Food and Chemical Toxicology 47 (2009) 2613–2619

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Physico-chemical change and heat stability of extra virgin olive oils flavoured by selected Tunisian aromatic plants

M.A. Ayadi [a,*,1], N. Grati-Kamoun [b,*,1], H. Attia [a]

[a] Unité d'Analyses Alimentaires, Ecole Nationale d'Ingénieurs de Sfax, B.P. 238, Sfax Tunisie, Tunisia
[b] Institut d'Olivier, B.P. 1087, 3018 Sfax Tunisie, Tunisia

ARTICLE INFO

Article history:
Received 13 May 2009
Accepted 21 July 2009

Keywords:
Olive oil
Physico-chemical change
Aromatic plants
Thermal oxidative stability

ABSTRACT

Objectives of this work were studying physico-chemical change and heat stability of olive oils flavoured by selected Tunisian aromatic plants. Flavoured olive oils were prepared by maceration of fresh plant materials (rosemary, lavender, sage, menthe, basil, lemon and thyme) with olive oil at a 5% w/w level for 15 days. A sensorial evaluation was applied to select more appreciate flavoured olive oils by consumers. An oxidative procedure was applied to test the stability of selected flavoured olive oils: oils samples were kept in glass bottles and heated at 60 and 130 °C during 55 days and 6 h, respectively. The resistance to oxidation of these selected flavoured oils was compared to a control samples by measuring PV, K232 and K270 values and change in chlorophyll, carotenes and polyphénols contents. Obtained results show that addition of aromatic plants causes a slight increase in free acidity and viscosity of aromatised olive oils. L^*, b^* and a^* values show that addition of thyme cause a great change in olive oil colours. Heat stability results shows that from selected aromatic plants, rosemary was effectiveness against oxidation followed by thyme and lemon. However, olive oil flavoured with basil exhibit a similar behaviour versus thermal oxidation then the natural olive oil.

1. Introduction

Virgin olive oil is a product widely produced and consumed throughout the ages in the Mediterranean cuisine and is highly appreciated for its delicious taste and aroma, as well as for its nutritional properties (Moldao-Martins et al., 2004). The nutritional benefits are primarily related to the fatty acid composition, mainly due to the high content of oleic acid and also to the balanced ratio of saturated and polyunsaturated fatty acids. In addition, olive oil presents considerable amounts of natural antioxidants and considered to be important in the prevention of many diseases (Massana et al., 1991; Mancini and Giacco, 1993; Antoun and Tsimidou, 1997; Grati-Kamoun, 2007). Its consumption is, therefore, gaining interest among consumers originating mainly from North Europe, USA and Canada. These potential consumers are not familiar with all the applications of olive oil and may be willing to buy ready preparations of olive oil enriched with other ingredients related to the Mediterranean diet. Such flavoured products would increase the use of olive oil among non-traditional consumers, at the same time, adding value to this precious agricultural product.

Aromatic plants have also been used since ancient times, in food flavouring, pharmaceutical, cosmetic and perfumery due to the presence of essential oils. Several biological activities, including antimicrobial and antioxidant properties are usually assigned to these oils or to some of their constituents (Piccaglia et al., 1993; Ijaz Hussain et al., 2008). Herbs, aromatic plants and spices are ingredients that could be used for the production of flavoured olive oils. It is well known that herbs, aromatic plants and spices maintain the nutritional value of the food, enhance the keeping qualities of food products and increase their shelf life (Chipault et al., 1956; Hirahara et al., 1974; Farag et al., 1989; Tsimidou et al., 1995). They are also used to enrich the flavour and aroma of various foods (Tsimidou and Boskou, 1994). Herbs and spices are widely added to Mediterranean dishes, for example, garlic in seafood dishes, and rosemary in barbecued meat or chicken.

A flavoured olive oil prepared with the addition of a certain herb or/and aromatic plants should not only satisfy the sensory requirements of consumers but also should present some other qualities appreciated in the food market, e.g. improved keeping quality compared to that of the plain oil.

Recently, several studies were devoted to the antioxidant properties of plain, flavoured and stored olive oil. Indeed, Nissiotis and Tasioula-Margari (2002) studied the changes in antioxidant concentration of virgin olive oil during thermal oxidation.

* Corresponding authors. Tel./fax: +216 74 675 761 (M.A. Ayadi).
E-mail addresses: ayadimedali@yahoo.fr (M.A. Ayadi), kamoun_naziha@yahoo.fr (N. Grati-Kamoun).
[1] These authors have participated equally to this work.

0278-6915/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.fct.2009.07.024

Grati-Kamoun (2006) investigated the evolution of physico-chemical characteristics, especially the phenolic compounds, of some Tunisian monovarietal olive oils during the storage at 40 °C.

Moldao-Martins et al. (2004) studied the effect of essential oils extracted from *Mentha x Pipertia* and *Thymus mastichia L.* on the quality of olive oil. Bouaziz et al. (2008) tested the stabilisation of olive oil by adding natural antioxidants from Chemlali olive leaves.

Flavoured olive oils are usually prepared by macerating the aromatic plants in the oil. Using this methodology flavour compounds are also co-extracted with other ones, such as natural antioxidants and pigments present in the aromatic plants, modifying the sensory characteristics and the stability during shelf life.

The aims of the present work were to produce several flavoured olive oils by maceration of selected Mediterranean aromatic plant and to evaluate the physico-chemical and heat stability change of these flavoured olive oils comparing to the natural one.

2. Materials and methods

2.1. Raw materials

Extra virgin olive oil used in this study was produced by the experimental oil mill (Taous) of Olive Tree Institute using "Chemlali" variety. Several Tunisian aromatic plants were used: thyme, rosemary, lavender, basil, lemon zests, white sage, garlic, menthe and geranium. All these aromatic plants were collected from south area of Tunisia (Kerkennah islands, Sfax, Gabes, Medenine area) and identified by a taxonomist.

2.2. Flavoured olive oil preparation

Plant materials were identified and authenticated by a plant taxonomist. Aerial parts of plants were collected between February and March 2006. Lemon zests were collected from fresh lemon purchased in a local supermarket.

Fresh plants were washed, gently dried at 40 °C and broken up into small branches. Plant materiel was added to a sample of oil at a rate of 5% (w/w). The mixture of each oil and aromatic plant was gently mixing using and agitator during 2 h. The mixture was kept in closed stainless bowls for 2 weeks. These bowls are kept in a fresh and sinks place to avoid any oxidation phenomena. After the maceration step flavoured olive oils samples were recuperated using a sieving operation.

2.3. Physico-chemical analysis

2.3.1. FFA determination

FFA content was determined in triplicate, by the titration method of AOAC (AOAC, 1999). About 7 g of well mixed oil was weighed into a 250 ml flask. Previously, neutralized hot ethyl alcohol (50 ml) and 1% phenolphthalein, as indicator, were added. The mixture was titrated with 0.1 N NaOH with vigorous shaking until permanent faint pink colour appeared and persisted at least 1 min. The FFA content was calculated as percentage of oleic acid according to the following equation:

$$\%\ \mathrm{FFA(as\ oleic\ acid)} = \frac{V \times N \times 28.2}{m}$$

where m is the mass of the test portion (g), N the normality of NaOH, and V the volume of NaOH consumed (ml).

2.3.2. Peroxide and specific extinction values

Peroxide value (PV) was determined by using the AOAC method (AOAC, 1999). About 5 g of oil was weighed into a 250 ml flask. Previously prepared acetic acid–chloroform solution (30 ml), saturated potassium iodide (0.5 ml), and distilled water (30 ml) were added with occasional shaking. The mixture was titrated with 0.1 N of $Na_2S_2O_3$ by shaking approximately, 0.5 ml of 1% starch solution was added, and titration was continued with shaking vigorously to release all iodine from $CHCl_3$ layer, until the blue colour just disappeared. PV was calculated by using the following equation:

$$\mathrm{PV\ (meq.Peroxide/kg\ sample)} = S \times N \times 1000/\mathrm{g\ sample}$$

where S is the ml $Na_2S_2O_3$ (blank corrected) and N is the normality $Na_2S_2O_3$ solution.

Specific absorptivity at 232 and 270 were determined using an UV spectrophotometer (Shimadzu Co., Kyoto, Japan) by measuring absorbance of 1% solution in cyclohexane at 232 and 270 nm with 1 cm of pass length.

2.3.3. Chlorophylls, carotenoids and phenols contents

Following the procedures described by Mosquera et al. (1991) a sample of olive oil (7.5 g) was placed in a Falcon tube and filled until 25 mL with cycle-hexane. The chlorophyll fraction was measured in a UV spectrophotometer (Shimadzu Co., Kyoto, Japan) at 670 nm and the carotenoids fraction at 470 nm. The concentration of pigments was expressed using the following equations:

$$[\mathrm{Chlorophylls}] = \frac{Abs_{670} \times 10^6}{613 \times 100 \times \mathrm{density}}\ \mathrm{mg/kg}$$

$$[\mathrm{Carotenoids}] = \frac{Abs_{470} \times 10^6}{2000 \times 100 \times \mathrm{density}}\ \mathrm{mg/kg}$$

The total phenol contents (TPC) of the olive oil extracts were determined by the Folin–Ciocalteau spectrophotometric method at 765 nm, in terms of gallic acid (mg GA/kg oil) (Montedoro et al., 1992).

2.3.4. Colour measurement

CieLab coordinates (L^*, a^* and b^*) were directly read with a spectrophotocolorimeter (Trintometre, Lovibond PFX 195 V 3.2, Amesbury, UK). In this coordinate system, the L^* value is a measure of lightness, ranging from 0 (black) to 100 (white), the a^* value ranges from −100 (greenness) to +100 (redness) and the b^* value ranges from −100 (blueness) to +100 (yellowness).

2.3.5. Viscosity and density determination

Viscosity was followed at 25 °C with a Stress Tech Rheologica Rheometer (Rheologica Instruments AB, Lund, Sweden) using a steel cone-plate (C40/4) under a constant shear rate of 100 s^{-1}. Oils density was measured at 25 °C using a densimeter (Mettler, ME-33360, Switzerland).

2.4. Sensory evaluation

Sensory evaluation of flavoured olive oils was carried out by 200 panelists (food engineering students, staff of biological department of engineering school of Sfax and staff of olive institute in Sfax). All of them, with experience of assessing olive oil, were non-smokers and their age ranged from 23 to 56 years old. The panelists were asked to evaluate the products for colour, taste and overall acceptability (Fresh bread was used as carrier and flavoured olive oil was added at a constant ratio). The ratings were on 2-point hedonic scale ranging from 1 (like) and 2 (dislike). All flavoured olive oil was evaluated and the tasting sessions occurred during 5 days after oils production. The mean sensory scores for various attributes of the flavoured oils were calculated.

2.5. Thermal stability tests

Thermal stability of flavoured and natural (control) olive oils was tested at 60 and 130 °C. Heating at 60 °C for a period of 55 days mimic an accelerate procedure of storage at ambient temperature and heating at 130 °C for 7 h mimic cooking and frying conditions. Oil samples (70 g) were kept in equal portions in open flasks (30 ml capacity, 30 mm diameter and 70 mm height) in the dark in an oven (Binder, No. 970465, Tuttlinger, Germany). Samples were removed each 15 days for oils heated at 60 °C and after 1 h for oils heated at 130 °C. Heat stability was evaluated by measuring peroxide value (PV), specific absorptivity at 232 and 270, Chlorophylls, carotenoids and total phenols contents.

2.6. Statistical analysis

All analytical determinations were performed at least in triplicate. Values of different parameters were expressed as the mean ± standard deviation ($\bar{x} \pm$ SD). Significant differences between mean ($P < 0.05$) were determined by Fisher's test using SPSS software for windows (SPSS. 11, USA).

3. Results

3.1. Physico-chemical change of flavoured olive oils

Table 1 presents physico-chemical change of olive oils after aromatisation. Values presented in Table 1 show that addition of aromatic plant causes a slight increase in flavoured oils free acidity. Indeed, acidity values of oils flavoured by rosemary, lavender, sage, lemon and thyme show a significant ($P < 0.05$) increase. In all case all values of acidity were lower than the limits set by the EU Regulation 2568/91 for extra virgin olive oil. Table 1 shows too that maceration with aromatic plants cause a change on viscosity and density of flavoured olive oils. Indeed, for the oil flavoured by thyme and basil the change in viscosity and density was not

Table 1
Physico-chemical change of flavoured olive oils.

Flavoured olive oils	FFA (%)	Viscosity (mPa/s)	Density (/)	Colour parameters		
				L^*	a^*	b^*
Control	0.42 ± 0.01a	9.03 ± 0.15^{a}	910.6 ± 0.35^{a}	90.83 ± 0.35^{a}	−13.00 ± 0.35^{a}	68.87 ± 0.35^{a}
Rosemary	0.57 ± 0.02^{b}	9.25 ± 0.11^{b}	917. 3 ± 0.45^{a}	82.21 ± 0.35^{b}	−12.1 ± 0.35^{a}	67.97 ± 0.35^{a}
Lavender	0.51 ± 0.01^{ab}	9.27 ± 0.09^{b}	921.3 ± 0.17^{b}	82.82 ± 0.35^{b}	−11.83 ± 0.35 ^{a}	67.43 ± 0.35^{a}
Sage	0.58 ± 0.03^{b}	9.25 ± 0.12^{b}	917.3 ± 0.29^{a}	80.02 ± 0.35^{b}	−11.62 ± 0.35^{a}	69.7 ± 0.35^{a}
Basil	0.46 ± 0.01^{a}	9.15 ± 0.20^{ab}	915.1 ± 0.45^{a}	79.09 ± 0.35^{b}	−10.73 ± 0.35^{a}	63.17 ± 0.35^{a}
Menthe	0.46 ± 0.03^{a}	9.35 ± 0.17^{b}	925.5 ± 0.55^{b}	84.78 ± 0.35^{b}	−13.01 ± 0.35^{a}	64.72 ± 0.35^{a}
Lemon	0.6 ± 0.05^{bc}	9.5 ± 0.12^{c}	920.1 ± 0.33^{b}	82.99 ± 0.35^{b}	−12.47 ± 0.35^{a}	67.23 ± 0.35^{a}
Thyme	0.56 ± 0.02^{b}	9.05 ± 0.21^{a}	917.1 ± 0.25^{a}	25.87 ± 0.35^{c}	0.62 ± 0.35^{b}	16.69 ± 0.35^{b}

Values given are the mean of three replicates ± standard deviation.
Means with the same letter in the same colon are not significantly different at $P > 0.05$.

significant. However, a significant increase of viscosity and density of oils flavoured by lemon, lavender and menthe was observed. Colour parameters show that the aromatisation cause a slight decrease of L^* leading to a darker olive oils except for oil flavoured by thyme where L^* decrease considerably from 90.83 to 25.87. Values of a^* and b^* show that no significant differences was observed for all flavoured olive oil except for oil flavoured by thyme where a^* increase from −13 to 0.62 and b^* decrease from 68.87 to 16.69.

All these physico-chemical change could be attributed to the migration of particulate compounds from aromatic plants to olive oil during maceration process. Such particulate compounds could be organic acids, phenolic compounds, pigments, antioxidants, essential oils...

3.2. Sensorial evaluation

Consumer's acceptability studies of olive oil-aromatic plants preparations are very important for the introduction of these products to the market. This acceptability could be evaluated by several parameters such as colour, taste and overall acceptability. Results of sensorial evaluation of flavoured olive oils were presented in Table 2. From this table we can classify the flavoured olive oils by ascending order based on consumer's preference. In deed, globally (based on the overall acceptability) panelists prefers olive oil flavoured by lemon, thyme, rosemary, basil, menthe, sage and lavender. It could be seen from Table 2 that panelists prefer oil colour of oils flavoured by rosemary and basil and prefer the taste of olive oils flavoured by lemon and thyme. Based on these results four flavoured olive oils were selected for thermal stability tests: olive oils flavoured by basil, rosemary, lemon and thyme.

3.3. Heat stability of the selected flavoured olive oils

3.3.1. Change of peroxide and specific extinction values

Fig. 1 illustrate the relationship between peroxide values and heating time at 60 (Fig. 1a) and 130 °C (Fig. 1b), respectively. The heating at 60 °C correlates better the oxidation under environmental conditions whereas the heating at 130 °C the oxidation under cooking conditions.

Table 2
Sensorial evaluation of flavoured olive oils.

Flavoured olive oils	Colour (%)	Taste (%)	Overall acceptability (%)
Rosemary	1.11 ± 0.03^{a}	1.35 ± 0.02^{b}	1.31 ± 0.02^{b}
Lavender	1.65 ± 0.02^{b}	1.75 ± 0.03^{b}	1.94 ± 0.04^{b}
Sage	1.45 ± 0.01^{c}	1.85 ± 0.01^{b}	1.85 ± 0.01^{b}
Basil	1.25 ± 0.01^{c}	1.47 ± 0.02^{b}	1.33 ± 0.02^{b}
Menthe	1.43 ± 0.04^{c}	1.75 ± 0.04^{b}	1.77 ± 0.03^{b}
Lemon	1.62 ± 0.02^{b}	1.13 ± 0.05^{b}	1.11 ± 0.02^{b}
Thyme	1.65 ± 0.02^{c}	1.10 ± 0.01^{b}	1.25 ± 0.01^{b}

Means with the same letter in the same colon are not significantly different at $P > 0.05$.

As can be observed, the peroxide values prior analysis (time zero) were less than 10 meq. O_2/kg. Before treatment all flavoured olive oils presented peroxide values below the maximum permitted for their classification as extra virgin oils (20 meq. O_2/kg) according to the European regulation for virgin olive oil. Fig. 1 shows that for all flavoured olive oil oxidation proceeds at a lower rate initially. After that the oxidation rate increases considerably. This first period of time is called the induction period (IP) or induction time (IT). During heating at 60 °C (Fig. 1a) and after 30 days (except for olive flavoured with rosemary), a steep rise in the slope was observed indicating that the oxidation process predominated, as the rate of peroxide formation was higher than that of decomposition. On the contrary, at 130 °C (Fig. 1b) the smooth slope between PV and heating time indicates that both oxidation and thermal decomposition occur. Several authors estimated the induction period for olive oil oxidation as the time necessary to reach a PV value of 70 meq. O_2/kg (Antoun and Tsimidou, 1997; Nissiotis and tasioula-Margari, 2002). In this study and based on present data above the induction time (IT) for each flavoured olive oil has been estimated as the time required for a sample to reach a peroxide value of 20 meq/kg, value overcoming the maximum permitted limit, and consequently losing the classification of virgin olive oil category. Induction time for each flavoured olive oil was summarised in Table 3.

From Fig. 1, Tables 1 and 3 we can conclude that despite similarity in their initial peroxide and acid values, flavoured olive oils show different deterioration patterns and different oxidative kinetics. Indeed, control oil and olive oil flavoured with basil show less thermal stability, followed by samples flavoured by lemon and thyme and finally the most stable oil is olive oil flavoured with rosemary. The peroxide value behaviour of these flavoured extra vierge olive oils could be explained by changes during oxidation process, reaching this value a maximum due to hydroperoxides formation. The results obtained are in accordance with those previously reported by Antoun and Tsimidou (1997), Caponio et al. (2003) and Malheiro et al. (2008).

The, K232 and K270, are mainly indicative, respectively, of the conjugation of trienes and the presence of carbonyl compounds. The maximum values permitted for K232 and K270 are, respectively, 2.50 and 0.20 for extra virgin olive oils and 2.60 and 0.25 for virgin olive oil, respectively.

Fig. 2 shows changes in K232 and K270 specific coefficients versus heating time. Before analysis flavoured olive oils present K232 values varied from 1.71 to 2.15 and K270 values varied from 0.07 to 0.1. In all case, before heating, K232 and K270 observed for each flavoured olive oil remain less than values recommended by the EU Regulation 2568/91 concerning quality characteristics of the virgin olive oil (K232 $\leqslant$ 2.5 and K270 $\leqslant$ 0.2). For oils heated at 60 °C the

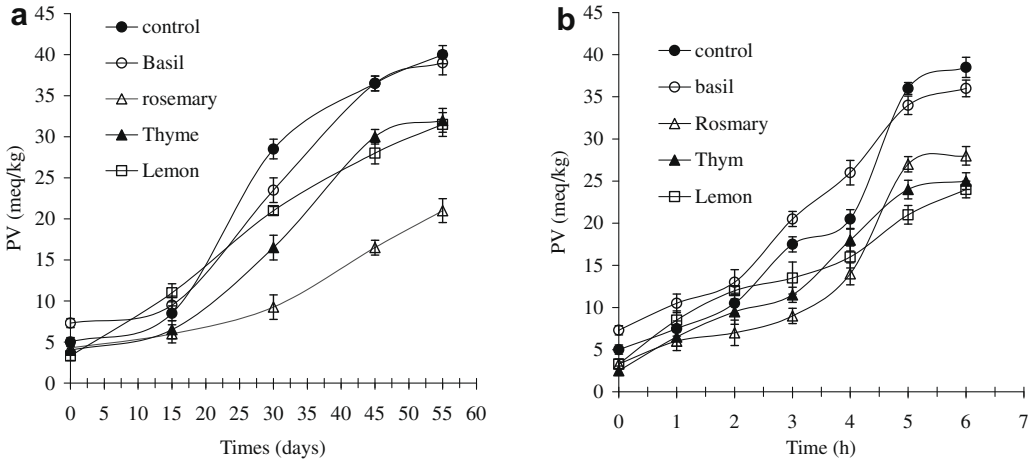

Fig. 1. Change in peroxide value of flavoured olive oils during thermal oxidation: (a) at 60 °C and (b) at 130 °C.

Table 3
Induction time of flavoured olive oil.

Temperature (°C)	Sample	Induction time
60	Control	22.5 days
	Basil	26 days
	Rosemary	53 days
	Thyme	34.5 days
	Lemon	28.5 days
130	Control	3.75 h
	Basil	3 h
	Rosemary	4.5 h
	Thyme	4.25 h
	Lemon	4.75 h

specific coefficient values (K232) increase in the initial heating stages (approximately the first 15 days), after that a slight increasing was observed. After 45 days of heating at 60 °C all oils present a K232 higher than 2.5 and a significant increase was observed for all flavoured olive oils. This phase (after reaching a K232 value of 2.5) indicates an accelerated degradation process. For oils heated at 130 °C, Fig. 2b shows a significant increase of K232 values after just 1 h. After this initial stage K232 increase slowly to reach values varying between 2.3 and 2.9 for olive oil flavoured with rosemary and control, respectively. This evolution means that heating at 130 °C causes great damage on the quality of all olive oils even during a short period.

The same evolution was also observed for K270 (Fig. 2c and d). In deed, Fig. 2c (heating at 60 °C) shows that K270 increase significantly after 45 days. This time correspond to the beginning of accelerated degradation period observed during K232 evolution. For oils heated at 130 °C the same evolution was observed: a significant increase after 1 h of heating indicates the sensitiveness of olive oil to high temperature even after aromatisation. Our results are in accordance to the obtained by Caponio et al. (2003).

The keep-ability of olive oil is a highly desirable attribute especially if the product is to be marketed in areas with warm climatic conditions (Muego-Gnanasekharan et al., 1993). Figs. 1 and 2 shows that aromatisation could prevent oxidation of olive oils. Indeed, the rosemary and thyme-treated samples exhibited a significant low value of peroxide, K232 and K270 than that of the control and oil flavoured with basil throughout the storage period. This result indicates the effectiveness of rosemary and thyme on heat stability of olive oil. For example, at the time the control reached a peroxide value of 40 meq/kg oil, rosemary, thyme and lemon flavoured olive oils samples reach 20, 32 and 31.5 meq/kg oil, respectively.

In the initial stages of oxidation, aromatic plants appear to be equally effective in stabilizing olive oil. After 55 days for samples heated at 60 °C and after 6 h for samples heated at 130 °C, rosemary was found to be more effective than thyme followed by lemon and basil. This effectiveness could be explained by the higher content of strong non-volatile antioxidants present in essential oil of each aromatic plant (Tsimidou and Boskou, 1994). Antoun and Tsimidou (1997) reported that rosemary was more effective to prevent olive oil oxidation then Oregano. These authors attribute this stability to the stability (non-volatile) of antioxidant compounds present in rosemary essential oils and to the partial evaporation of the essential oil of oregano, which is rich in carvacrol.

3.3.2. Change of chlorophyll, caratenoids and phenols contents

In addition to polyphénols, chlorophylls and carotenoids play an important role in the oxidative stability due to their antioxidant nature in the dark and prooxidant activity in the light and are mainly responsible for the colour of virgin olive oil, that varying from yellow–green to greenish gold (Criado et al., 2008).

Chlorophylls are present in olive oils and are the responsible for the greenish coloration of certain olive oils. Those pigments are also important in olive oil stability. Fig. 3 present the change of chlorophyll contents in flavoured olive oils during thermal oxidation. In the present work, before treatment, chlorophyll amount varied between 2.14 mg/kg, in the control and 3.89 mg/kg, in the olive oil flavoured with rosemary. A marked decrease was observed in its levels for all olive oils samples heated at 60 and 130 °C. This observation was more noticeable for samples heated at 130 °C. Indeed, practically chlorophylls content falls after 1 h of heating at 130 °C for all samples whereas this chlorophyll fall was observed after 30 days of heating at 60 °C.

Carotenes are present too in olive oil and are responsible for its yellow coloration. The behaviour of these pigments versus heating or oxidation process was similar to the chlorophylls one as shown in Fig. 4. The level of carotenoids in the unheated oils (time zero) was, respectively, 2.85, 4.18, 4.63, 5.1 and 6.81 mg/kg for control, olive oils flavoured with basil, lemon, thyme and rosemary. During heating at 60 °C, carotenes contents decrease drastically after 15 days, to a range of 2.5 mg/kg, for all flavoured olive oils. After this period the carotenoids contents remain practically constants to reach a value of 1.5 mg/kg. Same evolution was observed for oil samples heated at 130 °C.

Degradation of chlorophyll and carotenoids pigments present in olive oil during heating is very complex. The main difficulty in understanding the steps of these pigments degradation is that they

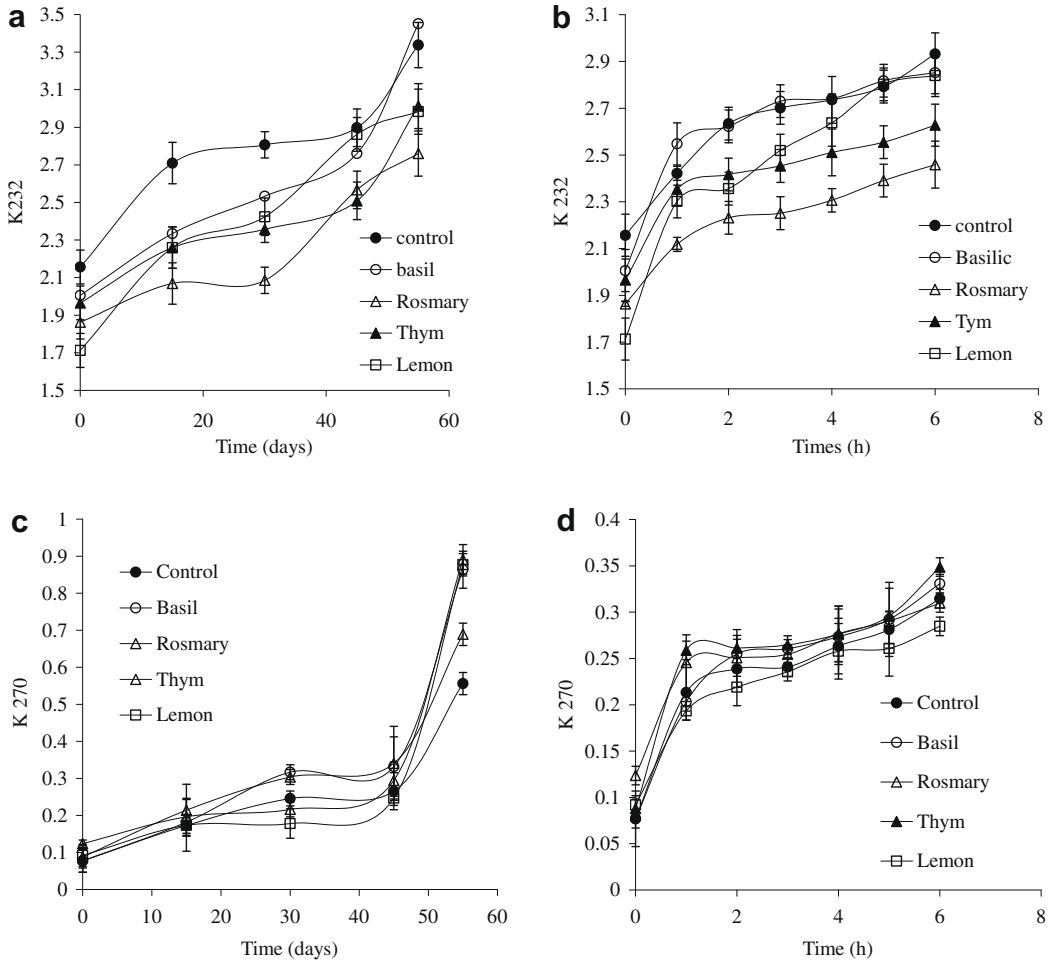

Fig. 2. Change in conjgated diene (K232 and K270) values of flavoured olive oils during heating, (a) and (c) at 60 °C; (b) and (d) at 130 °C.

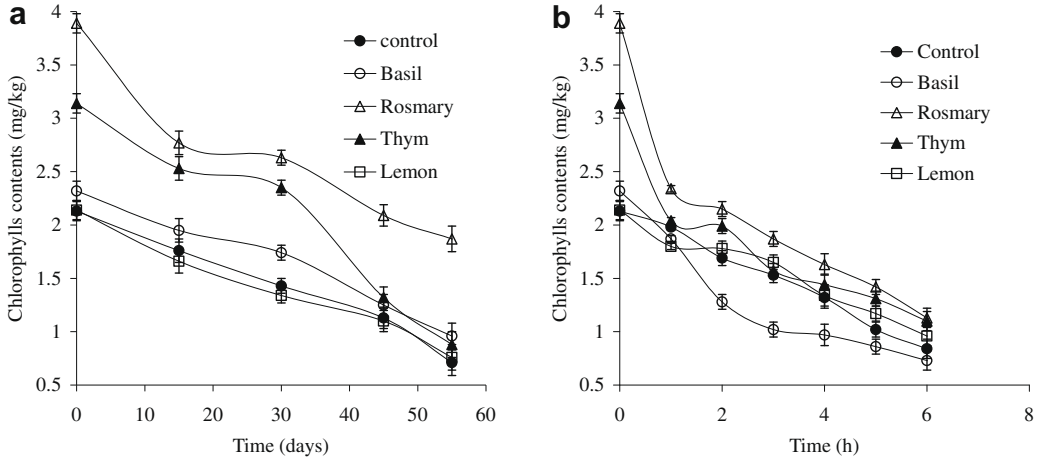

Fig. 3. Change of chlorophyll contents in flavoured olive oils during thermal oxidation: (a) at 60 °C and (b) at 130 °C.

yield different end-products, some of which are colourless. The overlapping of these products with degradation products adds to this difficulty (Criado et al., 2008). The oil pigments content, mainly the chlorophyll and carotenoids fractions concentration, decreased gradually during heating time (Figs. 2 and 3). However, the transformations of these components fractions during storage were different. In deed chlorophylls contents decrease continually versus heating time, however carotenoids contents decrease drastically after a short period of heating to reach an asymptotic value. These differences in degradation kinetics lead to conclude that carotenoids compounds apported by aromatic plants are more sensitive to the temperature than chlorophyll ones.

The presence of oxygen is a crucial factor in the carotene degradation. Gross (1991) reported that even a low concentration of

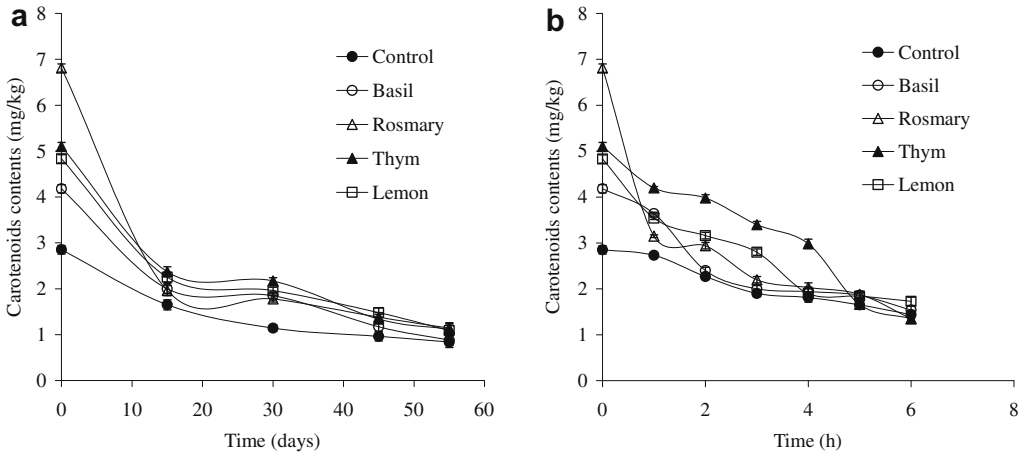

Fig. 4. Change of carotenoids contents in flavoured olive oils during thermal oxidation: (a) at 60 °C and (b) at 130 °C.

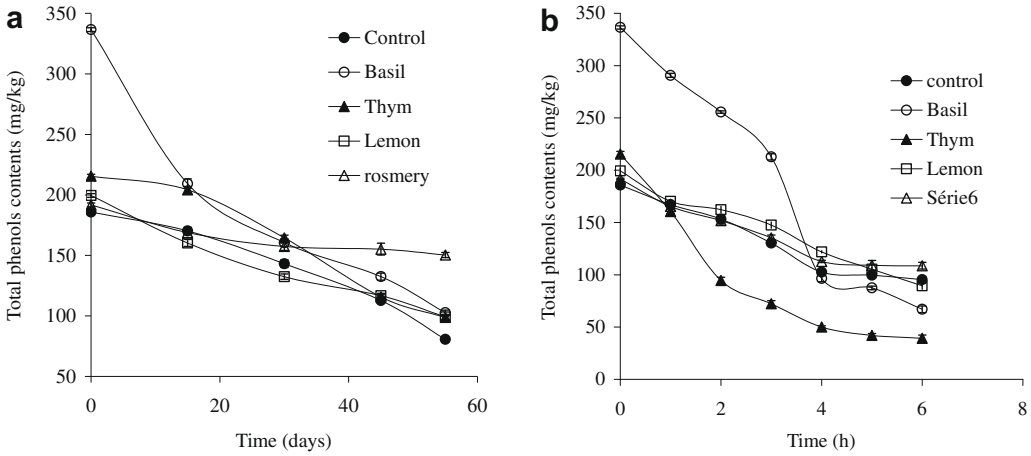

Fig. 5. Change of total phenols contents in flavoured olive oils during thermal oxidation: (a) at 60 °C and (b) at 130 °C.

oxygen leads to a significant loss of pigment. Criado et al. (2008) reported that, in parallel to oxygen presence, the presence of free radicals might also accelerate the degradation rate of carotenoids. It was understood that the oxidation of carotenes depended on the simultaneous oxidation of unsaturated fats. These unsaturated fats were probably oxidized in the first steps of the oil storage by lipoxygenase, and the oxidation product in turn oxidized the carotenoids. Both oxygen and presence of free radicals could explain the drastic decrease of carotenoids content after a short period of heating.

Polyphénols compounds are naturally present in olive oils and are the major compounds responsible for the stability of these oils during storage and heating. Dimitrios (2006) reported in a review of natural phenolic antioxidants sources that the major compounds present in natural olive oil are: hydroxytyrosol and derivatives and tyrosol and derivatives. Dimitrios (2006) reported too that the polyphénols content differs from oil to oil. Wide ranges have been reported (50–1000 mg/kg) but values are usually between 100 and 300 mg/kg. The cultivar, the stage of olive ripening, the system of extraction, and the conditions of processing and storage are critical factors for the content of polyphénols (Grati-Kammoun et al., 1999; Grati-Kamoun, 2007). Fig. 5 presents the change of polyphenols contents in control and flavoured olive oils during thermal oxidation. Initially, before heating, the amount of polyphenols ranged from 200 to 225 mg/kg for all flavoured oils except for oil flavoured with basil a value of 336.54 mg/kg was observed. Before heating at 60 °C a significant decrease of total phenols contents in olive oil flavoured with basil was observed. However a continuous slight decrease of total phenols contents was observed for control and the others flavoured olive oils. For oil flavoured with rosemary after a slight decrease total phenols content remain constant (~170 mg/kg) even after 55 days of treatment. For oil samples heated at 130 °C, an immediate decrease was observed for total phenols contents in olive oils flavoured with basil and thyme. However, total phenols present in the control and oils flavoured by lemon and rosemary decrease slightly during heating time.

4. General discussion and conclusions

Change in chlorophyll, carotenoids and total phenols could explain the differences observed in flavoured olive oils resistance to thermal oxidation. Indeed, even oil flavoured with basil had the higher amount of total phenols; it is the less effective against thermal oxidation. In opposition, oil flavoured with rosemary had a low initial value of total phenols contents and a high amount of chlorophyll and carotenoids, exhibit an effective resistance to thermal oxidation. These behaviours of flavoured olive oils could be explained by the effectiveness and the stability of particulate compounds which could migrate from aromatic plants to olive oil during maceration process. Indeed, several data were available in the literature concerning the composition of essential oils from Mediterranean aromatic plants such rosemary, thyme, basil and lemon (Piccaglia et al., 1993; Politeo et al., 2007; Mockute and Bernotiene, 2001; Almela et al., 2006). In our study, the abundance of

natural active substances present in each aromatic plant may have acted synergistically as free radical scavengers and/or contributed to the protection of initial total phenols such as tocopherol, the main antioxidant contained in original olive oil, against thermal oxidative degradation (Tomaino et al., 2005).

Almela et al., 2006 and Dimitrios (2006) reported that major antioxidant compounds present in rosemary were carnosic acid, carnosol and rosmarinic acid. Moreover Almela et al. (2006), when studying the phenolics compounds and free radical scavenging activity of rosemary extract, shows that pure α-tocopherol and fresh rosemary extracts were an effective antioxidants, with an almost identical kinetic of DPPH neutralization. These authors reported too that raw rosemary extract exhibits a great antioxidant activity higher than BHT (a known synthetic antioxidant) one. These data could explain the great stability of olive oil flavoured by rosemary against thermal oxidation. Politeo et al. (2007) studied chemical composition and antioxidant capacity of basil essential oil. In opposition to rosemary, basil essential oil exhibits a low antioxidant capacity. Indeed, Politeo et al. (2007) compared the antioxidant capacities of basil essential oil, basil volatile aglycones, BHT and pure eugenol and reported that eugenol is the more effectiveness followed by BHT followed by essential oil and finally the basil volatile aglycones. These authors reported too that among the compounds identified in the essential oil and volatile aglycones from basil, eugenol was considered the main contributor of the antioxidant capacity. They reported too that the antioxidant capacity of total essential oil is due only or mainly to the presence of eugenol (~6% of total essential oil) and that other constituents do not have significant effect on eugenol capacity. These data could explain our results relative to the richness of olive oil flavoured with basil on total phenols and its low stability against thermal oxidation.

In conclusion, the incorporation of some Mediterranean aromatic plants into olive oil relatively helped to improve their thermal resistance and stability. Indeed, a hierarchy in the thermal oxidation resistance of flavoured samples could be observed as well: oil flavoured with rosemary > with thyme > with lemon > - with basil.

This may be due to the abundance of natural antioxidants which were transferred into olive oils following the maceration process. In fact, these natural components (depending in the plant material) can react with free radicals of olive oil, thus effectively inhibiting the heat-induced loss of tocopherols being the most important natural antioxidant in olive oil. Through our study, we have demonstrated that rosemary, thyme and lemon, traditionally used for their aromatic properties in the preparation of Mediterranean food, exhibit good properties as free radical scavengers and/or antioxidants. This fact can support their use to control lipid oxidation during food processing. Further studies are warranted to better understand the factors influencing their antioxidant activity.

Conflict of interest

The authors declare that there are no conflicts of interest.

References

Almela, L., Sanchez-Munoz, B., Fernandez-Lopez, J., Roca, M.J., Rabe, V., 2006. Liquid chromatograpic–mass spectrometric analysis of phenolics and free radical scavenging activity of rosemary extract from different raw material. Journal of Chromatography A 1120, 221–229.

Antoun, N., Tsimidou, M., 1997. Gourmet olive oils: stability and consumer acceptability studies. Food Research International 30 (2), 131–136.

AOAC, 1999. Official Methods of Analysis, 16 ed. Association of Official Analytical Chemists, Washington, DC.

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 Chemistry 108, 253–262.

Caponio, F., Pasqualone, A., Gomes, T., 2003. Changes in fatty acid composition of vegetables oils in model doughs submitted to conventional or microwave heating. International Journal of Food Science and Technology 38, 481–486.

Chipault, J.R., Mizuno, G.R., Lundberg, W., 1956. The antioxidant properties of spices in foods. Food Technology 10, 209–211.

Criado, M.N., Romero, M.P., Casanovas, M., Motilva, M.J., 2008. Pigment profile and colour of monovarietal virgin olive oils from Arbequina cultivar obtained during two consecutive crop seasons. Food Chemistry 110, 873–880.

Dimitrios, B., 2006. Sources of natural phenolic antioxidants. Trends in Food Science and Technology 17, 505–512.

Farag, R.S., Badel, A.Z.M.A., Hewedi, F.M., El-Baroty, G.S.A., 1989. Antioxidant activity of some spice essential oils on linoleic acid oxidation in aqueous media. Journal of the American Oil Chemists' Society 66, 792–799.

Grati-Kammoun, N., Khlif, M., Hamdi, M.T., 1999. Evolution of oils characteristics with maturity of olives in Sfax: chemlali variety. Acta Horticulturae 474, 701–704.

Grati-Kamoun, N., 2006. Evolution of physico-chemical characteristics of some Tunisian olive oils during the storage. In: Proceedings of Olivebioteq 2006 (Biotechnology and Quality of Olive Tree Products Around the Mediterranean Basin) 5–10 november marsala-mazara del vallo (italy). pp. 405–410.

Grati-Kamoun, N., 2007. Étude de la diversité génétique de l'olivier cultivé en Tunisie : approche pomologique, chimique et moléculaire. Thesis, University of Sfax.

Gross, J., 1991. Pigments in Vegetables: Chlorophylls and Carotenoids. Van Nostrand Reinhold, New York.

Hirahara, F., Takai, Y., Iwao, H., 1974. Antioxidative activity of various spices on oils and fats. Japanese Journal of Nutrition 32 (1), l–8.

Ijaz Hussain, A., Farooq, A., Sherazi, S.T.H., Przybylski, R., 2008. Chemical composition, antioxidant and antimicrobial activities of basil (*Ocimum basilicum*) essential oils depends on seasonal variations. Food Chemistry 108, 986–995.

Malheiro, R., Oliveira, I., Vilas-Boas, M., Falcão, S., Bento, A., Pereira, J.P., in press. Effect of microwave heating with different exposure times on physical and chemical parameters of olive oil. Food and Chemical Toxicology. doi:10.1016/j.fct.2008.10.014.

Mancini, M., Giacco, R., 1993. Olive oil in the prevention of cardiovascular diseases. Olivae 47, 24–25.

Massana, L., Camprubi, M., Sarda, P., Sola, R., Joven, J., Turner, P.R., 1991. The Mediterranean-type diet: is there a need for further modification? American Journal of Clinical Nutrition 52, 886–889.

Mockute, D., Bernotiene, G., 2001. The α-terpenyl acetate chemotype of essential oil of *Thymus pulegioides* L. Biochemical Systematics and Ecology 29, 69–76.

Moldao-Martins, M., Beirao-da-Costa, S., Neves, C., Cavaleiro, C., Salgueiro, L., Beirao-da-Costa, M.L., 2004. Olive oil flavoured by the essential oils of *Mentha x piperita* and *Thymus mastichina* L. Food Quality and Preference 15, 447–452.

Montedoro, G., Servili, M., Baldioli, M., Miniati, E., 1992. Simple and hydrolysable phenolic compounds in virgin olive oil. 1. Their extraction, separation, and quantitative and semiquantitative evaluation by HPLC. Journal of Agricultural and Food Chemistry 40, 1571–1576.

Mosquera, M.M.L., Rejano, N.L., Gandul, R.B., Sánchez, G.A., Garrido, F.J., 1991. Colorpigment correlation in virgin olive oil. Journal of the American Oil Chemists' Society 68, 332–336.

Muego-Gnanasekharan, K.F., Resurrection, A.V.A., Garcia, V.V., Del Rosario, R., 1993. Consumer acceptance and storage stability of a cheese flavoured spread made from peanuts. Food Quality and Preference 4, 11l–117.

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

Piccaglia, R., Marottil, M., Giovanellil, E., Deans, S.G., Eaglesham, E., 1993. Antibacterial and antioxidant properties of Mediterranean aromatic plants. Industrial Crops and Products 2, 47–50.

Politeo, O., Jukic, M., Milos, M., 2007. Chemical composition and antioxidant capacity of free volatile aglycones from basil (*Ocimum basilicum* L.) compared with its essential oil. Food Chemistry 101, 379–385.

Tomaino, A., Cimino, F., Zimbalatti, V., Venuti, V., Sulfaro, V., De Pasquale, A., Saija, A., 2005. Influence of heating on antioxidant activity and the chemical composition of some spice essential oils. Food Chemistry 89, 549–554.

Tsimidou, M., Boskou, D., 1994. Antioxidant activity of essential oils from the plants of the Lamiaceae Family. In: Charalambous, G. (Ed.), Spices, Herbs and Edible Fungi. Elsevier, Amsterdam, pp. 273–284.

Tsimidou, M., Papavergou, E., Boskou, D., 1995. Evaluation of Oregano antioxidant in mackerel oil. Food Research International 28, 431–433.