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ORIGINAL PAPER

Oxidative Evolution of Virgin and Flavored Olive Oils Under Thermo-oxidation Processes

Manel Issaoui · Guido Flamini · Myriem Ellouze Hajaij · Pier Luigi Cioni · Mohamed Hammami

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Abstract Changes in the oxidative status of Chétoui olive oil were monitored to attest the efficiency of some bioactive compounds from aromatic plants to improve the stability of olive oils after a maceration process at different concentrations. Aromatized olive oils were prepared by addition of lemon and thyme extracts at four different concentrations (20–80 g kg^{-1} of oils) to virgin olive oils. The following parameters were monitored: free fatty acids, peroxide value, ultra violet absorption characteristics at 232 and 270 nm, fatty acid composition and aromatic profiles. After thermo-oxidation processes, the oleic/linoleic acid ratio remained stable (4.5). Oxidative stability slightly decreased during thermo-oxidation processes. The heating of the oils changed their volatile profile and led to the formation of new volatile compounds, such as the two isomers of 2,4-heptadienal after heating at 100 °C or (*E,Z*)-2,4-decadienal and (*E,E*)-2,4-decadienal after thermo-oxidation at 200 °C. The use of lemon and thyme extracts modified the aromatic and the nutritional value of the olive oil by the transfer of some bioactive compounds, such as limonene and carvacrol. In contrast, the oxidative stability of the product did not change. Furthermore, the aromatized oils may be employed in seasoning and cooking of some foods.

Keywords Chétoui olive oil · Oxidative statute · Thermo-oxidation · Hexanal/nonanal ratio · Flavored olive oil

M. Issaoui
High Institute of Applied Sciences and Technology of Medenine, University of Gabes, 4100 Medenine, Tunisia

M. Issaoui · M. E. Hajaij · M. Hammami (✉)
Laboratory of Biochemistry, UR: "Human Nutrition and Metabolic Disorder", Faculty of Medicine of Monastir, 5019 Monastir, Tunisia
e-mail: mohamed.hammami@fmm.rnu.tn

G. Flamini (✉) · P. L. Cioni
Dipartimento di Scienze Farmaceutiche, Sede di Chimica Bioorganica e Biofarmacia, via Bonanno 33, 56126 Pisa, Italy
e-mail: flamini@farm.unipi.it

Introduction

Virgin olive oil has been a widely used product throughout the ages in the Mediterranean cuisine and it is still highly appreciated for its delicious taste and aroma, as well as for its nutritional properties [1–5]. The nutritional benefits are primarily related to its fatty acid composition, characterized by a high content of oleic acid. Compared with other vegetable oils, virgin olive oil has been shown to be more resistant to oxidation thanks to its balanced composition and its richness in antioxidant compounds. However, despite its stability, virgin olive oil remains susceptible to rancidification [6]. Lipid oxidation constitutes a major factor for quality deterioration of olive oil.

Aromatized olive oils have been defined as oils that have been processed with plants, spices or herbs to improve their nutritional value, modify their sensory characteristics and, sometimes, increase their shelf-life [7–9]. Their origins seem to derive from ancient conservation practices with regard to vegetables. The resulting oils acquired the corresponding flavor and they were used for pasta, meat and salad dressings. Even if, according to EU regulation 1989 (2003) [10], they cannot be considered extra virgin olive oils, in other countries such as the USA, no federal laws are against it. However, some states such as California have enacted directives similar to those of the EU.

Methods used to evaluate olive oil quality include conventional as well as innovative techniques. Several methods have been used to assess the oxidative status of lipid substances, according to the detection of primary or secondary oxidation products and analysis of the oxidation substrate. Innovative methods include mainly Solid Phase Micro-Extraction–Gas Chromatography/Mass Spectroscopy (SPME–GC/MS) for the determination of volatiles [11–13]. SPME is a simple, fast and solventless sampling method for complex matrices [14].

The main objectives of this study were (1) to determine the changes undergone by olive oils after exposure to two high temperatures (100 and 200 °C), correlated with oxidation under cooking and frying conditions, respectively; and (2) to attest if the addition of some flavorings, such as lemon and thyme, both known for their high content of antioxidant compounds and their good flavor and aroma composition, could improve the stability of the olive oil samples submitted to the thermo-oxidation process.

Materials and Methods

Samples

An extra virgin olive oil produced in the year 2007/2008 by a three-phase continuous extraction system was used for the preparation of the flavored oils. The olives belonged to the Chétoui cultivar. It corresponded to the extra virgin olive oil commercial class according to EC regulations and it was characterized by high quality and antioxidant content. Lemon and thyme were purchased from a local market as dried plant material.

Herbs and Spices Extracts

In order to avoid turbidity and dosage troubles, the herbs and spices were not directly added to the oil, but they were previously extracted by maceration as follows: 1 kg of each herb was separately added to 5 L of extra virgin olive oil in sealed conical flasks, and stored at room temperature and in the dark, with daily shaking. Dilutions were made in order to determine the volume of extract to be used as minimum dose. After 2 months of storage, the extracts were considered suitable for the preparation of the flavored oils [10]. Flavored oils were prepared by adding different aliquots of filtered extract to the extra virgin olive oil in order to obtain different concentrations oil samples (20, 40, 60 and 80 g L^{-1} of lemon or thyme). The flavored oils obtained were then transferred in 1-L dark glass bottles, which were filled up to 98% of the volume, sealed and stored in the dark at room temperature [10].

Accelerated Oxidation Process

The accelerated storage test was carried out in the dark, in an opened container and in the presence of low percentages of headspace volume. The oxidation process was accelerated by heating at cooking (100 °C) and frying (200 °C) temperatures. The latter is the temperature at the smoke point, defined by AOCS (1997) as the temperature (°C) at which an oil begins to continuously smoke [15] for 4 h. After the thermo-oxidation process, samples were stored at −20 °C until use.

Chemical and Physical Analyses

Free Acidity, Peroxide and Specific Extinction Values

Free acidity, peroxide value (PV) and specific extinction values were determined according to the EEC (European Official Method of Analysis) [16].

Fatty acid Composition

Individual FAME were separated and quantified by gas chromatography using a model 5890 series II instrument (Hewlett-Packard Ca Palo Alto, CA, USA), equipped with a flame ionization detector and a fused silica capillary column HP-INNOWAX (30 m length × 0.25 mm i.d. and 0.25 μm of film thickness). Nitrogen was used as the carrier gas at a flow rate of 1 mL min^{-1}. The flame ionization detection temperature 280 °C; injector temperature 250 °C; oven temperature programmed from 180 to 250 °C. Results were expressed as the relative percentage of the total area. Iodine values were calculated from fatty acid percentages using the formula reported in reference [17]. The degree of oxidative susceptibility of the oils was estimated according to reference [18].

Shelf Life Prediction

The oxidative stability of each olive oil sample was determined using a Rancimat instrument (Model 734; Metrohm Ltd., Herisau, Switzerland) according to a procedure previously described [19]. Results were expressed as the induction time (h) of each sample, that is the time needed to obtain a significant measurable rancidity in an oil sample, when the oxidation becomes rapidly accelerated [20]. Three grams of each olive oil sample were placed in a glass reaction tube and oxidation was carried out at 120 °C with an airflow rate of 20 L h^{-1}. The oxidative stability index (OSI) for each oil sample was determined with a 743 Rancimat Control (version 1.00, Build 79; Metrohm Ltd., Herisau, Switzerland) software application for Windows by extrapolating the two linear parts of the conductivity plot

and identifying their intersection point [21]. Results were also expressed as oxidative stability days per kg of oil, according to the following formula:

$$\left[\left(h_{\text{inductiontime}} \times 1000 \text{ g kg}^{-1}\right) / \left(\text{g oil x 24 h day}^{-1}\right)\right]$$

as previously described [22].

Pigments

Carotenes and chlorophylls were determined as described by [23], using 7.5 g of oil dissolved in cyclohexane. Carotene and chlorophyll pigments were determined by measuring the absorbance at 470 and 670 nm, respectively. Results were expressed as mg of pheophytin "a" and lutein per kg of oil, respectively.

Total Phenols

The phenolic extract was obtained as previously reported [24]. Briefly, 10 mL of a methanolic solution [methanol/water (80:20, v/v) and 20 mg of Tween 20 (2%, v/w)] were homogenized with 10 g of olive oil, using an Ultra-Turrax T25 (IKA Labortechnik, Janke & Kunkel, Staufen, Germany) apparatus for 1 min at 15,000 *g* and then centrifuged at 5,000*g* for 10 min at 4 °C; the extraction was repeated twice. To eliminate the oil droplets, the methanol extract was kept −20 °C for 24 h. Total phenols and *o*-diphenols were determined colorimetrically and results were expressed as hydroxytyrosol equivalents.

Volatile Compound Analyses

Sampling

Solid phase micro extraction (SPME) was used as the technique for headspace sampling of virgin olive oils. Supelco SPME devices coated with polydimethylsiloxane (PDMS, 100 μm) were used to sample the headspace of 2 mL of virgin olive oil inserted into a 5-mL glass vial and allowed to equilibrate for 30 min. After the equilibration time, the fiber was exposed to the headspace for 50 min at room temperature. Once sampling was finished, the fiber was withdrawn into the needle and transferred to the injection port of the GC–MS system [25].

Identification

GC–EIMS analyses were performed with a Varian CP 3800 gas-chromatograph equipped with a DB-5 Capillary column (30 m × 0.25 mm; coating thickness = 0.25 μm) and a Varian Saturn 2000 ion trap mass detector. Analytical conditions were as follows: injector and transfer line temperature were 250 and 240 °C, respectively; oven temperature was programmed from 60 to 240 °C at 3 °C min^{-1}; carrier gas, helium at 1 mL min^{-1}; splitless injection. Identification of the constituents was based on comparison of the retention times with those of authentic samples, comparing their linear retention indices relative to the series of *n*-hydrocarbons, and on computer matching against commercial (NIST 98 and ADAMS) and home-made library mass spectra built from pure substances and components of known mixtures and MS literature data [26–30]. Moreover, the molecular weights of all the identified substances were confirmed by GC-CIMS, using MeOH as CI ionizing gas [31, 32].

Statistical Analysis

All parameters were determined in triplicate for each sample. Variance (ANOVA) and linear regression analyses were processed by SPSS statistical package (Version 12.00 for Window, SPSS Inc. Chicago, IL, USA, 2003). The significance of differences at a 5% level among means was determined by one-way ANOVA, using Tukey's test. ANOVA was applied to evaluate the influence of the storage period on the stability and on the aroma composition of olive oil. The analysis of variance was also applied in order to attest the influence of the addition of thyme and lemon at different concentration (from 20 to 80 g kg^{-1} of oil) to olive oil.

Results and Discussion

Evolution of fatty acid composition, free fatty acids, peroxide value, conjugated dienes, conjugated trienes, pigments and total phenols compounds of virgin olive oil during the oxidation process.

In terms of fatty acids composition (Table 1), significant variations were detected during the oxidation test, mainly for C16:0 and C18:3. A progressive increase of free acidity was observed (Table 1). However, this value did not exceed the maximum limit for extra virgin olive oil established by EC regulations (0.8 g for oleic acid 100 g^{-1} of oil).

Table 1 also illustrates the results of peroxide values after heating at 100 and 200 °C. The latter temperature, corresponding to the smoke point of the oil, was used because the majority of consumers believe that when oil smokes, it becomes unhealthy. The heating at 100 °C better correlates the oxidation undergone by the oil during cooking conditions, while the heating at 200 °C simulates oxidation under frying conditions. As can be observed, the peroxide values prior to analysis (time zero) were less than 10 mequiv O_2 kg^{-1}, that is values below the maximum

Table 1 Influence of heating conditions on acidity, fatty acid composition and oxidative stability of Chétoui Borj Toumi olive oil

Analytical parameters	Room temperature	4 h at 100 °C	4 h at 200 °C
Acidity[$]	0.3[b]	0.7[a]	0.7[a]
Peroxide value[&]	9.2[c]	13.3[b]	19.8[a]
K232[!]	1.7[c]	1.9[b]	2.3[a]
K270*	0.07[c]	0.09[b]	0.15[a]
C16:0(%)	14.5[a]	11.2[b]	11.5[b]
C16:1	0.3[b]	0.4[a]	0.4[a]
C18:0	2.6[b]	3.1[a]	3.1[a]
C18:1	69.3[a]	69.0[a]	68.9[a]
C18:2	13.2[b]	15.4[a]	15.2[a]
C18:3	0.6[a]	0.7[a]	0.7[a]
O/L	5.2[a]	4.5[a]	4.5[a]
SFA	17.1[a]	14.3[b]	14.6[b]
MUFA	69.6[a]	69.4[a]	69.4[a]
PUFA	13.8[c]	16.1[a]	16.0[a]
OS	719.9[b]	839.0[a]	828.0[a]
IV	100.5[a]	92.5[b]	92.0[b]
OSI[*]	9.5[b]	5.6[c]	11.1[a]
OSI[¥]	131.9[b]	78.3[c]	155.2[a]
OSI[§]	0.4[a]	0.2[b]	0.4[a]
Phenols (mg kg^{-1})	580.0[a]	265.0[b]	115.0[c]
Chlorophylls (mg kg^{-1})	13.0[a]	7.0[b]	3.0[c]
β-Carotene (mg kg^{-1})	7.0[a]	3.1[b]	1.5[c]

Analytical parameters with different superscripts are significantly different ($P < 0.05$) between heating conditions. [$] (%); [&] (mequiv O_2 kg^{-1}); [!] conjugated dienes value; * conjugated trienes value; IV: iodine value; OS: oxidative susceptibility; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; OSI: oxidative stability index; [*] Hours; [¥] day kg^{-1}; [§] year kg^{-1}

permitted for their classification as extra virgin oils (20 mequiv O_2 kg^{-1}) according to the European regulation. However, during the oxidative process, the peroxide value increased (Table 1) because of the high concentrations of oxygen available to react with alkyl radicals to form peroxyl radicals and then hydroperoxides.

Table 1 shows changes in K232 and K270 specific coefficients versus heating time. The K232 is correlated with the formation of conjugated dienes of polyunsaturated fatty acids, while the K270 values are indicative of the presence of primary and secondary oxidation products, including conjugated trienes and carbonyl compounds. Before analysis, olive oils presented K232 values of 1.7 and K270 values of about 0.07, within the values recommended by the EU Regulation 2568/91. After treatment, an increase in both dienes and trienes values was noted. The K270 coefficient notoriously increased in oils submitted to thermo-oxidation, which can be explained by the peroxide degradation.

Polyphenols compounds are naturally present in olive oils and are the major compounds responsible for the stability of these oils during storage and heating. Table 1 reports their changes during thermal oxidation of the oil. Before heating, the amount of polyphenols was about 580 mg kg^{-1}. After heating at 100 °C, a significant decrease was observed (from 580 to 265 mg kg^{-1}, respectively). As expected, a sharper decrease of the total phenols content was observed after heating at 200 °C (from 580 to 115 mg kg^{-1}, respectively).

The color of olive oil is usually due to the lipophilic chlorophyll and pheophytin pigments contained in the olive fruit, where pheophytin A is usually the main determining factor [33]. Chlorophylls are responsible for the greenish coloration of certain olive oils. Table 1 presents the change of chlorophyll contents in olive oils during thermal oxidation. Before treatment, the chlorophyll amount was about 13.0 mg kg^{-1}. A marked decrease was observed in its levels for olive oils heated at 100 °C and it was even more noticeable after heating at 200 °C. Because of their conjugated molecular structure, carotenoids are excellent singlet oxygen quenchers and can protect oil from photo-oxidation [34]. The trend observed for these pigments versus heating or oxidation processes was similar to that of chlorophylls, as shown in Table 1. The level of carotenoids in the unheated oils (time zero) was 7 mg kg^{-1}. During heating at 100 °C, they drastically decreased, after 4 h, to 3.1 mg kg^{-1}. A more pronounced reduction, as expected, was observed for oil samples heated at 200 °C (1.5 mg kg^{-1}).

The Oxidative Stability Index (OSI) value is well correlated to the longevity of an oil sample. It is defined as the induction period, that is the time needed before the rate of lipid oxidation of an oil sample rapidly accelerates [15]. This is indicated by an extremely rapid increase in the

concentration of low molecular weight rancid by-products (i.e. aldehydes, ketones, alcohols, carboxylic acids, and esters) [35]. The degree of rancidity is determined by continuously measuring an increase in conductivity due primarily to the ionizable short-chain acids, formic acid [20] and acetic acid [36], resulting from the induced oxidation of the oil sample. This measurement is vital for olive oil samples because olive oils contain lipoxygenase enzymes that catalyze the peroxidation of polyunsaturated fatty acids and carotenes [36]. These oxidative reactions may result in a decreased shelf life of the oil. Our results showed that oxidative stability decreased under heating at 100 °C from 9.5 to 5.6 h.

Evolution of Volatile Compounds of Virgin Olive Oil During the Thermal Oxidation Process

The aroma of an olive oil is mainly due to its content of many low molecular weight, relatively non-polar compounds that are easily volatilized at room temperature. Table 2 shows the volatiles detected in virgin olive oil. Most of them are produced through the lipoxygenase pathway (LOX) and are always present in the headspace of virgin olive oils. Chétoui Borj Toumi olive oils exhibited a very complex bouquet of volatiles (Table 2). (*E*)-3-hexen-1-ol, which is related to the grassy and fruity sensory notes (Table 2), was the most important one of this olive oil (24.5%), followed by two esters, (*E*)-3-hexen-1-yl acetate (23.5%) and hexyl acetate (8.6%), which are responsible for sweet, floral and fruity notes [37].

When samples were subjected to oxidation, the initial volatiles disappeared in a few hours and then the oxidation process started producing a great amount of volatile compounds, some of them already detected in the initial volatile profiles (Table 2). The evolution of the total volatile compounds responsible for virgin olive oil flavor during the thermoxidation process is reported in Table 2. At accelerated conditions of 100 °C, a significant modification in the matrix of volatiles, mainly an increase in the percentage of hexanal (from trace levels to 11.6%) was apparent. In contrast, the hexyl acetate content, an ester produced through the enzymatic degradation of the linoleic acid, completely disappeared after the thermo-oxidation process. The same behavior was observed for (*E*)-2-hexen-1-ol, a derivative of the enzymatic degradation of linolenic acid. Furthermore, some new volatile compounds were detected after thermoxidation at 100 °C for 4 h, such as (*E,Z*)-2,4-heptadienal (21.1%), (*Z*)-2-heptenal (7.8%), (*E,E*)-2,4-heptadienal (3.9%), 1-heptanol, (*E,Z*)-2,4-decadienal, and (*E,E*)-2,4-decadienal (all trace amounts). Hence, 2,4-heptadienal isomers presented a higher correlation with the temperature of oxidation than 2,4-decadienal ones. Alcohols were produced in variable amounts during oxidation.

Table 2 Influence of heating conditions on aromatic compounds (%) of Chétoui Borj Toumi olive oil

	Room temperature	4 h at 100 °C	4 h at 200 °C
Hexanal (%)	Tr^{c}	11.6^{a}	5.8^{b}
(*E*)-3-Hexen-1-ol	24.5^{a}	23.9^{a}	Tr^{b}
(*E*)-2-Hexen-1-ol	5.4		
3-heptanone			1.7
Unknown	0.7		
α-Pinene	2.1		1.3
(*Z*)-2-heptenal		7.8^{a}	5.5^{b}
1-heptanol		Tr	
3-octanol		3.5^{a}	0.5^{b}
(*E,Z*)-2,4-heptadienal		21.1^{a}	3.1^{b}
Octanal			0.4
6-Methyl-5-hepten-2-one	1.3		
6-Methyl-5-hepten-2-ol	5.0		
(*E*)-3-Hexenyl acetate	23.5^{a}	9.7^{b}	
Hexyl acetate	8.6		
(*E, E*)-2,4-heptadienal	0.0^{c}	3.9^{a}	1.6^{b}
1-*p*-menthene			0.8
1,8-cineole			0.9
(*E*)–*β*-ocimene	1.2		
(*E*)-2-octenal			2.7
Nonanal	1.3^{c}	2.8^{b}	14.9^{a}
(*E*)-2-nonenal			0.8
1-Dodecene	2.2^{b}	4.2^{a}	
Decanal		Tr	Tr
(*E*)-2-decenal			7.5
(*E, Z*)-2,4-decadienal		Tr^{b}	16.3^{a}
(*E, E*)-2,4-decadienal		Tr^{b}	33.7^{a}
(*E*)-2-undecenal			1.8
α-Copaene	4.3^{a}	3.4^{ab}	0.4^{c}
Unknown	0.6		
(*E,E*)-α-farnesene	3.3		
Methyl tridecanoate	13.3		

Flavored compounds with different superscript letters are significantly different ($P < 0.05$) between heating conditions

Tr trace

Compounds such as 1-heptanol and 3-octanol that were absent in the initial aromatic fraction, increased during the oxidation process (Table 2). In fact, 1-heptanol and 3-octanol derived from the oxidative degradation of oleic and linoleic acids, respectively [6]. On the contrary, other alcohols of the lipoxygenase pathway (LOX) such as (*E*)-3-hexen-1-ol and (*E*)-2-hexen-1-ol showed a negative trend during the process (Table 2). These results are in good agreement with those of Vichi et al. on Bianchera cultivar [6]. In fact, most of the new aromatic compounds detected

in olive oils treated at 100 °C for 4 h were seven carbon atoms derivatives. It can be hypothesized that C-7 volatiles could represent good markers of thermo-oxidized olive oils.

The influence of the thermo-oxidation was detrimental when oils were treated at 200 °C for 4 h (Table 2). The levels of the (*E*)-3-hexen-1-ol underwent a further sharp decrease from 23.9%, at 100 °C, to trace amounts after a treatment at 200 °C for 4 h. (*E*)-2-Hexen-1-ol completely disappeared after each treatment (Table 2). However, another LOX product, hexanal, exhibited a different behaviour (Table 2). Its levels, barely detectable in immediately obtained oils, increased to 11.6% after thermo-oxidation at 100 °C and than dropped to 5.8% after the treatment at 200 °C. Probably, the higher temperature contributed to the loss of this quite volatile derivative. (*E*)-3-Hexenyl acetate showed the same behavior as that of (*E*)-3-hexen-1-ol, i.e. the high temperature treatment (200 °C) was detrimental for this compound (Table 2).

Furthermore, (*E,Z*)-2,4-decadienal and (*E,E*)-2,4-decadienal were formed in appreciable amounts only when the oil was treated with the most severe conditions (4 h at 200 °C). Hence, these two chemicals may represent markers of severe degradation of the oil. Some new volatiles appeared during the oxidative deterioration process at 200 °C, such as (*E*)-2-decenal (7.5%), (*E*)-2-octenal (2.7%), (*E*)-2-undecenal (1.8%), (*E*)-2-nonenal (0.8%), and octanal (0.4%). It can be seen that after the thermo-oxidation at 200 °C, the main detected compounds were C-10 derivatives. Hence, during the oxidation process in conditions near to the smoke point (≈210 °C), the production of large quantities of well-known off-flavor compounds (nonanal, 2,4-decadienal, (*E*)-2-decenal and (*E*)-2-undecenal), can explain the unpleasant odor of the frying olive oil [37].

Some of the volatiles positive contribute to the flavor of olive oil, i.e. hexanal contributes to the sweet perception [37] and is positively correlated with the overall acceptability of potential and habitual consumers. In the present study, hexanal was detected at trace levels in the freshly obtained oil. These levels markedly increased during the oxidation process. In fact, the amount of hexanal is due to both autoxidation and the lipoxygenase cascade, through the formation of 13-linoleic acid. Consequently, hexanal cannot represent an adequate marker of oxidation in the case of virgin olive oil, in agreement with the observations of Morales et al. for the oil obtained from the cultivar Arabequina [38]. In contrast, nonanal, which derives from the autoxidation of oleic acid, exhibited a different behaviour. Initially, nonanal was not detected, but in the course of the oxidation process its percentage increased markedly (Table 2). The ratio hexanal/nonanal is a very

Table 3 Influence of treatment conditions on acidity, fatty acid composition and oxidative stability of flavored Chétoui Borj Toumi olive oil with lemon

	Chétoui Borj Toumi olive oil flavored with lemon											
	20 g kg^{-1}			40 g kg^{-1}			60 g kg^{-1}			80 g kg^{-1}		
	0 h	4 h at 100 °C	4 h at 200 °C	0 h	4 h at 100 °C	4 h at 200 °C	0 h	4 h at 100 °C	4 h at 200 °C	0 h	4 h at 100 °C	4 h at 200 °C
Acidity[$]	0.5^{a}	0.6^{a}	0.6^{a}	0.5^{a}	0.6^{a}	0.6^{a}	0.5^{a}	0.6^{a}	0.6^{a}	0.5^{a}	0.6^{a}	0.6^{a}
C16:0[❶]	11.2^{a}	11.4^{a}	11.8^{a}	11.1^{a}	11.6^{a}	11.1^{a}	11.1^{a}	11.6^{a}	12.0^{a}	11.3^{a}	11.7^{a}	11.6^{a}
C16:1	0.4^{a}	0.4^{a}	0.5^{a}	0.4^{a}	0.4^{a}	0.4^{a}	0.4^{a}	0.4^{a}	0.5^{a}	0.4^{a}	0.5^{a}	0.4^{a}
C18:0	3.0^{a}	3.1^{a}	3.1^{a}	3.0^{a}	3.1^{a}	3.1^{a}	3.2^{a}	3.0^{a}	3.0^{a}	3.2^{a}	3.1^{a}	3.1^{a}
C18:1	69.1^{a}	68.8^{a}	68.7^{a}	67.3^{b}	68.7^{a}	69.2^{a}	68.9^{a}	68.6^{a}	68.4^{a}	68.5^{a}	68.6^{a}	68.9^{a}
C18:2	15.3^{a}	15.4^{a}	15.1^{a}	15.0^{a}	15.3^{a}	15.2^{a}	15.2^{a}	15.4^{a}	15.1^{a}	15.6^{a}	15.3^{a}	15.1^{a}
C18:3	0.7^{a}	0.7^{a}	0.7^{a}	0.7^{a}	0.7^{a}	0.7^{a}	0.7^{a}	0.7^{a}	0.7^{a}	0.7^{a}	0.7^{a}	0.7^{a}
O/L	4.5^{a}	4.5^{a}	4.5^{a}	4.5^{a}	4.5^{a}	4.5^{a}	4.5^{a}	4.4^{a}	4.5^{a}	4.4^{a}	4.4^{a}	4.5^{a}
SFA	14.2^{a}	14.5^{a}	14.9^{a}	14.3^{a}	14.7^{a}	14.3^{a}	14.3^{a}	14.7^{a}	15.1^{a}	14.5^{a}	14.7^{a}	14.7^{a}
MUFA	69.5^{a}	69.3^{a}	69.2^{a}	67.8^{b}	69.1^{a}	69.6^{a}	69.3^{a}	69.1^{a}	68.9^{a}	68.9^{a}	69.0^{a}	69.3^{a}
PUFA	16.0	16.2	15.9	15.8	16.1	16.0	15.9	16.1	15.8	16.3	15.5	15.9
OS	832.9^{a}	832.0^{a}	831.9^{a}	820.9^{b}	836.9^{a}	830.6^{a}	827.2^{b}	838.1^{a}	823.5^{b}	846.7^{a}	835.6^{a}	825.1^{b}
IV	92.3^{a}	92.4^{a}	92.4^{a}	90.3^{b}	92.1^{a}	92.3^{a}	91.9^{a}	92.2^{a}	91.5^{a}	92.4^{a}	92.0^{a}	91.9^{a}
OSI[*]	5.8^{b}	6.1^{b}	10.9^{a}	6.0^{b}	5.6^{b}	11.3^{a}	5.6^{b}	5.7^{b}	11.3^{a}	6.3^{b}	6.1^{b}	10.7^{a}
OSI[¥]	80.4^{b}	85.1^{b}	151.0^{a}	84.4^{b}	78.2^{b}	157.0^{a}	77.7^{b}	80.1^{b}	157.0^{a}	87.7^{b}	85.0^{b}	149.0^{a}
OSI[§]	0.2^{b}	0.2^{b}	0.4^{a}	0.2^{b}	0.2^{b}	0.4^{a}	0.2^{b}	0.2^{b}	0.4^{a}	0.2^{b}	0.2^{b}	0.4^{a}

Analytical parameters with different superscript letters are significantly different ($P < 0.05$) between heating conditions. [$](%); IV: iodine value; OS: oxidative susceptibility; [❶]: %; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; OSI: oxidative stability index; [*] Hours; [¥] day kg^{-1}; [§] year kg^{-1}

important indicator of the oxidative statute of olive oil, as proposed by [6] and [38]. As depicted in Fig. 5, in Chétoui oil, this ratio was about 4.1 after treatment at 100 °C and about 0.4 after treatment at 200 °C. According to [38], this ratio passes abruptly from thousands, for gourmet oil, to a value lower than two for oxidized oils (Fig. 5).

The volatile compounds responsible for the flavor of virgin olive oil are quite different from those responsible of off-flavors. The cause may depend on their different origins, namely biochemical for flavor and chemical for off-flavor. The main differences that characterize defective olive oils are the absence of C6 aldehydes, alcohols (produced from linolenic acid), and some sesquiterpenes that contribute to their typical green flavor, the lack of esters contributing to the fruity flavor, and the presence of many aldehydes with low odor thresholds responsible of the typical rancid odor of oxidized oils [6, 38].

Evolution of Fatty Acid Profiles and Analytical Quality Indices of flavored Olive Oil under Thermo-oxidation Process

To improve both aromatic and antioxidant profiles during the frying process, some aromatic and antioxidant plants have been added to the olive oil. Aromatic plants have been used since ancient times in food flavoring, pharmaceutics, cosmetics and perfumery. It is well known that olive oil is an important captor of flavors. For this reason, many people added different kind of spices and aromatic herbs in order to give different seasoning properties to the oil. Some herbs also have, beside their organoleptic properties, an important antioxidant capacity.

Acidity is used as a criterion for the classification of olive oils. Despite this fact, acidity is not a good indicator of olive oil quality since an olive oil with a relatively high acidity may possess a highly desirable aroma, whereas an olive oil with a low acidity may lack aroma [39]. A smaller increase in acidity after thermo-oxidation at 100 and 200 °C was detected in flavored olive oils independently of the concentration of herbs infused (Tables 3, 4).

No significant differences were found between the flavored oils and the control, to demonstrate that lemon and thyme fused did not affect the oxidative stability of oils measured by the Rancimat apparatus. This result indicated that oxidative stability was mainly correlated with the ratio of oleic/linoleic acid and secondly by minor compounds [10, 40].

Evolution of Aromatic Compounds of Flavored Olive Oil Under Thermo-oxidation Processes

Some typical compounds such as limonene, carvacrol, myrcene, α-thujene and α-pinene were found in the oils

Table 4 Influence of treatment conditions on acidity, fatty acid composition and oxidative stability of flavored Chétoui Borj Toumi olive oil with thyme

	Chétoui Borj Toumi olive oil flavored with thyme											
	20 g kg^{-1}			40 g kg^{-1}			60 g kg^{-1}			80 g kg^{-1}		
	0 h	4 h at 100 °C	4 h at 200 °C	0 h	4 h at 100 °C	4 h at 200 °C	0 h	4 h at 100 °C	4 h at 200 °C	0 h	4 h at 100 °C	4 h at 200 °C
Acidity	0.5^{a}	0.6^{a}	0.6^{a}	0.5^{a}	0.6^{a}	0.6^{a}	0.5^{a}	0.6^{a}	0.6^{a}	0.5^{a}	0.6^{a}	0.6^{a}
C16:0[❶]	11.1^{a}	11.4^{a}	11.5^{a}	11.4^{a}	11.4^{a}	11.6^{a}	11.4^{a}	11.5^{a}	11.4^{a}	11.6^{a}	11.6^{a}	11.7^{a}
C16:1	0.4^{a}	0.3^{a}	0.4^{a}	0.4^{a}	0.4^{a}	0.4^{a}	0.4^{a}	0.5^{a}	0.4^{a}	0.5^{a}	0.5^{a}	0.5^{a}
C18:0	3.2^{a}	3.1^{a}	3.2^{a}	3.4^{a}	3.1^{a}	3.1^{a}	3.0^{a}	3.1^{a}	3.1^{a}	3.1^{a}	3.1^{a}	3.1^{a}
C18:1	69.1^{a}	68.9^{a}	68.9^{a}	68.8^{a}	68.8^{a}	68.8^{a}	68.5^{a}	68.6^{a}	69.0^{a}	68.5^{a}	68.7^{a}	68.8^{a}
C18:2	15.3^{a}	15.4^{a}	15.1^{a}	15.2^{a}	15.3^{a}	15.1^{a}	15.7^{a}	15.4^{a}	15.2^{a}	15.3^{a}	15.4^{a}	15.1^{a}
C18:3	0.7^{a}	0.7^{a}	0.7^{a}	0.7^{a}	0.8^{a}	0.7^{a}	0.8^{a}	0.8^{a}	0.7^{a}	0.8^{a}	0.8^{a}	0.8^{a}
O/L	4.5^{a}	4.4^{a}	4.5^{a}	4.5^{a}	4.5^{a}	4.5^{a}	4.3^{a}	4.4^{a}	4.5^{a}	4.5^{a}	4.5^{a}	4.5^{a}
SFA	14.3^{a}	14.5^{a}	14.7^{a}	14.9^{a}	14.5^{a}	14.8^{a}	14.4^{a}	14.6^{a}	14.6^{a}	14.7^{a}	14.7^{a}	14.8^{a}
MUFA	69.5^{a}	69.2^{a}	69.4^{a}	69.2^{a}	69.3^{a}	69.3^{a}	68.9^{a}	69.1^{a}	69.4^{a}	69.0^{a}	69.1^{a}	69.3^{a}
PUFA	16.0^{a}	16.2^{a}	15.8^{a}	15.9^{a}	16.1^{a}	15.9^{a}	16.5^{a}	16.2^{a}	15.9^{a}	16.1^{a}	16.1^{a}	15.9^{a}
OS	829.8^{a}	837.7^{a}	819.0^{b}	830.8	840.5^{a}	826.0^{b}	858.7^{a}	840.3^{a}	828.1^{b}	837.5^{a}	836.2^{b}	824.8^{b}
IV	92.3^{a}	92.3^{a}	91.7^{a}	92.0^{a}	92.4^{a}	91.9^{a}	92.8^{a}	92.3^{a}	92.1^{a}	92.0^{a}	92.2^{a}	91.8^{a}
OSI[&]	5.8^{b}	6.1^{b}	10.9^{a}	6.0^{a}	5.6^{a}	11.3^{a}	5.6^{b}	5.7^{b}	11.3^{a}	6.3^{b}	6.1^{b}	10.8^{a}
OSI[¥]	80.4^{b}	85.1^{b}	151.2^{a}	84.4^{b}	78.2^{b}	156.8^{a}	77.7^{b}	80.1^{b}	157.2^{a}	87.7^{b}	84.7^{b}	149.7^{a}
OSI[§]	0.2^{b}	0.2^{b}	0.4^{a}	0.2^{b}	0.2^{b}	0.4^{a}	0.2^{b}	0.2^{b}	0.4^{a}	0.2^{b}	0.2^{b}	0.4^{a}

Analytical parameters with different superscript letters are significantly different ($P < 0.05$) between heating conditions. [$] (%); [&] (mequiv O_2 kg^{-1}); IV:; [❶]: %; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; OSI: oxidative stability index; [&] Hours; [¥] day kg^{-1}; [§] year kg^{-1}

Table 5 Influence of treatment conditions on volatiles of Chétoui Borj Toumi olive oil flavored with lemon

	Chétoui Borj Toumi olive oil flavored with lemon											
	20 g kg^{-1}			40 g kg^{-1}			60 g kg^{-1}			80 g kg^{-1}		
	0 h	4 h at 100 °C	4 h at 200 °C	0 h	4 h at 100 °C	4 h at 200 °C	0 h	4 h at 100 °C	4 h at 200 °C	0 h	4 h at 100 °C	4 h at 200 °C
Hexanal (%)	11.4^{a}	11.9^{a}	7.6^{b}	11.8^{a}	12.6^{a}	5.6^{b}	12.0^{a}	11.1^{a}	6.3^{b}	10.9^{a}	9.2^{a}	5.9^{b}
(*E*)-3-Hexen-1-ol	25.0^{a}	21.5^{a}	0.7^{b}	24.4^{a}	21.9^{a}	Tr^{b}	17.8^{ab}	20.9^{a}	0.8^{c}	21.5^{a}	20.1^{a}	0.6^{b}
1-Hexanol	4.0^{a}	4.3^{a}	Tr^{b}	5.0^{a}	4.4^{a}	Tr^{b}	Tr^{b}	3.2^{a}	Tr^{b}	6.2^{a}	Tr^{b}	Tr^{b}
(*Z*)-2-heptenal	5.2	7.5^{a}	5.6^{b}	5.0^{ab}	6.8^{a}	5.3^{ab}	5.5^{a}	5.9^{a}	5.3^{a}	4.7^{ab}	5.7^{a}	4.9^{ab}
1-octen-3-ol	0.0^{b}	0.0^{b}	0.3^{a}	0.0^{b}	0.0^{b}	0.8^{a}	0.0^{b}	0.0^{b}	0.5^{a}	0.0^{b}	0.0^{b}	0.6^{a}
1-Decene	2.0^{a}	0.0^{b}	0.0^{b}	Tr^{a}	0.0^{b}	0.0^{b}	1.8^{a}	0.0^{b}	0.0^{b}	2.2^{a}	0.0^{b}	0.0^{b}
2-pentylfuran			Tr			1.2			0.5			1.1
(*E,Z*)-2,4-heptadienal	17.6^{b}	19.0^{a}	3.8^{c}	14.1^{b}	16.5^{a}	3.0^{c}	16.5^{a}	16.1^{a}	2.2^{b}	14.2^{a}	15.3^{a}	3.0^{b}
Octanal			0.0			0.8			0.7			0.8
(*Z*)-3-Hexenyl acetate	9.6^{a}	9.2^{a}	0.0^{b}	8.3^{a}	8.1^{a}	0.0^{b}	9.1^{a}	9.1^{a}	Tr^{b}	8.4^{a}	8.1^{a}	0.4^{b}
(*E,E*)-2,4-heptadienal	3.9^{b}	5.3^{a}	1.4^{c}	3.5^{a}	3.5^{a}	1.8^{b}	3.5^{a}	3.7^{a}	1.6^{b}	3.0^{a}	3.7^{a}	1.7^{b}
1-*p*-menthene			1.0			1.0			0.9			1.0
1,8-cineole			0.8			0.6			0.5			0.6
(*E*)–*β*-ocimene	Tr	Tr		Tr	Tr		Tr	1.6		Tr	2.1	
(*E*)-2-octenal			2.5			2.5			2.7			2.2
Nonanal	2.7^{b}	3.0^{b}	12.9^{a}	2.9^{b}	3.1^{b}	14.8^{a}	4.3^{b}	2.9^{c}	16.0^{a}	2.3^{b}	3.0^{b}	14.4^{a}
(E)-2-nonenal			0.7			0.8			0.7			0.7
1-Dodecene	3.1^{a}	3.9^{a}	Tr^{b}	3.9^{a}	4.1^{a}	Tr^{b}	4.0^{a}	3.5^{a}	0.4^{b}	3.7^{a}	3.7^{a}	Tr^{b}
(Z)-4-decenal			0.0			0.0			0.5			0.4
(E)-4-decenal			0.0			0.0			0.3			Tr
Decanal	Tr	Tr	Tr	2.2^{a}	Tr^{b}	Tr^{b}	2.1^{a}	2.0^{a}	0.5^{b}	Tr^{b}	1.9^{a}	Tr^{b}
(E)-2-decenal			7.3			8.0			7.6			7.2
(E, Z)-2,4-decadienal			17.3			15.8			14.9			15.6
(E, E)-2,4-decadienal			34.3			33.7			31.8			32.4
(E)-2-undecenal			1.9	2.7		2.2			2.2			1.9
α-Copaene	2.1^{b}	3.1^{a}	0.5^{c}		3.2	Tr	3.6^{a}	2.7^{ab}	0.4^{c}	3.3^{a}	3.0^{a}	Tr^{b}
1-tetradecene		Tr			Tr			Tr			Tr	

Flavored compounds with different superscript letters are significantly different ($P < 0.05$) between heating conditions

Tr trace

after maceration of the herbs (Figs. 1, 2). Hence, the maceration process allowed the transfer of some volatiles into the oils. In fact, lemon released into the olive oil a new compound, limonene, known for its citron and lemon-like attributes (Table 5) [41]. The presence of limonene in oils depended on the herb amount used in the maceration process (Fig. 1). The infusion with thyme, released into the oil other compounds known for their aromatic and antioxidant properties, such as carvacrol α-thujene, α-pinene, myrcene, α-terpinene, γ-terpinene, *cis*-sabinene hydrate, 4-terpineol, thymoquinone, and *β*-caryophyllene (Fig. 2). The percentage of each aromatic compound in the flavored olive oils was again correlated with the amount of herb used (Fig. 2). Hence, the highest percentage of limonene (13.8%) was detected in flavored olive oil when 80 g kg^{-1} of lemon was used. Similarly, the highest percentage of carvacrol (37.6%) was detected in flavored olive oil treated with 80 g kg^{-1} of thyme.

The thermo-oxidation process at 100 °C of the flavored olive oils on the bioactive compounds was not detrimental

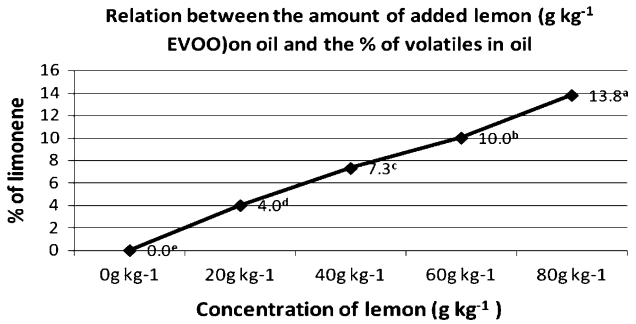

Fig. 1 Relation between the amount of added lemon (g kg^{-1}) in the extra virgin olive oil (EVOO) and the occurrence of limonene in oil (%) (Limonene percentages with different superscripts are significantly different ($P < 0.05$) between concentration of added lemon in virgin olive oil

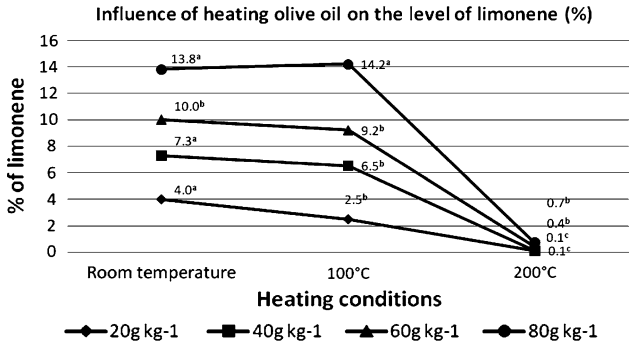

Fig. 3 Influence of heating process of the oil on the % of limonene (limonene percentages with different superscripts are significantly different ($P < 0.05$) between heating conditions

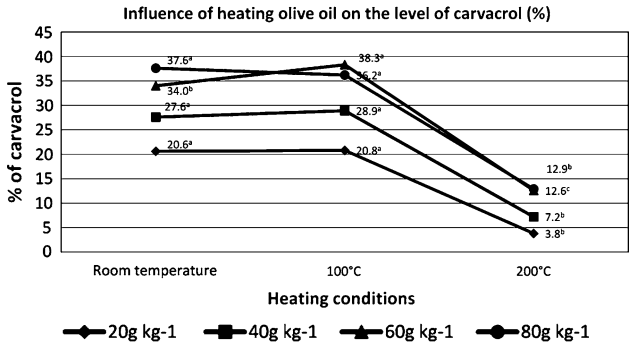

Fig. 4 Influence of heating process on the level of carvacrol (%) (Carvacrol percentages with different superscripts are significantly different ($P < 0.05$) between heating conditions

(Figs. 3, 4). As depicted in Fig. 3, the percentage of limonene decreased slightly from room temperature to 100 °C. At the highest concentration of lemon used for the preparation of flavored olive oil, the level of limonene remained stable as indicated in Fig. 3. The same behaviour can be observed in Fig. 4, which depicts the effect of thermo-oxidation on the level of carvacrol. On the contrary, thermo-oxidation at 200 °C was very detrimental (Figs. 3, 4). As indicated in Fig. 3, the percentage of limonene in flavored olive oil decreased markedly at 200 °C (i.e. from 13.8% at room temperature to 0.7% at 200 °C). It is also noteworthy that the level of carvacrol in olive oil flavored with thyme decreased markedly three times less than the initial value (Fig. 4). The ratio of hexanal/nonanal can be used to differentiate between oxidized and good-quality olive oils as indicated by [37] (Fig. 5). The ratio changes abruptly from six, for a control oil sample, to four for samples heated at 100 °C. The value of the ratio hexanal/nonanal of flavored oils at different concentrations is inferior to 2, which means that flavored olive oils at 200 °C were completely oxidized (Fig. 5, Table 6).

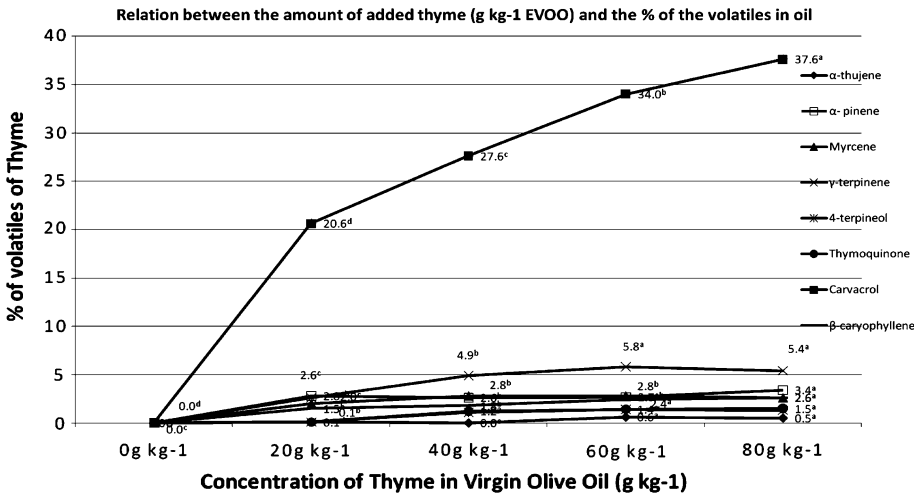

Fig. 2 Relation between the amount of added thyme (g kg^{-1}) in the extra virgin olive oil and the occurrence of the corresponding volatiles in oil (%) (Volatile percentages with different superscripts are significantly different ($P < 0.05$) between concentration of added thyme in virgin olive oil

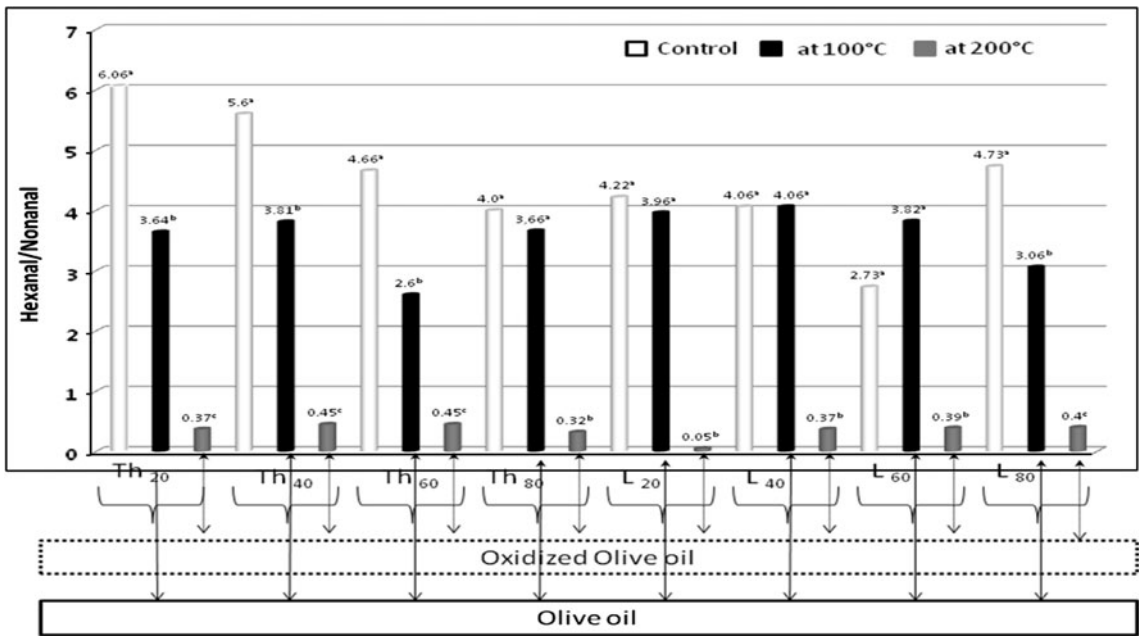

Fig. 5 Evolution of the ratio hexanal/nonanal under thermo-oxidation process of different flavored olive oil at different concentration (hexanal/nonanal ratios with different superscripts are significantly different ($P < 0.05$) between heating conditions; Th20; Th40; Th 60 and Th 80: thyme with different concentration: g of thyme kg^{-1} of VOO; L20; L40. L60. L80: lemon with different concentration: g of lemon kg^{-1} of VOO

Table 6 Influence of treatment conditions on volatiles of Chétoui Borj Toumi olive oil flavored with thyme

	Chétoui Borj Toumi olive oil with flavored thyme											
	20 g kg^{-1}			40 g kg^{-1}			60 g kg^{-1}			80 g kg^{-1}		
	0 h	4 h at 100 °C	4 h at 200 °C	0 h	4 h at 100 °C	4 h at 200 °C	0 h	4 h at 100 °C	4 h at 200 °C	0 h	4 h at 100 °C	4 h at 200 °C
Hexanal (%)	9.1^{a}	6.2^{b}	4.6^{c}	5.6^{a}	4.2^{b}	5.5^{a}	4.2^{a}	2.9^{b}	4.5^{a}	3.6^{a}	3.3^{b}	4.4^{a}
(*E*)-3-Hexen-1-ol	20.0^{a}	14.1^{b}	Tr^{c}	13.5^{a}	9.7^{b}	Tr^{c}	5.4^{b}	6.6^{a}	0.5^{c}	7.5^{a}	7.1^{a}	Tr^{b}
3-heptanone	1.4^{b}	2.6^{a}	1.6^{b}		1.0	Tr		0.6	0.5		Tr	Tr
1-heptanol	3.3^{b}	4.6^{a}	4.3^{a}	2.1^{b}	2.9^{b}	4.0^{a}	1.7^{b}	1.8^{b}	4.2^{a}	2.0^{b}	1.8^{b}	4.7^{a}
1-octen-3-ol		Tr	0.8		1.2	0.7	0.7	0.8	0.5		0.8	0.3
3-octanol	12.6^{a}	12.6^{a}		8.2^{a}	7.8^{ab}		6.2^{a}	5.3^{ab}		5.1^{a}	5.8^{a}	
2-pentylfuran			0.8			1.3			0.9			0.4
(E, Z)-2,4-heptadienal			2.5			3.7			2.6			2.2
Octanal			0.5			Tr			0.5			0.5
(*Z*)-3-Hexenyl acetate	6.6^{a}	5.7^{a}	0.5^{b}	4.9^{a}	4.0^{a}	0.5^{b}	3.1^{a}	2.6^{ab}	nd	3.3^{a}	3.2^{a}	nd
1-hexyl acetate	2.7^{a}	2.4^{a}	Nd	1.3^{a}	1.4^{a}	nd	1.0^{a}	0.7^{ab}	nd	0.9^{a}	1.0^{a}	nd
(E, E)-2,4-heptadienal			1.4			1.7			1.6			1.1
1,8-cineol			0.6			1.0			1.2			0.6
Linalool	1.6^{a}	1.9^{a}		2.5^{a}	2.5^{a}	Tr^{b}	3.2^{a}	2.9^{a}	1.2^{b}	3.0^{a}	3.1^{a}	0.3^{b}
Nonanal	1.5^{b}	1.7^{b}	12.2^{a}	1.0^{b}	1.1^{b}	12.2^{a}	0.9^{b}	1.0^{b}	9.8^{a}	0.9^{b}	0.9^{b}	13.6^{a}
(*E*)-2-nonenal			0.8			0.8			0.6			0.6
1-Dodecene	2.3^{a}	2.0^{a}	0.5^{b}	1.8^{a}	1.8^{a}	0.3^{b}	1.4	1.4		1.4^{a}	1.4^{a}	Tr^{b}
(*Z*)-4-decenal			0.8			0.4						

Table 6 continued

	Chétoui Borj Toumi olive oil with flavored thyme											
	20 g kg^{-1}			40 g kg^{-1}			60 g kg^{-1}			80 g kg^{-1}		
	0 h	4 h at 100 °C	4 h at 200 °C	0 h	4 h at 100 °C	4 h at 200 °C	0 h	4 h at 100 °C	4 h at 200 °C	0 h	4 h at 100 °C	4 h at 200 °C
Decanal			Tr		Tr	0.3		0.8	Tr		0.6	0.5
(*E*)-2-decenal			7.1			6.8			5.1			6.4
(*E, Z*)-2,4-decadienal			15.8			14.3			11.8			13.4
(*E, E*)-2,4-decadienal			32.3			30.1			22.8			27.1
(*E*)-2-undecenal			2.3			1.8			1.2			1.6
α-Copaene	2.0[a]	2.3[a]	0.5[b]	1.3[a]	1.5[a]	0.4[b]	1.1[a]	1.3[a]	Tr[b]	1.0[a]	1.0[a]	0.4[b]
1-tetradecene	Tr	Tr		Tr	Tr		Tr	Tr		Tr	Tr	
Tetradecane		3.3			3.7	0.4		4.3			1.3	
(*E, E*)-α-farnesene	Tr			Tr			Tr	Tr		Tr	Tr	

Flavored compounds with different superscript letters are significantly different ($P < 0.05$) between heating conditions

Tr trace

Conclusion

The appearance of "smoke" behavior of olive oil was correlated to the formation of new biochemical compounds, such as nonanal, (*E,Z*)-2,4-decadienal and (*E,E*)-2,4-decadienal (which are unhealthy compounds). So far, the "smoking point" has been a very important indicator of the limit to use olive oil in frying conditions (this is < to 200 °C). The addition of herbs modified the sensorial characteristics of the original virgin olive oil, thanks to the transfer of some aromatic compounds. Because of their peculiar sensorial and nutritional characteristics, the flavored virgin olive oils obtained could represent a product which could also be employed in different fields, i.e. as a condiment for some foods, and therefore meets the acceptance of also non-traditional consumers. However, the use of these aromatic plants in order to improve the oxidative properties of virgin olive oil treated at the smoke temperature was not very efficient until a concentration of 80 g kg^{-1}.

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