained with EEVOO supposed that firstly, there is other oxidation compounds that may interfere with the triene conjugated evaluated at 270 nm. Secondly, it may be supposed that the decomposition of hydroperoxides formed was very fast at the highest temperatures (Velasco and Dobarganes,

Table 4
Comparison of flame, oven and microwave heating effect in quality parameters (K_{232} and K_{270}) of extra virgin olive oils with and without myrtle extracts.

Flame heating					
Time (min)	0	2	3	5	10
K_{232}					
Control	2.07 ± 0.00^{de}	2.46 ± 0.00^{d}	2.48 ± 0.01^{cd}	2.88 ± 0.09^{c}	5.96 ± 0.07^{a}
Myrtle MAE	2.04 ± 0.04^{e}	2.13 ± 0.02^{de}	2.27 ± 0.04^{de}	2.28 ± 0.00^{de}	3.13 ± 0.11^{b}
Myrtle CE	2.02 ± 0.00^{e}	2.17 ± 0.08^{de}	2.16 ± 0.05^{de}	2.25 ± 0.02^{de}	3.33 ± 0.11^{b}
K_{270}					
Control	0.10 ± 0.00^{d}	0.14 ± 0.01^{d}	0.15 ± 0.01^{d}	0.58 ± 0.09^{b}	1.05 ± 0.01^{a}
Myrtle MAE	0.11 ± 0.00^{d}	0.12 ± 0.00^{d}	0.16 ± 0.01^{d}	0.51 ± 0.04^{bc}	1.09 ± 0.00^{a}
Myrtle CE	0.12 ± 0.01^{d}	0.13 ± 0.00^{d}	0.17 ± 0.04^{d}	0.52 ± 0.10^{c}	1.03 ± 0.02^{a}

Oven heating				
Time (hour)	0	1	2	3
K_{232}				
Control	2.07 ± 0.00^{efg}	2.25 ± 0.00^{d}	2.58 ± 0.02^{b}	2.76 ± 0.03^{c}
Myrtle MAE	2.04 ± 0.04^{fg}	2.12 ± 0.03^{e}	2.43 ± 0.01^{c}	2.63 ± 0.03^{b}
Myrtle CE	2.02 ± 0.00^{g}	2.10 ± 0.00^{ef}	2.42 ± 0.04^{c}	2.65 ± 0.00^{b}
K_{270}				
Control	0.11 ± 0.00^{f}	0.48 ± 0.01^{e}	0.66 ± 0.05^{c}	0.75 ± 0.00^{b}
Myrtle MAE	0.11 ± 0.00^{f}	0.46 ± 0.00^{e}	0.58 ± 0.00^{d}	0.69 ± 0.01^{c}
Myrtle CE	0.12 ± 0.01^{f}	0.55 ± 0.00^{d}	0.71 ± 0.03^{bc}	0.83 ± 0.02^{a}

Microwave heating					
Time (min)	0	5	10	15	20
K_{232}					
Control	2.07 ± 0.02^{gh}	2.04 ± 0.04^{h}	2.21 ± 0.04^{def}	2.24 ± 0.02^{cde}	2.33 ± 0.14^{bcd}
Myrtle MAE	1.98 ± 0.04^{h}	2.13 ± 0.01^{efgh}	2.30 ± 0.07^{cde}	2.37 ± 0.02^{b}	2.48 ± 0.02^{ab}
Myrtle CE	2.02 ± 0.00^{h}	2.10 ± 0.00^{fgh}	2.19 ± 0.01^{defg}	2.34 ± 0.00^{bc}	2.53 ± 0.01^{a}
K_{270}					
Control	0.10 ± 0.00^{h}	0.14 ± 0.00^{efg}	0.18 ± 0.03^{cde}	0.21 ± 0.01^{cd}	0.27 ± 0.00^{ab}
Myrtle MAE	0.11 ± 0.00^{gh}	0.17 ± 0.01^{def}	0.20 ± 0.00^{cd}	0.22 ± 0.00^{bc}	0.28 ± 0.01^{a}
Myrtle CE	0.12 ± 0.01^{fgh}	0.17 ± 0.00^{de}	0.17 ± 0.00^{def}	0.21 ± 0.01^{cd}	0.29 ± 0.00^{a}

The values were expressed as the mean of three measurements ($n = 3$) ± standard deviation (SD). Means within a same heating procedure, in each parameter studied, with different letters differ significantly ($p \leq 0.05$).

2002). Thus, no correlation between this both parameters could be established.

Regarding the heating procedure at 180 °C, K_{232} values increased during the thermal stress to achieve a value of 2.76 after 3 h which is very low compared to the result obtained with flame heating. ROOH are the products of the action of alkylperoxy radicals with the antioxidant, their immediate decomposition at the higher temperatures would even lead to chain propagation rather than chain breaking under these conditions (Velasco and Dobarganes, 2002) and this may explain the difference of K_{232} values found in these both heating procedures and a time effect may be thus taken in consideration since a longer heating time induced lower oxidative stability of the oil (Carrasco-Pancorbo et al., 2007). Enriched oil showed a slight protective effect on hydroperoxide formation (K_{232}) of 4.79 and 3.83% for MAE-and CE-EEVOO respectively at the end of the heating (after 3 h) compared to the control which in the contrast with the result of flame heating. As shown above, drastic losses of myrtle phenolic compounds (as myricitrin) occur during oven heating in comparison to flame heating and thus reducing the antioxidant activity of the enriched oil and their stability becomes close to the control one. For the changes on K_{270} values, it can be seen in Table 4 that the production rate of secondary products from oil oxidation was faster during the first hour for all the oil types (control and enriched oils) but thereafter, the K_{270} values increased slowly which may be explained but the strong volatilization of secondary oxidized product as formic acid and aldehydes in these drastic conditions (Carrasco-Pancorbo et al., 2007). The control and enriched oils showed similar evolution patterns during oven heating but CE-EEVOO showed slightly higher value at the end of the treatment than the control and MAE-EEVOO.

Table 4 presents the changes on specific coefficient absorbance during microwave heating of oils. The microwave heating during all exposure times showed less damage effect on EVOO in relation with hydroperoxides production (K_{232}) compared to the above heating procedures, flame and oven heating, and which is in agreement with the degradation effect on phenolic compound contents, i.e. lower polyphenol losses, lower hydroperoxides formation and lower oxidation status. Our results are in accordance with other work previously reported by Brenes et al. (2002) but in contrast with the work of Albi et al. (1997) who reported a more drastic effect of microwave heating than of oven one. These conflicting results may be related to the conditions of heating and the instrument used. K_{232} values increased slowly during microwave heating with similar trends for the control and myrtle-enriched oils up to 15 min, but at the end of the treatment, a significant ($p < 0.05$) higher value was observed for enriched oil than for the control and their values were slightly higher than the limit value (2.5). Our result is in agreement with the finding of Malheiro et al. (2012) studying the enrichment of EVOO by green tea. This may be related to the photo-oxidation of chlorophyll where it may act as prooxidant substances which lead to increase the production of oxidation primary product. This hypothesis may be supported by the work of Wanasundara and Shahidi (1998) who reported that green tea extract exhibited a prooxidant effect in edible oils, namely marine oils, but dechlorophyllized green tea extract presented an excellent antioxidant activity in the oil studied.

Regarding K_{270}, the absorbance values of conjugated trienes (CT) were gradually increased with the increase of heating time but after 15 min, the K_{270} values become higher than the limit value to achieve 0.27, 0.28 and 0.29 for the control, MAE-or CE-EEVOO respectively and thus disqualifying the oil studied from EVOO category. This parameter is in accordance with K_{232} result which indicated that microwave heating caused minor changes on olive oil quality compared to the above heating procedures. According

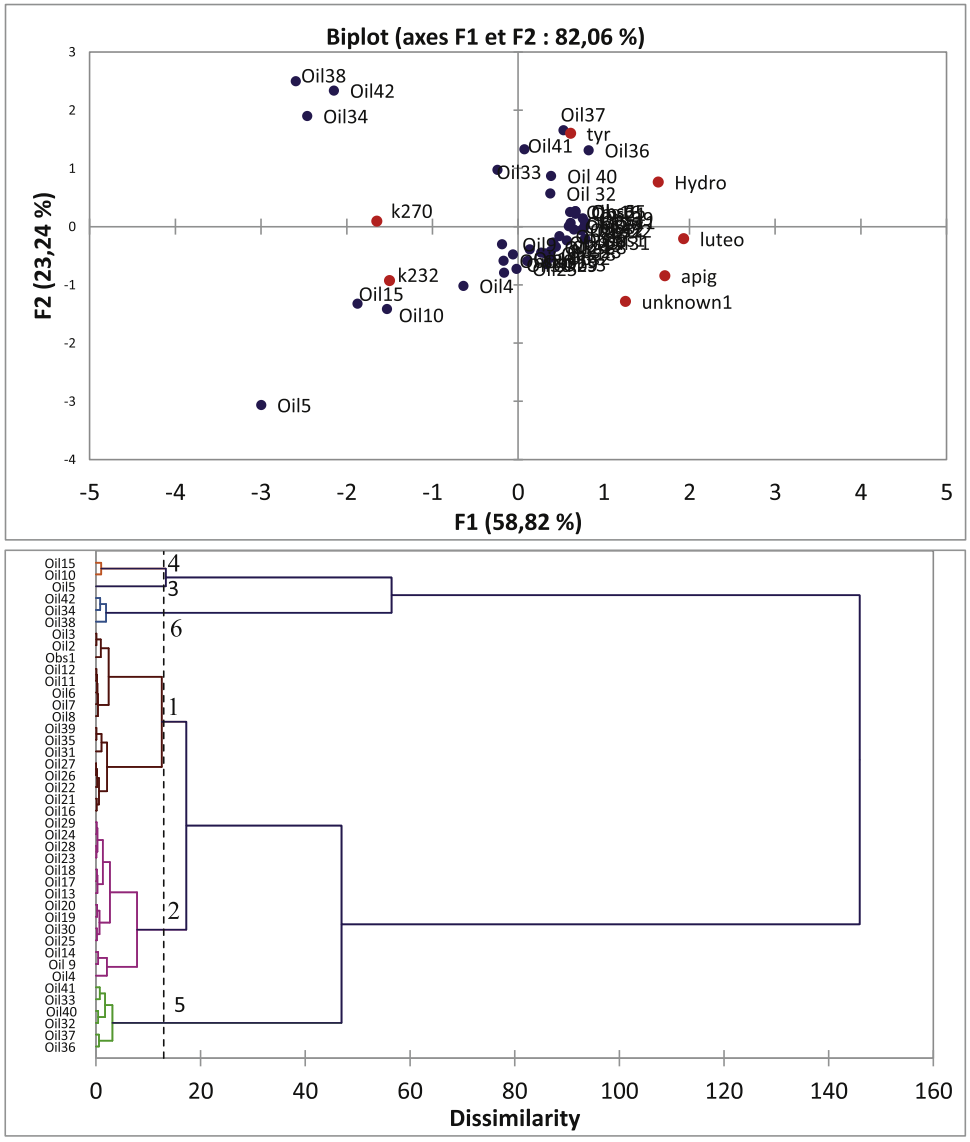

Fig. 8. Principal component analysis (A) and hierarchical cluster analysis (B) of EVOO samples based on the main important factors (red color) (hydroxytyrol, tyrosol, luteolin, apigenin and unknown 1 content, K_{232} and K_{270}). Oil 1–5: oil control heated by flame (0, 2, 3, 5 and 10 min); Oil 6–10: Oil + myrtle MAE heated by flame (0, 2, 3, 5 and 10 min); Oil 11–15: oil + myrtle CE heated by flame (0, 2, 3, 5 and 10); Oil 16–20: oil control heated by microwave (0, 5, 10, 15 and 20 min); Oil 21–25: oil + myrtle MAE heated by microwave (0, 5,10, 15 and 20 min); Oil 26–30: oil + myrtle CE heated by microwave (0, 5, 10, 15 and 20 min): Oil 31–34: oil control heated by oven (0, 1, 2 and 3 h); Oil 35–38 oil + myrtle MAE heated by oven (0, 1, 2 and 3 h): Oil 39–42: oil + myrtle CE heated by oven (0, 1, 2 and 3 h). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

to the statistical analysis, the K_{270} values during microwave heating did not show significant difference ($p < 0.05$) for all oils studied at the different exposition times, i.e. the addition of myrtle extract to the EVOO did not affect significantly the K_{270} values which is in contrast with the works of Malheiro et al. (2012, 2013) who reported that green tea and olive leaves increased K_{270} values in EVOO and soybean oil under microwave heating respectively at the end of treatment (15 min, 1000 W) which means that these extracts acted as prooxidant by favoring secondary oxidation compounds formation which is not observed with myrtle extract.

3.4. Principal component analysis (PCA) and hierarchical cluster analysis

Once the information from all parameters analysis of the oils had been collected, a statistical study was carried out to see the distribution that followed the data. Principal component analysis (PCA) was considered as a good tool in establishing relationships between variables. Fig. 8a shows the biplot of the two first principal components that explain 82.06% of the total variance in the data collected from oils heated by the different procedures. As can be clearly seen in the general plot of the parameters, there is a positive relationship between luteolin, apigenin and secoiridoid 1, and negative correlation of these variables with K_{232} and K_{270}. It can be also observed that tyrosol and hydroxytyrosol showed a weak correlation with the others factors that means that these compounds had different behaviors than other ones as observed during oven heating for tyrosol.

In order to observe similarities or dissimilarities between the oil samples, hierarchical cluster analysis was used as a complementary tool to PCA. The dissimilarity of different clusters was calculated by Ward's method. Fig. 8b shows the dendrogram obtained from the oil samples and six clusters are formed. As can be seen, cluster 1 was formed principally by oil heated by microwave (0–5 min) and flame (0–3 min); and the cluster 2 was formed by oil heated by microwave (5–20 min) and flame (5 min). This result means that the oil heated by flame (after 5 min) present similarity with the oil heated by microwave (after 20 min) which suggest that flame

heating is more severe than microwave one. The oil heated for 20 min by flame formed two distinct clusters, 3 and 4, which contained oil control (without enrichment) and enriched oil respectively. This result suggest that these oils have a difference within the composition and phytochemical profile which is in agreement with the chemical analysis, in the other hand, the enriched oil was less degraded than oil control. The clusters 5 and 6 were principally formed by oils heated by oven at 180 °C. A clear distinction between the oil control and enriched oil was not determined because the treatment applied is quite severe compared to other previous treatment and thus, there is a close composition and oxidation status for all oil studied. According to all these observation, the enrichment of olive oil by myrtle extract induces a significant effect principally during flame heating.

4. Conclusions

Our results demonstrate that heating treatment could induce significant losses of EVOO phenolic compounds which lead to the deterioration of its nutritional and organoleptic qualities. The more safety heating procedure was microwave treatment in comparison with flame or oven ones. A chemical structure and degradation rate relationship was observed and therefore hydroxytyrol was the main important antioxidant to ensure oil stability but also flavonoids fraction have positive effects. The addition of myrtle extracts in EVOO prevent strongly the consumption of its endogenous phenolic compounds as hydroxytyrol, tyrosol and flavonoids (luteolin and apigenin) and the greatest protective effect was observed when microwave and flame heating were applied. In addition, a higher inhibition in the formation of primary oxidation compounds due to a higher capacity to protect PUFA was observed in enriched oil during flame and oven heating. Myrtle extracts may benefit the oils by improving their composition in antioxidant compounds and their oxidative stability. The development of a new functional food with EVOO and myrtle extract should be explored.

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References

Albi, T., Lanzón, A., Guinda, A., Leon, M., Pérez-Camino, M.C., 1997. Microwave and conventional heating effects on thermoxidative degradation of edible fats. J. Agri. Food Chem. 45 (10), 3795–3798.

Allouche, Y., Jimenez, A., Gaforio, J.J., Uceda, M., Beltran, G., 2007. How heating affects extra virgin olive oil quality indexes and chemical composition. J. Agric. Food Chem. 55, 9646–9654.

Artajo, L.S., Romero, M.P., Morelló, J.R., Motilva, M.J., 2006. Enrichment of refined olive oil with phenolic compounds: evaluation of their antioxidant activity and their effect on the bitter index. J. Agric. Food Chem. 54, 6079–6088.

Bendini, A., Cerretani, L., Carrasco-Pancorbo, A., Gómez-Caravaca, A.M., Segura-Carretero, A., Fernández-Gutiérrez, A., Lercker, G., 2007. Phenolic molecules in virgin olive oils: a survey of their sensory properties, health effects, antioxidant activity and analytical methods. An overview of the last decade Alessandra. Molecules 12, 1679–1719.

Bengana, M., Bakhouche, A., Lozano-Sánchez, J., Amir, Y., Youyou, A., Segura-Carretero, A., Fernández-Gutiérrez, A., 2013. Influence of olive ripeness on chemical properties and phenolic composition of Chemlal extra-virgin olive oil. Food Res. Int. 54, 1868–1875.

Bešter, E., Butinar, B., Bučar-Miklavčič, M., Golob, T., 2008. Chemical changes in extra virgin olive oils from Slovenian Istra after thermal treatment. Food Chem. 108, 446–454.

Biesaga, M., 2011. Influence of extraction methods on stability of flavonoids. J. Chromatogr. A 1218, 2505–2512.

Bouaziz, M., Fki, I., Jemai, H., Ayadi, M., Sayadi, S., 2008. Effect of storage on refined and husk olive oils composition: stabilization by addition of natural antioxidants from Chemlali olive leaves. Food Chem. 108, 253–262.

Brenes, M., García, A., Dobarganes, M.C., Velasco, J., Romero, C., 2002. Influence of thermal treatments simulating cooking processes on the polyphenol content in virgin olive oil. J. Agric. Food Chem. 50, 5962–5967.

Capriotti, A.L., Cavaliere, C., Crescenzi, C., Foglia, P., Nescatelli, R., Samperi, R., Lagana, A., 2014. Comparison of extraction methods for the identification and quantification of polyphenols in virgin olive oil by ultra-HPLC-QToF mass spectrometry. Food Chem. 158, 392–400.

Carrasco-Pancorbo, A., Cerretani, L., Bendini, A., Segura-Carretero, A., Del Carlo, M., Gallina-Toschi, T., Lercker, G., Compagnone, D., Fernandez-Gutierrez, A., 2005. Evaluation of the antioxidant capacity of individual phenolic compounds in virgin olive oil. J. Agric. Food Chem. 53, 8918–8925.

Carrasco-Pancorbo, A., Cerretani, L., Bendini, A., Segura-Carretero, A., Lercker, G., Fernandez-Gutierrez, A., 2007. Evaluation of the influence of thermal oxidation on the phenolic composition and on the antioxidant activity of extra-virgin olive oils. J. Agric. Food Chem. 55, 4771–4780.

Cerretani, L., Bendini, A., Rodriguez-Estrada, M.T., Vittadini, E., Chiavaro, E., 2009. Microwave heating of different commercial categories of olive oil: Part I. Effect on chemical oxidative stability indices and phenolic compounds. Food Chem. 115, 1381–1388.

Dahmoune, F., Boulekbache, L., Moussi, K., Aoun, O., Spigno, G., Madani, K., 2013. Valorization of Citrus limon residues for the recovery of antioxidants: evaluation and optimization of microwave and ultrasound application to solvent extraction. Ind. Crop. Prod. 50, 77–87.

Dairi, S., Madani, K., Aoun, M., Kee Him, J.L., Bron, P., Lauret, C., Cristol, J.-P., Carbonneau, M.-A., 2014. Antioxidative properties and ability of phenolic compounds of *Myrtus Communis* leaves to counteract in vitro LDL and phospholipid aqueous dispersion oxidation. J. Food Sci., http://dx.doi.org/10.1111/1750-3841.12517.

European Union Commission Regulation EEC 2568/91, 1991. On the characteristics of olive and olive pomace oils and their analytical methods. Off. J. Eur. Commun., L248.

Godoy-Caballero, M., Acedo-Valenzuela, M., Galeano-Díaz, T., 2012. Simple quantification of phenolic compounds present in the minor fraction of virgin olive oil by LC-DAD-FLD. Talanta 101, 479–487.

Godoy-Caballero, M.P., Acedo-Valenzuela, M.I., Galeano-Diaz, T., 2013. New reversed phase dispersive liquid–liquid microextraction method for the determination of phenolic compounds in virgin olive oil by rapid resolution liquid chromathography with ultraviolet-visible and mass spectrometry detection. J. Chromatogr. A 1313, 291–301.

Huang, C.L., Sumpio, B.E., 2008. Olive oil, the mediterranean diet, and cardiovascular health. J. Am. Coll. Surg. 207, 407–416.

Krichene, D., Allalout, A., Mancebo-Campos, V., Salvador, M.D., Zarrouk, M., Fregapane, G., 2010. Stability of virgin olive oil and behaviour of its natural antioxidants under medium temperature accelerated storage conditions. Food Chem. 121, 171–177.

Liazid, A., Palma, M., Brigui, J., Barroso, C.G., 2007. Investigation on phenolic compounds stability during microwave-assisted extraction. J. Chromatogr. A 1140 (1), 29–34.

Lozano-Sánchez, J., Bendini, A., Quirantes-Piné, R., Cerretani, L., Segura-Carretero, A., Fernández-Gutiérrez, A., 2013. Monitoring the bioactive compounds status of extra-virgin olive oil and storage by-products over the shelf life. Food Control 30, 606–615.

Malheiro, R., Casal, S., Lamas, H., Bento, A., Pereira, J.A., 2012. Can tea extracts protect extra virgin olive oil from oxidation during microwave heating? Food Res. Int. 48, 148–154.

Malheiro, R., Rodrigues, N., Manzke, G., Bento, A., Pereira, J.A., Casal, S., 2013. The use of olive leaves and tea extracts as effective antioxidants against the oxidation of soybean oil under microwave heating. Ind. Crop. Prod. 44, 37–43.

Nissiotis, M., Tasioula-Margari, M., 2002. Changes in antioxidant concentration of virgin olive oil during thermal oxidation. Food Chem. 77, 371–376.

Romani, A., Campo, M., Pinelli, P., 2012. HPLC/DAD/ESI-MS analyses and anti-radical activity of hydrolyzable tannins from different vegetal species. Food Chem. 130, 214–221.

Rubio, L., Macia, A., Castell-Auvi, A., Pinent, M., Blay, M.T., Ardevol, A., Romero, M.P., Motilva, M.J., 2014. Effect of the co-occurring olive oil and thyme extracts on the phenolic bioaccessibility and bioavailability assessed by in vitro digestion and cell models. Food Chem. 149, 277–284.

Rubio, L., Motilva, M.J., Macia, A., Ramo, T., Romero, M.P., 2012. Development of a phenol-enriched olive oil with both its own phenolic compounds and complementary phenols from thyme. J. Agric. Food Chem. 60, 3105–3112.

Santos, C.S.P., Cruz, R., Cunha, S.C., Casal, S., 2013. Effect of cooking on olive oil quality attributes. Food Res. Int. 54, 2016–2024.

Suárez, M., Romero, M.-P., Ramo, T., Motilva, M.-J., 2011. Stability of a phenol-enriched olive oil during storage. Eur. J. Lipid Sci. Technol. 113, 894–903.

Tamendjari, A., Laincer, F., Laribi, R., Arrar, L., Rovellini, P., Venturini, S., 2014. Olive oils from Algeria: phenolic compounds, antioxidant and antibacterial activities. Grasas y Aceites 65, e001.

Tuck, K.L., Hayball, P.J., 2002. Major phenolic compounds in olive oil: metabolism and health effects. J. Nutr. Biochem. 13, 636–644.

Valli, E., Bendini, A., Cerretani, L., Fu, S., Segura-Carretero, A., Cremonini, M.A., 2010. Effects of heating on virgin olive oils and their blends: focus on modifications of phenolic fraction. J. Agric. Food Chem. 58, 8158–8166.

Velasco, J., Dobarganes, C., 2002. Oxidative stability of virgin olive oil. Eur. J. Lipid Sci. Technol. 104, 661–676.

Wanasundara, U.N., Shahidi, F., 1998. Antioxidant and pro-oxidant activity of green tea extracts in marine oils. Food Chem. 63 (3), 335–342.