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Study on the Effects of Heating of Virgin Olive Oil Blended with Mildly Deodorized Olive Oil: Focus on the Hydrolytic and Oxidative State

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In this study, mildly deodorized olive oil (DEO) and its admixtures with extra virgin olive oil (EVOO) have been analyzed after heating treatments by microwave and conventional oven. Different patterns in oxidative and hydrolytic degradation of lipids in genuine and sophisticated olive oils have been evaluated by chromatographic and spectroscopic methods and related to heating treatments. The experimental plan focused on the assessment of the (a) hydrolytic degree of the samples by the free acidity and the 1,2- and 1,3-diacylglycerols (DAG) determinations; (b) oxidative status of the samples by the assessment of the peroxide value (POV) and oxidized fatty acid (OFA), the specific absorption at 270 nm (k_{270}), the accelerated aging test (OSI) and volatile compounds. In general, the thermal treatment by conventional oven led to a higher content of 1,3-DAG and secondary oxidation products than microwave heating. A duo−trio sensory test was also performed: tasters were not able to discriminate between EVOO and DEO heated by conventional oven whereas they were when oils were microwaved.

KEYWORDS: Extra virgin olive oil; microwave; mildly deodorized olive oil; alkyl esters; oxidative; hydrolytic indices

INTRODUCTION

It is well-known that EVOO has higher nutritional and sensory quality in comparison to other vegetable oils (*1*), especially due to the presence of both high ratio monounsaturated/polyunsaturated fatty acids and antioxidant fraction (lipophilic and hydrophilic phenolic compounds) (*2*). Unfortunately, it is also well-known that probably a great number of olive oils fraudulently sold as EVOOs are actually illegally mixed with other cheaper olive oils of lower quality and poorer characteristics (*3*). The so-called “mildly deodorized” olive oils (DEOs) are nowadays the most used adulterants, because of their difficulty of being detected when added to EVOO (*4*). These oils commonly show physicochemical characteristics similar to those of genuine EVOO. In addition, the mild conditions illegally applied for their deacidification and deodorization do not lead to the formation of chemical markers that could be exploited as forensic proofs of the fraud. Several methods have been proposed based on the determination of compounds formed during mild deodorization such as isomerization of 1,2-diacylglycerols to 1,3-diacylglycerols (*5*), but these can also be found in aged EVOOs (*6*).

Recently other authors (*4*) studied water content and volatile profile of several olive oil samples, evidencing some interesting modifications related to the mild deodorization treatment.

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At present, the determination of alkyl esters seems the most promising method to detect the DEOs as suggested by data recently published in two scientific works (*3*, *7*). According to these authors, methyl and ethyl esters of fatty acids can be considered good markers of low quality of virgin olive oil subjected to mild deodorization: in particular, ethyl esters prevail on methyl esters and, as suggested by Mariani and Bellan (*8*), the concentration of ethyl oleate exceed 15 mg per kg of oil and the sum of the fatty acid alkyl esters (FAAEs) exceed 30−40 mg kg^{-1}.

Nowadays, a great number of cheaper EVOOs sold in supermarkets and discounts are probably illegal blends between EVOOs and DEOs. These virgin olive oils may be consumed raw in toasts, salads and other foodstuffs (*9*), but often they are also consumed after domestic heating, such as frying, boiling, conventional and microwave heating (*10*); these thermal treatments are commonly utilized for home-cooking, food catering and industrial processes (*10*, *11*). Several studies published in the literature up to now have compared the effects of conventional and microwave heating by physical and chemical parameters (*10*, *12*, *13*). Microwave heating is very popular for high speed and convenience, as compared to conventional heating treatments (*14*), and was reported to induce significantly higher amount of compounds formed by oxidation and hydrolysis of lipids in comparison with traditional cooking treatments, probably as the result of a different extent of radical formation. In fact, higher levels of polar compounds, triglyceride oligopolymers and oxidized triglycerides were found in microwaved than in

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conventionally heated oils (*13*). The phenolic fraction is affected by different heating treatments; Cerretani et al. (*15*) studied the effect of microwave heating treatments on phenols compared to oxidation or heating by conventional oven, particularly for lignans which show high stability to thermal treatments due to their weak antioxidant properties (*11*).

The main parameters that influence the extent of oxidation and the degradation of oils during heating are the oil composition, time and temperature of heating, the food (in the case that there is some food in contact with the oil) and the surface-to-oil volume ratio (*16*). Strong interactions exist among these variables, so they are difficult to control and define (*17*).

The purpose of this investigation was to study how the heating treatments by microwave or conventional oven under common home-cooking conditions may affect the hydrolytic degree and the oxidation state of a genuine EVOO and its blends with a DEO, by using several chemical approaches. Moreover, a duo−trio test was carried out to verify if the final consumer is able to sensorially distinguish between EVOO and DEO after heating. To the best of our knowledge there is no data recorded about heating behavior of DEOs or their admixtures with EVOOs.

MATERIALS AND METHODS

Apparatus. The determination of k_{270} was carried out using an UV−vis 1610 instrument (Shimadzu Co., Kyoto, Japan), which had a six slot shuttle and a system for temperature control of working conditions. The oxidative stability of samples was evaluated by the OSI, using an eight-channel oxidative stability instrument (Omnion, Decatur, IL). All gas chromatography analyses were performed using a Carlo Erba MFC 500 (Carlo Erba, Milan, Italy) gas chromatograph equipped with a flame ionization detector (FID) or an Agilent 6890N Network gas chromatograph, coupled with a quadrupolar mass-selective spectrometry Agilent 5973 Network detector (Agilent Technologies, Palo Alto, CA). High-performance liquid chromatography (HPLC) analyses of tocopherols were performed with a HP 1100 series (Agilent Technologies) provided with a binary pump delivery system, a degasser, an autosampler, diode array UV−vis detector (DAD) and mass spectrometer detector (MSD).

Materials, Reagents and Standards. The SPE cartridges (6 mL) STRATA Si-1 Silica (55 μm, 70 Å) packed with silica gel phase (1000 mg), were obtained from Phenomenex (Torrence, CA). The standards used for diacylglycerols (DAG) quantification (dilaurin), for oxidized fatty acid (OFA) quantification (tricaproin and triheptadecanoin) and for fatty acid alkyl esters (FAAEs) quantification (pentadecanoic acid ethyl ester (C15:0 EE) and heptadecanoic acid methyl ester (C17:0 ME)) were acquired from Sigma-Aldrich (St. Louis, MO), as were sodium benzyloxyde in benzyl alcohol and α-tocopherol. Hydranal-Titran 2 and Hydranal-solvent oil (solvents used to measure the water content with the volumetric titration of Karl Fischer) were purchased from Sigma-Aldrich, too. The internal standard used for quantification of volatile compounds (4-methyl-2-pentanone) and for their identification (hexanal, (*E*)-2-hexenal, (*Z*)-3-hexen-1-ol, (*E*)-2-hexen-1-ol, 1-hexanol, hexylacetate, (*Z*)-2-pentenol, 3-pentanone, (*E*)-2-pentenal, 1-penten-3-ol, 1-penten-3-one, farnesene, limonene, β-ocimene, α-pinene, α-copaene, propanoic acid, butanoic acid, pentanoic acid, hexanoic acid, heptanoic acid and octanoic acid) were purchased from Fluka (Buchs, Switzerland). Methyl pentadecanoate, ethyl pentadecanoate, methyl heptadecanoate, methyl palmitate, ethyl palmitate, methyl oleate, ethyl oleate, methyl linoleate, and ethyl linoleate were used to identify the alkyl esters and to calculate their response constants; they were obtained from Sigma-Aldrich. All solvents used were analytical grade (Merck & Co. Inc., Darmstadt, Germany).

Samples and Thermal Treatment. Genuine EVOO and a sample of oil strongly suspected for mild deodorization (DEO) were analyzed as well as two blends of these oils, prepared at seventy−thirty percent (70−30, v/v) and fifty−fifty percent (50−50, v/v) of EVOO and DEO respectively. Not heated oils (termed TQ) were sampled as control. For analytical purposes, 300 g of each kind of sample was inserted in a opened glass container and subjected to microwave (MW) or conventional (CO) heating; the heated samples were respectively named as MW and CO. The surface/volume ratio was constant for the samples (256 cm^2/330 mL, total capacity of glass container 1.5 L, oil thickness 1.6 cm). The amount of olive oil subjected to thermal treatment was enough to carry out all the analyses in triplicate, especially for the extraction of phenolic compounds (*18*). The time−temperature conditions for both heating treatments were similar to home-cooking or food catering: all the samples were heated either for 1 h in a conventional oven (type M20-VN, Instruments s.r.l, Bernareggio (MI), Italy) at 180 °C or for 9 min at 750 W of power in a microwave oven (model no. AMW214/WH of Whirlpool, Benton Harbor, MI), with a frequency of radiation of 2450 MHz. These combinations of time and temperature for each type of heating system were necessary to reach similar final temperatures. During heating, the temperature was registered at fixed intervals of thermal treatment by a thermocouple HI 98804 (Hana Instrument, Woonsocket) inserted at approximately the geometrical center of the samples. Both unheated and heated samples were stored at 12 °C in a thermostat and some aliquots in a freezer at −43 °C before the analyses. All chemical analyses were performed in triplicate.

Determination of Fatty Acid Alkyl Esters (FAAEs). Isolation of fatty acid alkyl esters (FAAEs) was carried out according to Pérez-Camino et al. (*3*), but slight modifications have been introduced, especially concerning the internal standards used. A 0.2 ± 0.0001 g amount of oil sample was mixed with 250 μL of standard solutions of C15:0 EE and C17:0 ME, both 50 $\mu g\ g^{-1}$, and *n*-hexane was added to reach the volume of 2 mL. This oil solution was split in two fractions of 1 mL and eluted separately. Silica SPE cartridges (1000 mg) were placed in an automatic vacuum elution apparatus and conditioned by passing 12 mL of *n*-hexane. Then 1 mL of the oil solution was charged, and the solvent was pulled through at 0.5 mL min^{-1}, leaving the samples and the standards on the cartridge. The elution was made with 7 mL of the solvent mixture *n*-hexane:toluene (85:15, v/v), and this fraction was rejected. Then the alkyl esters were collected, by elution with 10 mL of the same mixture at a flow rate of 1 mL min^{-1}. The eluate was evaporated in a rotary evaporator at room temperature under vacuum until dry. The residue was dissolved with 200 μL of heptane, and a volume (1 μL) of this solution was injected into a GC port set at 280 °C. A Zebron ZB-1 ms (Phenomenex) silica capillary column (30 m length × 0.25 mm i.d. × 0.25 μm film thickness) was utilized. A flow rate of 1 mL min^{-1} of helium was used as carrier gas. The FID detector was set at 280 °C. The initial oven temperature was kept at 140 °C for 3 min and increased to 200 °C at a rate of 5 °C min^{-1}, then raised to 280 °C with a rate of 10 °C min^{-1}. Response constant were calculated for each alkyl ester to determine the correct concentrations of each compound, using standard solutions of alkyl esters at three different concentration (0.050, 0.025, and 0.010 mg mL^{-1}). The amount of ethyl esters was expressed as mg of C15:0 EE kg^{-1} of oil, while methyl esters were reported as C17:0 ME kg^{-1} of oil.

Hydrolytic Degree of the Samples. The hydrolytic degree of the samples was evaluated by the analysis of the free acidity (FA) and by the content of total diacylglycerols (DAG) and major 1,2- and 1,3-DAG carried out by a GC method. FA was determined according to the European Communities methods (*19*) and the following amendments and expressed as g of oleic acid per 100 g of oil.

Gas Chromatographic (GC) Determination of Total Diacylglycerols (DAG) and Major 1,2-DAG and 1,3-DAG. DAG were determined according to a modified version of the method suggested by Serani et al. (*5*). 500 μL of a solution of dilaurin, used as internal standard (20 mg of dilaurin in 10 mL of chloroform), was added to 100 mg of oil. The mixture was vortexed for about 30 s and taken to dryness under a nitrogen stream. The residue was silylated according to Sweeley et al. (*20*) with 0.2 mL of silylation reactive, obtained from pyridine, hexamethyldisilazane and chlorotrimethylsilane (3:1:9, v/v) and kept for 5 min at room temperature. Then, this mixture was taken to dryness under a nitrogen stream and the residue was dissolved in 200 μL of *n*-hexane centrifuged at 2000*g* for 1 min. A volume (1 μL) of the solution was injected into a GC equipped with a flame ionization detector (FID). The GC was a Carlo Erba MFC 500 which was coupled to a Rtx-65TG (Rested, Bellefonte, PA) fused silica capillary column (30 m length × 0.25 mm i.d. × 0.10 μm film thickness) coated with 35% dimethyl−65% diphenyl polysiloxane. Oven temperature was programmed from 250 to 320 °C at a rate of 2 °C min^{-1} then raised to 365 °C at a rate of 5 °C min^{-1}. The final temperature was kept for 21 min. The injector and detector temperatures were both set at 360 °C. Helium was used as carrier gas at a pressure of 130 kPa. The split ratio was 1:70. Identification of DAG was carried out by comparing the

peak retention times and the GC traces with those of the DAG standards and chromatograms reported in literature. Results were quantified respect dilaurin as internal standard and expressed as g of dilaurin in 100 g of oil.

Determination of Water Content in Virgin Olive Oil. The water content of the samples was analyzed with a TitroMatic 1S instrument. This measurement uses a Karl Fischer titration based on 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, and Hydranal-Titran 2 was used as a titrating reagent. Each sample was introduced three times, and the quantity of sample was measured with the back weighing technique. The sample was dissolved in the solution of chloroform:Hydranal-solvent (2:1, v/v) oil and the titrating reagent was added until the equivalence point. The quantity of water was expressed as mg of water per kg of oil ($n = 3$).

Oxidative Status of the Samples. Primary Autoxidation Products. Evaluation of primary autoxidation products was performed by the determination of the peroxide value (POV). POV was determined according to the European Communities official methods and the following amendments (*19*). POV was expressed as mequiv of O_2 kg^{-1} of oil.

Oxidative Status of the Samples. Secondary Autoxidation Products. In this work the presence of secondary oxidation products was carried out by the determination of specific absorption at 270 nm (k_{270}), by the study of volatile compounds (SPME−GC/MS) and the analysis of OFA (oxidized fatty acids).

Determination of k_{270}. The UV-spectrophotometric index was determined according to the European Communities official methods and the following amendments (*19*). To calculate the k_{270} value, the oil samples were diluted in isooctane in a 1% solution, placed into a 1 cm cuvette, and analyzed at the wavelength of 270 nm, against a blank of isooctane.

Volatile Compounds Analysis. A 1.5 g amount of the sample was weighed into a 10 mL vial. The oil sample was spiked with 0.15 g of 4-methyl-2-pentanone (internal standard) to a concentration of 5 $\mu g\ g^{-1}$. The vial was fitted with a silicone septum and immersed in a water bath at 37 °C; the sample was maintained under magnetic stirring. After 2 min of sample conditioning a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber (50/30 μm, 2 cm long from Supelco Ltd., Bellefonte, PA) was exposed to the sample headspace for 30 min and immediately desorbed for 3 min at 250 °C in the gas chromatograph. Volatile compounds were identified and quantified by gas chromatography coupled with quadrupolar mass-selective spectrometry, using an Agilent 6890N Network gas chromatograph and an Agilent 5973 Network detector (Agilent Technologies). Analytes were separated on a ZB-WAX column 30 m × 0.25 mm i.d., 1.00 μm film thickness (Phenomenex). Column temperature was held at 40 °C for 10 min and increased to 200 at 3 °C min^{-1}. The FID temperature was set at 250 °C and the ion source and the transfer line were at 180 and 230 °C, respectively. Electron impact mass spectra were recorded at 70 eV ionization energy in the 15−250 amu mass range, 2 scans s^{-1}. The identification of the volatile compounds was first carried out by mass spectrometry and later checked with standards (*21*). Moreover, the volatile identification was obtained by a comparison of their mass spectral data with the information from the National Institute of Standards and Technology (NIST) library (2005 version) and MS literature data. Volatile compounds were also identified using the relative retention times of the standards with respect to the internal standard (4-methyl-2-pentanone) and expressed as mg of internal standard per kg of oil.

Analysis of Oxidized Fatty Acids (OFA). The determination of OFA was carried out according to Rovellini and Cortesi (*22*), after transesterification with 1.0 M sodium benzyloxyde in benzyl alcohol followed by HPLC−DAD analysis at $\lambda = 255$ nm. The results were referred to tricaproin, added as internal standard, and expressed as percentage (g of OFA 100 g^{-1} of oil). OFA were tentatively identified based on their UV−vis and mass spectra and literature data (*23*).

Chromatographic Analysis of Tocopherols. One gram of oil sample was dissolved in 10 mL of *n*-hexane, and extracts were filtered through a 0.45 μm nylon filter. The amount of α-tocopherol was determined by HPLC equipped with a DAD set at 295 nm. The column used was a Phenomenex, Luna, CN 100A (150 mm, 4.6 mm i.d.) in isocratic conditions with *n*-hexane:dichloromethane:methanol (95:4:1, v/v), as the mobile phase, at a flow rate of 1.0 mL min^{-1}. The injection volume was 5 μL. Analyses were carried out at room temperature. A calibration curve was constructed with standard solutions of α-tocopherol ($r^2 = 0.999$) and used for quantification. Results are given in mg of α-tocopherol per kg of oil.

Spectrophotometric Determination of Total Phenol Content. The total phenol (TP) content of the extracts was carried out spectrophotometrically using Folin-Ciocalteu reagent and the absorbance was determined at 750 nm (*23*). Total phenols were quantified using a gallic acid calibration curve ($r^2 = 0.994$). The results were expressed as mg of gallic acid kg^{-1} of oil.

Evaluation of Oxidative Stability under Forced Conditions. An eight-channel oxidative stability instrument (OSI) (Omnion) was used. To obtain the OSI time, a stream of purified air (120 mL min^{-1} air flow rate) was passed through 5.0 ± 0.1 g oil sample heated at 110 °C, under atmospheric pressure. The effluent air contains especially short chain acids as formic acid and other volatile compounds formed during thermal oxidation of the oil; these substances were recovered and measured in deionized water, as an increase of conductivity. The OSI time was expressed in hours and hundredths of hours, which was defined as a measure of the stability of an oil.

Fatty Acid Composition. The fatty acid composition of oil samples was determined as FAMEs after alkaline treatment, obtained by mixing 0.05 g of oil dissolved in 2 mL of *n*-hexane with 1 mL of 2 N potassium hydroxide in methanol according to Christie (*24*). A volume (1 μL) of this solution was injected into a GC equipped with a flame ionization detector (FID), following the same conditions described for the determination of FAAEs.

Olfactory Discriminant Analysis by "Duo−Trio" Test. To establish if the samples were different for the sensory characteristics, a duo−trio test was performed by a fully trained sensory panel group of the Department of Food Sciences of the University of Bologna (Italy) recognized by Italian Ministry of Agriculture (Mipaaf) as required by European Union (EU) law 2568/91 (*19*) and following amendments. Duo−trio test analysis was applied according to Meilgaard et al. (*25*). An identified reference sample was presented to each subject (a group of 10 trained tasters), followed by two coded samples, one of which matches the reference sample. Tasters had to indicate which coded sample matches the reference, on the basis of a simple olfactory analysis; two samples were discriminated if the number of correct replies was at least 9 (0.05 as significance level). All oils were compared so 30 possible combinations were prepared using a balanced reference mode (that is all samples being compared were used at random as the reference).

Statistical Analysis. Means and standard deviations were calculated with Statistica 6.0 (2001, Starsoft, Tulsa, OK) statistical software. Statistica was used to perform one-way-analysis of variance and Tukey's honest significant difference test at a 95% confidence level ($p < 0.05$) to identify differences among groups.

RESULTS AND DISCUSSION

Determination of Fatty Acid Alkyl Esters (FAAEs). According to Pérez-Camino et al. (*3*) and Biedermann et al. (*7*), methyl and ethyl esters of free fatty acids can be considered as good markers of virgin olive oil subjected to mild deodorization: in particular, the limits proposed by Mariani and Bellan (*8*) and Pérez-Camino et al. (*3*) were checked. The histograms (**Figures 1** and **2**) show that the oil strongly suspected for mild deodorization (DEO) and its admixtures at 50% and 70% with EVOO (50−50; 70−30) were over these limits. In particular, the total amount of FAAEs was higher than the limit, fixed at 30−40 mg kg^{-1} of oil, for these samples (*8*). DEO was the only sample characterized by a total amount of FAAEs over 70 mg kg^{-1} of oil, which was the limit fixed by Pérez-Camino et al. (*3*). On the other hand, EVOO was largely under these limits. No significant variations were evidenced in samples subjected to heating. As shown in **Figure 2**, the amount of ethyl oleate exceeded the quantity of 15 mg kg^{-1} of oil in DEO, 70−30 and 50−50. Moreover, the FAMEs/FAEEs ratio was lower than 0.9 for DEO, confirming that these samples could be (or contain) mildly deodorized olive oil.

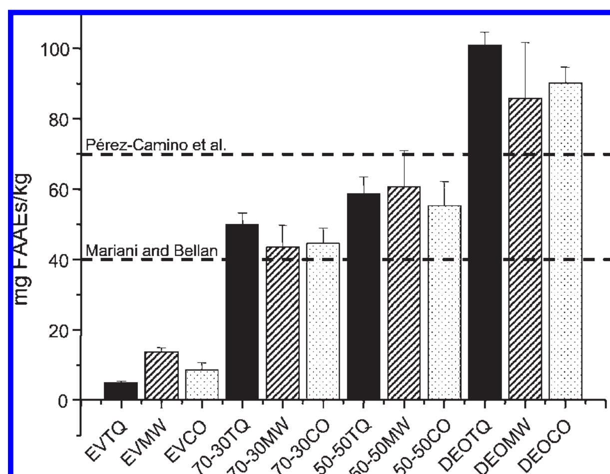

Figure 1. Total amount of FAAEs (in mg kg^{-1}) for all the analyzed samples, before and after the thermal treatments by microwave and conventional oven. The limits proposed (40 and 70 mg kg^{-1}) by Mariani and Bellan (*8*) and Pérez-Camino et al. (*3*) respectively are indicated as dotted lines.

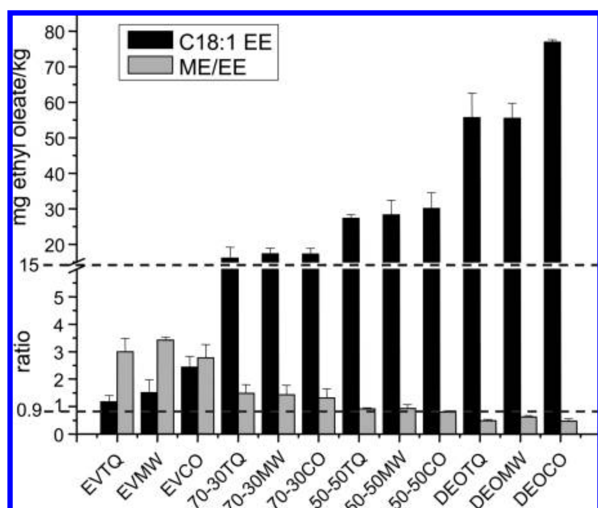

Figure 2. C18:1 EE content (in mg kg^{-1}) and methyl esters/ethyl esters ratio (ME/EE) for all the analyzed samples, before and after the thermal treatments by microwave and conventional oven. The limits for ME/EE and for C18:1 EE respectively of 0.9 and 15 proposed by Mariani and Bellan (*8*) are indicated as dotted lines.

Time−Temperature Profile and Fatty Acid Composition. The final temperature, registered by a thermocouple into the mass of oil, was similar for both heating systems, but the raising of the temperature was more rapid for the microwave heating (**Table 1**). In this work, the surface/volume ratio and the time−temperature conditions were constant for all oil samples. The fatty acids composition was similar for all samples, too. In fact, as shown in **Table 2**, the ratio between oleic acid and linoleic acid ranged from 8.3 to 11.0 for EVOO and DEO respectively, with middle values for the admixtures, and not significative variations were observed in samples subjected to the heating treatments.

Hydrolytic Degree of the Samples. The hydrolytic degree of oil samples was determined by the titrimetric determination of the free acidity (FA) and the gas chromatographic analysis of 1,2- and 1,3-diacylglycerols (DAG). As shown in **Table 3**, FA did not significantly increase after microwave or conventional heating of the samples. Moderate FA increase was generally found in vegetable oils after heating (*15*, *26*). On the other hand, Albi et al. (*12*) did not find FA increase after 120 min of microwave heating at half power (170 °C) in EVOO. FA was much higher for DEO than EVOO (**Table 3**): the high value of FA found in DEO could suggest that this sample was not probably subjected to a previous neutralization, which is sometimes used before the mild deodorization treatment of low quality virgin olive oils with organoleptic defects and moderate free acidity (*3*). As was expected, **Figure 3** shows a higher amount of 1,3-DAG in DEO than in EVOO probably due to its heating during the mild deodorization step. The content of 1,3-DAG significantly increased with heating treatments of oils, and in particular, this effect was more evident for conventional oven than microwave. In fact, the 1,2-DAG isomers are reported to be mainly present in a fresh EVOO due to an incomplete biosynthesis of triacylglycerols, whereas the 1,3-DAG increase in oils stored for a long period of time or in oils subjected to heating because of their higher stability in comparison with the 1,2-forms (*5*). DEOTQ showed a lower amount of water in microemulsion than EVTQ (**Table 3**), according to Cerretani et al. (*4*). A decrease of water was evidenced after thermal treatments, probably because of the evaporation effect due to the high temperature reached, without significant variations between the two heating system.

Table 1. Mean Temperatures of Oil Sample at Different Treatment Times for the Two Heating Conditions[a]

microwave oven		conventional oven	
time (min)	*T* (°C)	time (min)	*T* (°C)
0	25	0	25
5	102	20	106
6	115	30	126
7	129	40	143
8	143	50	160
9	165	60	168

[a] Data are expressed as mean of three determinations.

Oxidative Status of the Samples. It is well-known that the primary derivatives of fatty acids oxidation can be evaluated by the POV determination. During autoxidation, hydroperoxides undergo decomposition reactions that give rise to several secondary oxidation compounds (*27*). POV showed different trend according to the two types of heating systems: POV increased for microwaved samples (**Table 4**), according to Cossignani et al. (*28*), reaching values slightly higher than the legal limit of 20 mequiv O_2 kg^{-1} (*29*). Albi et al. (*12*) found a small POV increase for EVOO after microwave heating at 170 °C for 120 min. POV was significantly higher for heated EVOO after thermal treatments at 100 °C for 142 h with a flow of air of 10 L h^{-1} (*26*). In DEO and 70−30 samples, POV decreased after thermal treatment by conventional oven (**Table 4**), as found by Carrasco-Pancorbo et al. (*11*) for the first hour of heating in conventional oven at 180 °C. The significant decrease of POV especially measured in oils subjected to heating by conventional oven is probably due to a faster oxidative degradation. This leads to higher values of secondary oxidation products evidenced by the specific UV-absorbance and also by the content of (*E*)-2-heptenal. In fact, in this work the presence of secondary oxidation products was measured by specific absorption at 270 nm, by OFA values and by volatile aldehydes. Before thermal treatments, the samples showed k_{270} values that were below the limit established by the EC Regulation for EVOO category (*29*), which corresponded to 0.22. However, after microwave or conventional heating, all the samples exceeded this limit, except for the 70−30MW (**Table 4**). In fact, a k_{270} increase is probably related to the presence of conjugated trienes (due to isomerization and

Table 2. OSI Values, Oleic/Linoleic Acid Ratios, α-Tocopherol and Total Phenols Amount in All the Analyzed Samples before and after the Thermal Treatments by Microwave and Conventional Oven[a]

	OSI (h)		C18:1/C18:2		α-tocopherol (mg kg^{-1} oil)		total phenols (mg kg^{-1} oil)	
	mean	sd	mean	sd	mean	sd	mean	sd
EVTQ	20.6 a	0.1	8.3 f	0.03	207.47 a	1.83	309.87 a	12.51
EVMW	13.8 c	0.1	8.3 f	0.10	155.89 d,e	15.85	221.02 b	18.02
EVCO	15.3 b	0.1	8.4 e	0.05	181.07 a,b,c,d	6.54	223.43 b	13.06
70−30TQ	20.5 a	0.5	9.0 d	0.03	189.41 a,b,c	10.76	231.07 b	18.13
70−30MW	11.2 d	0.1	9.1 d	0.02	146.4 e	8.66	168.28 c	7.69
70−30CO	13.9 c	0.2	9.1 d	0.02	163.17 c,d,e	3.04	167.15 c	11.05
50−50TQ	11.3 d	0.1	9.5 c	0.03	191.81 a,b	3.82	208.52 b	18.95
50−50MW	9.2 e	0.1	9.6 b,c	0.01	104.51 f,g	9.64	130.20 d	3.90
50−50CO	9.7 e	0.1	9.7 b	0.06	111.14 f	11.47	117.07 d,e	4.09
DEOTQ	7.6 f	0.1	10.9 a	0.04	166.63 b,c,d,e	2.5	103.00 e	0.38
DEOMW	5.0 g	0.1	11.0 a	0.04	75.04 h	14.79	73.99 f	2.95
DEOCO	4.8 g	0.1	11.0 a	0.02	83.04 g,h	2.41	66.20 f	2.99

[a] Data are expressed as mean of three determinations, standard deviations given in columns. Same letters within each column do not significantly differ ($p < 0.05$). OSI, oxidative stability index expressed as hours; C18:1/C18:2, oleic/linoleic acid ratio; TP, total phenols determined by spectrophotometric analysis and expressed as mg gallic acid per kg of oil; α-tocopherol determined by HPLC-DAD analysis and expressed as mg α-tocopherol per kg of oil.

Table 3. FA, 1,3-DAG and Water Contents in All the Analyzed Samples before and after the Thermal Treatments by Microwave and Conventional Oven[a]

			1,3-DAG (g 100 g^{-1} oil)		water (mg kg^{-1} oil)	
	mean	sd	mean	sd	mean	sd
EVTQ	0.18 b	0.02	0.09 f	0.02	734 a	39
EVMW	0.17 b	0.00	0.21 e,f	0.03	437 e,f	25
EVCO	0.19 b	0.03	0.36 d,e,f	0.06	459 d,e,f	35
70−30TQ	0.15 b	0.02	0.46 c,d,e,f	0.05	665 a,b	49
70−30MW	0.22 b	0.02	0.53 c,d,e	0.01	504 d,e,f	32
70−30CO	0.20 b	0.04	0.72 b,c,d	0.01	462 d,e,f	17
50−50TQ	0.21 b	0.02	0.66 b,c,d	0.02	624 b,c	65
50−50MW	0.21 b	0.02	0.55 c,d,e	0.10	530 d,e	24
50−50CO	0.18 b	0.02	0.98 a,b	0.03	556 c,d,e	24
DEOTQ	0.54 a	0.03	0.56 c,d,e	0.08	567 b,c,d	29
DEOMW	0.54 a	0.06	0.85 a,b,c	0.23	411 f	28
DEOCO	0.52 a	0.03	1.11 a	0.11	400 f	12

[a] Data are expressed as mean of three determinations, standard deviations given in columns. Same letters within each column do not significantly differ ($p < 0.05$). FA, free acidity determined by a titrimetric analysis and expressed as g of oleic acid in 100 g of oil; 1,3-DAG, 1,3-diacylglycerols determined by GC-FID and expressed as g of dilaurin per 100 g of oil; water content determined by a Karl-Fischer titration and expressed as mg of water per kg of oil.

conjugation of double bonds in oxidation products of linolenic acid) and secondary products of oxidation, such as aldehydes and ketones (*26*). A similar trend was evidenced by Allouche et al. (*30*) after conventional heating at 180 °C for 36 h and after thermal treatments for 142 h at 100 °C with a flow of air of 10 L h^{-1} (*26*). The increase of k_{270} was greater after heating by conventional oven than microwave oven, probably because of a stronger oxidative process. As reported in **Table 4**, the OFA values of oils tendentially increased in EVOO and its admixtures (50−50; 70−30) after heating in microwave oven while they decreased in DEO. On the other hand, it is possible to observe lower values of OFA for samples subjected to conventional oven heating than for the corresponding microwaved oils that were in each case lower than those of DEOTQ. These results indicate a stronger decomposition of OFA as a consequence of conventional oven than microwave heating. The most advanced oxidation stages, due to thermal treatments, are characterized by a drastic reduction of compounds arising from the LOX pathway (C_6 aldehydes, alcohols and ketones) and very high concentrations of C_5−C_{11} saturated and unsaturated aldehydes, as shown in **Figure 4**. In particular, (*E*)-2-heptenal can be considered a useful marker of secondary oxidation products (*31*). This compound is characterized by a very low odor threshold (1.5 μg per kg in the virgin olive oil) and a penetrating fatty rancid odor, too. According to the above-reported comments for the OFA values, the speed of formation of secondary oxidation products was particularly accelerated by conventional oven heating; in fact, the amount of (*E*)-2-heptenal was higher after heating by conventional oven than by microwave oven (**Table 4**), as previously found for k_{270} values.

In olive oils, tocopherols are mainly represented by α-tocopherol, which is biologically the most active. Together with polar phenolic compounds and carotenoids, they are responsible by the oxidative stability of olive oil (*2*). **Table 2** reported the α-tocopherol content of the studied samples. A considerable loss of α-tocopherol was observed after heating, by conventional or microwave oven, as reported by several works (*10*, *26*, *30*, *32*, *33*). The decrease was more evident for DEO than EVOO, and this behavior could be partially attributed to the difference of the amount of phenolic compounds, which was lower for DEOTQ than EVTQ (**Table 2**). It is well-known that phenols are able to reduce the oxidized forms of tocopherols, such as tocopheryl radicals and quinones (*23*). As DEOTQ was very poor in phenolic compounds (**Table 2**), α-tocopherol protects the oil from thermal oxidation by decreasing quickly. In HPLC chromatograms, it has been possible to observe the presence of some unknown peaks that eluted before the α-tocopherol under normal phase conditions; they were also formed by heating especially by conventional oven. To date, these compounds (characterized by a UV maximum respectively of 270 and 265 nm) have not been identified yet. They may be considered as oxidative or degradation forms of α-tocopherol, as for example α-tocopherylquinone (*34*).

Evaluation of Oxidative Stability under Forced Conditions. In general, OSI time values depend on the qualitative and quantitative composition of fatty acids, tocopherols and phenols of the samples, with different contributions (*2*). **Table 2** shows the OSI time values of the examined samples, and for each one is also reported the ratio between oleic and linoleic acid, the α-tocopherol and total phenol contents. DEO showed OSI time values lower than EVOO; this difference is linked to the amounts of antioxidants, as total phenol and α-tocopherol, and to the starting oxidative status. In fact, the ratio between oleic and linoleic acid was higher for DEO than for EVOO. Notable decreases were observed after thermal treatments, by both conventional and microwave oven, as described by Carrasco-Pancorbo et al. (*11*), following the degradation of the antioxidant compounds but generally no variations were evidenced between the two types of heating systems.

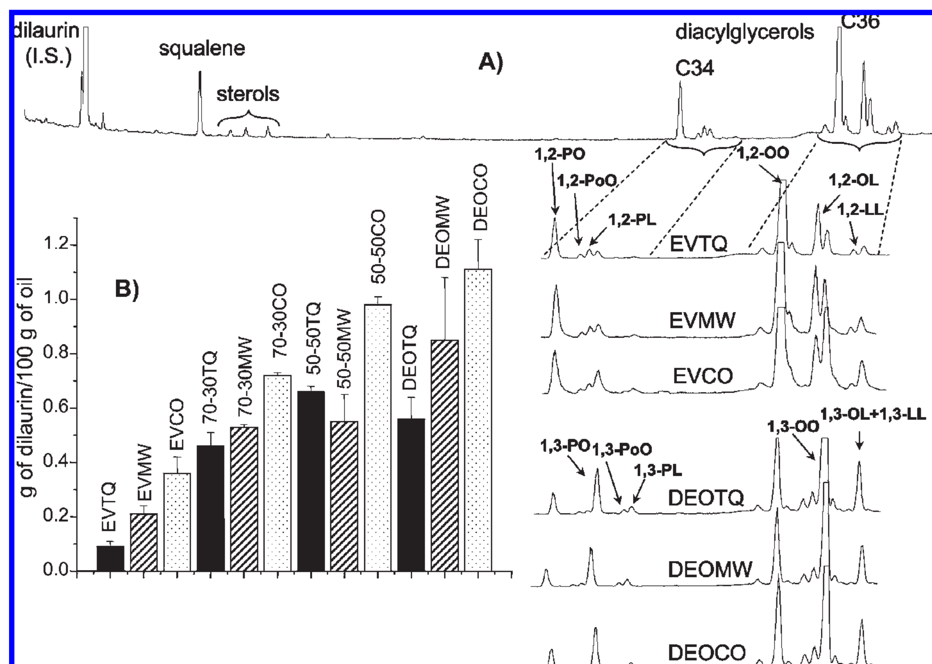

Figure 3. GC chromatograms (**A**) of DAG profile for EVOO and DEO before and after the thermal treatments by microwave and conventional oven. The histogram (**B**) shows the amount of 1,3-DAG in all samples. 1,2-PO, 1-palmitoyl-2-oleoyl-*sn*-glycerol; 1,2-PoO, 1-palmitoleoyl-2-oleoyl-*sn*-glycerol; 1,2-PL, 1-palmitoyl-2-linoleoyl-*sn*-glycerol; 1,2-OO, 1,2-diolein; 1,2-OL, 1-oleoyl-2-linoleoyl-*sn*-glycerol; 1,2-LL, 1,2-dilinolein; 1,3-PO, 1-palmitoyl-3-oleoyl-*sn*-glycerol; 1,3-PoO, 1-palmitoleoyl-3-oleoyl-*sn*-glycerol; 1,3-PL, 1-palmitoyl-3-linoleoyl-*sn*-glycerol; 1,3-OO, 1,3-diolein; 1,3-OL, 1-oleoyl-3-linoleoyl-*sn*-glycerol; 1,3-LL, 1,3-dilinolein.

Table 4. Oxidative Indices (POV, k_{270}, (*E*)-2-Heptenal and OFA) in All the Analyzed Samples before and after the Thermal Treatments by Microwave and Conventional Oven[a]

	POV (mequiv of O_2 kg^{-1} oil)		k_{270}		(*E*)-2-heptenal (mg kg^{-1} oil)		OFA (g 100 g^{-1} oil)	
	mean	sd	mean	sd	mean	sd	mean	sd
EVTQ	8.6 e	0.6	0.15 g	0.01	nd d	nd	1.33 a,b,c	0.09
EVMW	19.9 b	0.2	0.24 e	0.01	0.14 c,d	0.02	1.80 a	0.01
EVCO	14.3 d	0.6	0.33 c	0.02	0.42 b	0.06	1.64 a,b	0.13
70−30TQ	19.5 b,c	0.6	0.15 g	0.02	nd d	nd	1.08 a,b,c	0.11
70−30MW	23.8 a	0.2	0.19 f	0.01	0.36 b,c	0.08	1.42 a,b,c	0.18
70−30CO	17.4 c	1.6	0.44 b	0.02	0.50 a	0.03	0.67 c	0.09
50−50TQ	19.4 b,c	0.1	0.18 f,g	0.01	nd d	nd	1.28 a,b,c	0.03
50−50MW	23.3 a	1.8	0.28 d	0.01	0.42 b,c	0.04	1.52 a,b	0.22
50−50CO	19.5 b,c	0.2	0.58 a	0.01	0.54 b,c	0.08	0.85 b,c	0.13
DEOTQ	17.6 c	1.2	0.19 f	0.01	nd d	nd	1.84 a	0.34
DEOMW	19.4 b,c	0.6	0.3 c,d	0.01	0.45 b,c	0.02	1.51 a,b	0.2
DEOCO	12.6 d	0.2	0.45 b	0.01	0.68 b	0.07	0.79 b,c	0.09

[a] Data are expressed as mean of three determinations, standard deviations given in columns. Same letters within each column do not significantly differ (p < 0.05). POV, peroxide value determined by a titrimetric analysis and expressed as mequiv of O_2 kg^{-1} of oil; k_{270}, determined by specific absorption at 270 nm; (*E*)-2-heptenal determined by SPME−GC/MS and expressed as mg of internal standard per kg of oil; OFA, oxidized fatty acids determined by HPLC−DAD analysis and expressed as g of OFA 100 g^{-1} of oil.

Olfactive Discriminant Analysis by "Duo−Trio" Test. As was expected, the aroma of nonheated oils resulted different from those heated. In fact, high temperatures speed up the oxidative rate of olive oil (*1*, *35*), inducing a progressive formation of hydroperoxide, which are odorless and tasteless. However, they are susceptible to decomposition into secondary oxidation products. Among them, several volatile compounds (saturated and unsaturated aldehydes, ketones, hydrocarbons, alcohols, acids, lactones, esters and furans) are formed (*36*): because of the low flavor threshold of most of these compounds (*37*), they affect the sensory characteristics of oils and fat-containing products and contribute more with the rancid flavor. In general, for each kind of oil, tasters discriminated between samples heated by microwave and conventional oven, suggesting that different oxidative degrees were obtained with these different heating systems. Moreover, the aroma of EVOO and its admixture at 70% (70−30) resulted different from DEO both for not heated and microwaved samples, whereas tasters were not able to discriminate between oils after heating by conventional oven. This is in agreement with a contemporary higher formation of volatile aldehydes from the oxidation process and a more drastic reduction of pleasant compounds from LOX pathway after heating treatment by conventional oven. The mixture with 50% of DEO (50−50TQ) resulted not different from DEOTQ, even after they were both subjected to heating treatments.

In conclusion, the determination of FAAEs confirmed that the oil strongly suspected for mild deodorization (DEO) was effectively obtained by this illegal treatment. As suggested by the analysis carried out in this study, thermal treatments by conventional oven led to a more intense oxidative and hydrolytic

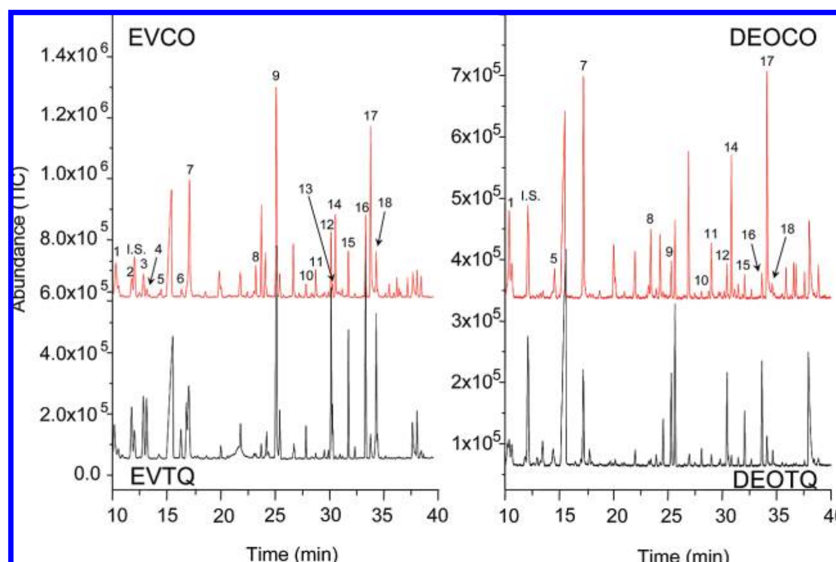

Figure 4. GC chromatograms of volatile compounds for EVOO and DEO before and after the thermal treatments by conventional oven. 1, pentanal; 2, pentene dimer; 3, pentene dimer; 4, 1-penten-3-one; 5, (*E*)-2-butenal; 6, pentene dimer; 7, hexanal; 8, heptanal; 9, (*E*)-2-hexenal; 10, hexyl acetate; 11, octanal; 12, (*Z*)-3-hexenyl acetate; 13, (*Z*)-2-penten-1-ol; 14, (*E*)-2-heptenal; 15, 1-hexanol; 16, (*Z*)-3-hexen-1-ol; 17, nonanal; 18, (*E*)-2-hexen-1-ol.

degradation of the samples than by microwave heating. Probably this evidence could be attributed to the longer heating time with conventional oven. This trend was confirmed by the results of an olfactory duo−trio test: in general tasters were able to discriminate between each kind of sample heated by microwave and conventional oven. On the contrary, tasters were not able to discriminate between EVOO and DEO both treated by conventional oven (EVCO and DEOCO), suggesting a real difficulty for consumers to identify a DEO after this kind of heating. Moreover, heating treatments caused strong depletions in antioxidant compounds, as seen for total phenols and α-tocopherol. Further studies should be carried out on a larger set of DEO samples to confirm results obtained in this experimentation taking into account different surface/volume ratio and/or different home-cooking conditions such as deep-frying.

ABBREVIATIONS USED

EVOO, extra virgin olive oil; DEO, mildly deodorized olive oil; FAAEs, fatty acid alkyl esters; FID, flame ionization detector; HPLC, high-performance liquid chromatography; DAD, diode array UV−vis detector; MSD, mass spectrometer detector; SPE, solid phase extraction; DAG, diacylglycerols; OFA, oxidized fatty acid; C15:0 EE, pentadecanoic acid ethyl ester; C17:0 ME, heptadecanoic acid methyl ester; FA, free acidity; POV, peroxide value; SPME, solid phase microextraction; FAMEs, fatty acid methyl esters; FAEEs, fatty acid ethyl esters; LOX, lipoxygenase pathway; C18:1 EE octadecenoic acid ethyl ester.

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