J Am Oil Chem Soc (2015) 92:701–708
DOI 10.1007/s11746-015-2636-1

ORIGINAL PAPER

Impact of Aromatization by *Citrus limetta* and *Citrus sinensis* Peels on Olive Oil Quality, Chemical Composition and Heat Stability

Ibtihel Khemakhem[1] · Chiraz Yaiche[1] · Mohamed Ali Ayadi[1,2] · Mohamed Bouaziz[2,3]

Received: 20 November 2014 / Revised: 2 February 2015 / Accepted: 14 March 2015 / Published online: 29 March 2015

Abstract The objectives of this work were to study the impact of aromatization by sweet lemon and sweet orange peels on olive oil quality, chemical composition and heat stability. Flavored olive oils were prepared by maceration of sweet lime and sweet orange zests at 1, 3 and 5 %. The oil samples were kept at 60 °C for 40 days. Physicochemical tests (FFA, PV, oxidative stability, polyphenols and pigments contents) were carried out. The antioxidant activity (β-carotene-linoleate bleaching assay, DPPH radical scavenging and reducing power) was also tested. Results indicated that the addition of citrus zests contributed to the increase in polyphenols and carotenoids contents. Moreover, it led to an increase in the DPPH-radical scavenging activity (99.50 % for flavored olive oil with sweet orange zests at 5 %). Flavored olive oils with sweet orange zests were more stable to heat treatment than those flavored with sweet lemon zests. The degradation rate of bioactive compounds was lower for virgin olive oil comparing to flavored oils.

Keywords Olive oil aromatization · Sweet orange · Sweet lime · Oxidative stability · Antioxidant activity

✉ Mohamed Bouaziz
mohamed.bouaziz@fsg.rnu.tn

[1] Laboratoire d'Analyse, Valorisation et Sécurité des Aliments, Ecole Nationale d'Ingénieurs de Sfax (ENIS), Université de Sfax, BP 1175, 3038 Sfax, Tunisie

[2] Institut Supérieur de Biotechnologie de Sfax (ISBS), Université de Sfax, BP 1175, 3038 Sfax, Tunisie

[3] Laboratoire d'Electrochimie et Environnement, Ecole Nationale d'Ingénieurs de Sfax (ENIS), Université de Sfax, BP 1175, 3038 Sfax, Tunisie

Introduction

Virgin olive oil (VOO) is the most characteristic fat used in the Mediterranean region. It is known for its specific taste and aroma, as well as for its medicinal properties. Health-protective effects of olive oil are attributed to its high content of monounsaturated fatty acids especially oleic acid and also to the balanced ratio of saturated and polyunsaturated fatty acids [1]. Therefore, these characteristics provide protective effects against cardiovascular diseases by preventing low density lipoprotein (LDL) oxidation [2, 3].

Extra virgin olive oil is considered to be the best oil for its oxidative stability due to the presence of minor components, such as phytosterols, pigments, squalene, tocopherols, flavonoids and phenols. The main phenolic compounds present in the VOO are phenolic acids such as phenylethyl alcohols, benzoic and cinnamic acids, secoiridoids and lignans [4]. These phenols are powerful antioxidants. Actually, they scavenge free radicals such as lipid alkyl radicals or lipid peroxy radicals, control transition metals (iron and copper) and quench singlet oxygen [5]. Oxidative stability, usually evaluated by the Rancimat method, reveals the resistance of the oil to the initiation of the oxidation process characterized by free radical reactions [6].

Recently, the consumption of VOO is gaining interest all over the world. In order to orient the international market towards Tunisian olive oil, the enrichment of oils by ingredients related to the Mediterranean diet should be adopted.

Aromatic plants, herbs and spices have been used since ancient times, in food flavoring, pharmaceuticals, cosmetics and perfumery due to their biological activities, including antimicrobial and antioxidant properties. Flavored olive oils (FOO) are usually prepared by macerating the aromatic plants in the oil [1] or by using essential oils extracted from plants as a flavoring agent

[7]. Using the methodology of maceration, aromatic compounds, natural antioxidants and pigments present in the aroma are extracted, modifying, therefore, the sensory proprieties, nutritional characteristics and stability of olive oil during storage [1]. Currently, orange peels are known as a major source of natural antioxidants used in food in order to prevent rancidity, ensuring thus the stability of oils and fats [8]. Orange peels are known for their high concentration of flavonoids and their richness in volatile compounds [9]. For these reasons, they can also be used to produce FOO.

The objectives of this study were to produce FOO by maceration zests of two types of citrus (sweet orange and sweet lime) and to evaluate the quality and physicochemical properties of FOO compared to the natural one and finally to examine their heat stability.

Materials and Methods

Plant Material

Extra virgin olive oil used in the present work was obtained from "chemlali" variety. Olive oil sample was supplied by a local producer. Sweet orange (*Citrus sinensis*) and sweet lemon (*Citrus limetta*) are plants that belong to the Rutaceae family. Fresh fruits used in this study originated from the Cap-Bon region (North East of Tunisia).

Olive Oil Aromatization Process

Citrus peels [*Citrus sinensis* (CS) and *Citrus limetta* (CL)] were cut into small pieces then homogenized in the oil each at a rate of 1, 3 and 5 % (w/w). The mixture was kept in closed stainless bowls for 10 days in a cool place to avoid any oxidation phenomena. After maceration, FOO were filtered (peels were removed from the oil). The maceration process was performed in triplicate.

Physicochemical Analysis

FFA (free fatty acids) and PV (peroxide values) of olive oils were determined according to the methods described in AOAC [10].

Chlorophylls and Carotenoids Contents

Carotenoids and chlorophylls compounds were performed according to the procedures described by Mosquera *et al.* [11]

$$\left[\text{Chlorophylls}\right] = \frac{A_{670} \times 10^6}{613 \times 100 \times d}\ \text{mg/kg}$$

$$[\text{Carotenoids}] = \frac{A_{470} \times 10^6}{2000 \times 100 \times d}\ \text{mg/kg}$$

where A is the absorbance and d is the spectrophotometer cell thickness (1 cm).

Chlorophylls and carotenoids contents were expressed as mg of pheophytin "a" and lutein per kg of oil, respectively.

Determination of Total Phenol Contents

Total phenols were measured using the Folin Ciocalteu method according to the procedure described by Bouaziz *et al.* [12]. Briefly, 5 mL of *n*-hexane and 5 mL of methanol–water (80:20, v/v) were added to 5 mL of the sample. The mixture was stirred for 30 min and centrifuged at 3500 rpm for 10 min. Total phenol contents were resolved by measuring the absorbance at 727 nm on a spectrophotometer and its values were expressed in terms of gallic acid equivalents (mg GAE/kg oil).

Oxidative Stability by Rancimat

The oxidative stability was estimated by measuring the oxidation induction time. Oil stability was assessed by the Rancimat method at 120 °C on a Rancimat apparatus (Metrohom Series 743), using an oil sample of 3 g at air velocity 20 L/h.

Thermal Stability Tests

VOO and FOO were stored at 60 °C for 40 days. Equal portions of oil samples were kept in open 1-L flasks in an oven. Heat stability was evaluated by measuring FAA, PV, oxidative stability, chlorophylls, carotenoids and total phenols contents.

Antioxidant Activity Determination

The antioxidant capacity of olive oil samples was investigated by using three tests: DPPH radical-scavenging, β-carotene-linoleate bleaching assay and reducing power assay.

DPPH Radical-Scavenging Activity Assay

The DPPH radical (1,1-diphenyl-2-picrylhydrazyl) scavenging effect was evaluated as described by Bersuder *et al.* [13]. Five hundred microliters of a sample was added to 500 µL of ethanol and 125 µL of DPPH-ethanol solution (0.02 %). The reaction mixture was shaken vigorously and it was maintained in the dark for 1 h. After that, a steady state was reached. The absorbance of the remaining DPPH radical was measured at 517 nm against a blank solution. A control sample was prepared. The inhibition rate (IR) of the free radical DPPH was calculated as follows:

$$\text{IR}\ (\%) = \frac{\text{Control} + \text{DPPH°(Blank)} - \text{DPPH°(Sample)}}{\text{Control}} \times 100$$

where control was the DO at 517 nm of 500 µL of ethanol and 125 µL of DPPH-ethanol solution (0.02 %). DPPH° (Blank) was the DO at 517 nm of sample added to 625 µL of ethanol. DPPH° (Sample) was the DO at 517 nm of sample added to 500 µL of ethanol and 125 µL of DPPH-ethanol solution (0.02 %).

β-Carotene-Linoleate Bleaching Assay

Antioxidant activity of olive oil was determined using the β-carotene bleaching inhibition method described by Ben Taârit *et al*. [14] with some modifications. 0.5 mg of β-carotene was dissolved in 1 mL of chloroform, linoleic acid (25 µL) and Tween 40 (200 µL) were added. Chloroform was evaporated under vacuum at 40 °C. Then 100 mL of ultra-pure water was added and the emulsion was vigorously shaken. 2.5 mL of the emulsion was added to a tube containing 0.5 mL of sample. The absorbance was immediately measured at 470 nm and the mixture was incubated in a water bath at 50 °C. After 120 min, the absorbance was measured again. Antioxidant activity (AA) was evaluated using the following formula:

$$\text{AA}\ (\%) = \left(1 - \frac{A_S^0 - A_S^{120}}{A_C^0 - A_C^{120}}\right) \times 100$$

where A_S^{120} and A_C^{120} are the absorbance values of the sample and the control, respectively, after incubation for 120 min, and A_S^0 and A_C^0 are the absorbance values of the sample and control, respectively, measured at zero time.

Reducing Power Assay

The ability of olive oil to reduce iron (III) was evaluated according to the protocol of Yildirim *et al*. [15]. Sample solutions (1 mL) were mixed with phosphate buffer (1.25 mL, 0.2 M pH 6.6) and potassium ferricyanide (1.25 mL, 10 g/L). The mixtures were incubated at 50 °C for 30 min. Then 1.25 ml of 10 % (w/v) trichloroacetic acid were added. Then, the mixtures were centrifuged at 3000×*g* for 10 min. 1.25 mL of the supernatant solution were mixed with 1.25 mL of distilled water and 0.25 mL of ferric chloride (1 g/L). After 10 min reaction, the resulting solution absorbance was measured at 700 nm.

Statistical Analyses

All analytical determinations were performed at least in triplicate. Values of different parameters were expressed as the mean ± standard deviation ($x \pm$ SD). Analyses of variance (ANOVA) were carried out using the software SPSS statistics 17. Significant differences ($p < 0.05$) were detected using Duncan's multiple range tests.

Table 1 Physicochemical change of control and flavored olive oils

Samples	FAA (%)	PV(mequiv O_2/kg)	Oxidative stability (h)
Control	0.53 ± 0.03^{a}	8.00 ± 0.5^{a}	4.23 ± 0.2^{b}
CL 1 %	0.53 ± 0.03^{a}	11.50 ± 0.5^{b}	3.95 ± 0.05^{a}
CL 3 %	$0.55 \pm 0.00^{a,b}$	8.50 ± 0.5^{a}	3.93 ± 0.10^{a}
CL 5 %	0.58 ± 0.03^{b}	8.00 ± 1.0^{a}	3.99 ± 0.02^{a}
CS 1 %	0.53 ± 0.03^{a}	10.50 ± 0.5^{b}	3.99 ± 0.08^{a}
CS 3 %	0.58 ± 0.03^{b}	7.50 ± 1.0^{a}	4.03 ± 0.11^{ab}
CS 5 %	0.58 ± 0.03^{b}	7.50 ± 0.5^{a}	3.96 ± 0.04^{a}

Values given are the means of three replicates ± standard deviations

Means with the same superscript letter in the same column are not significantly different at $p > 0.05$

Results and Discussion

Physicochemical Change of Flavored Olive Oils

Table 1 presents the physicochemical change of olive oils after aromatization. Results presented in Table 1 show that the maceration with citrus peel caused a slight increase in free acidity in flavored oils for concentrations 3 and 5 % of each aroma. Though, there is no significant difference between control and FOO for the concentration 1 %. These results are in disagreement with those of Ayadi *et al*. [1] who demonstrated that the addition of lemon zest led to a significant increase ($p < 0.05$) in acidity. In all cases, all acidity values were lower than the limits set by the EU Regulation 1348/2013 for extra virgin olive oil [16]. The PV of oil samples ranged between 7 and 11.5 mequiv O2/kg. Therefore, all peroxide values were lower than the limits set by the EU Regulation 1348/2013 for extra virgin olive oil [16]. No significant ($p > 0.05$) difference was found between VOO and FOO with CL and CS at the concentrations 3 and 5 %. Only a significant ($p < 0.05$) increase in PV of oil flavored with CS 1 % and that flavored with CL 1 % was observed. Oxidative stability values measured by Rancimat show that the addition of aroma caused a significant ($p < 0.05$) decrease in the oxidative resistance of FOO with CL 1 %, CL 3 %, CL 5 %, CS 1 % and CS 5 %. For example, the oxidative stability of olive oil decreased from 4.23 to 3.93 h after its enrichment with CL 3 %. The decrease in the oxidative stability of flavored oils compared to VOO could be linked to the presence of water in zests. However, results showed that there was no significant ($p > 0.05$) difference between control and FOO with CS 3 %. No significant ($p > 0.05$) difference was found between FOO with sweet orange peels and those flavored with sweet lemon peels.

Table 2 Antioxidant activity of control and flavored olive oils

Samples	DPPH radical scavenging (%)	β-Carotene-linoleate bleaching (%)	Reducing power (DO 700 nm)
Control	42.70 ± 1.75^{a}	19.25 ± 0.35^{a}	0.134 ± 0.016^{a}
CL 1 %	71.40 ± 1.37^{c}	25.47 ± 0.71^{b}	0.148 ± 0.009^{a}
CL 3 %	92.10 ± 1.18^{d}	59.05 ± 3.06^{e}	0.141 ± 0.008^{a}
CL 5 %	92.60 ± 1.25^{d}	71.79 ± 1.12^{f}	0.169 ± 0.003^{b}
CS 1 %	55.2 ± 4.70^{b}	48.13 ± 1.87^{c}	0.219 ± 0.007^{c}
CS 3 %	59.07 ± 3.90^{b}	53.66 ± 1.23^{d}	0.250 ± 0.008^{d}
CS 5 %	98.57 ± 1.37^{e}	63.06 ± 3.05^{g}	0.269 ± 0.010^{e}

Values given are the means of three replicates ± standard deviations

Means with the same superscript letter in the same column are not significantly different at $p > 0.05$

Antioxidant Activity Determination

DPPH Radical-Scavenging Assay

The DPPH radical-scavenging assay was used to evaluate the ability of bioactive compounds to scavenge the free radical DPPH° [17]. Scavenging activities of FOO were measured and compared to control (Table 2). Results show that FOO have a radical scavenging activity significantly higher than control. In fact, scavenging activity reached 42.7 % for the control, while it ranged between 55.2 and 98.57 % for FOO. Besides, the scavenging activity increased as zests concentration were raised. According to Table 2, FOO with CS 5 % displayed the highest antioxidant activity. Moreover, the enrichment of VOO with lemon peels and orange peels at a concentration of 5 % gave rise to av increase in the antioxidant activity. It has been established that sweet orange and sweet lemon zests have a high content of compounds with antioxidant properties [9, 18].

β-Carotene-Linoleate Bleaching Assay

The β-carotene bleaching assay indicates the level of lipophilic compounds with antioxidant proprieties [19]. FOO had an antioxidant activity higher than the control as observed in DPPH radical-scavenging. In fact, results presented on Table 2 show that the antioxidant activity reached 19.25 % for the control, while it varied from 25.47 to 71.79 % for FOO. The inhibition of β-carotene bleaching may be related to the presence of lipophilic antioxidants in sweet lemon and sweet orange zests which had been transferred to the oil during the maceration. This lipophilic antioxidants could be carotenoids and polyphenols contained in FOO. In addition, the antioxidant activity increased by the increase in peel concentration put in the oil. Indeed, the highest antioxidant capacity was obtained for the oil flavored with CL 5 % (71.79 %).

Reducing Power Assay

The antioxidant activity of studied olive oils, evaluated by the measurement of their reducing power is shown in Table 2. The tested flavored oils were endowed with the antioxidant activity. These results may be attributed to the migration of bioactive compounds from peels to olive oil during the maceration process. In the present study, the addition of CL peels caused a slight increase in reducing power values. However, a significant increase was observed for FOO with CS zests. The highest reducing power was obtained for oil flavored with CS 5 %.

Heat Stability of the Flavored Olive Oils

Free Fatty Acids Profile

The evolution of FFA of the flavored oils and the control during heat treatment is presented in Fig. 1a. As it can be observed, FFA of all the samples increased during storage at 60 °C without exceeding the limits set by the EU Regulation 1348/2013 for extra virgin olive oil [16]. In this context, Ben Youssef *et al.* [19] reported that FFA of the oil from 'Chemlali' variety increased during storage at room temperature in an open flask. FFA of flavored oils were higher than that of VOO during heat treatment. This could be explained by the presence of water in the zests which may cause the hydrolysis of triglycerides. After 40 days of storage, the highest acidity was detected in the samples with high concentrations of added aroma. Indeed, it reached 0.95 % for FOO with CL 5 and 0.93 % for that flavored with CS 5 %. This result could be explained by the increase in water content by the increase in the zests concentration.

Changes of Peroxide Value (PV)

PV is a very important parameter which monitors the oxidative process in its early stages. The change of PV of

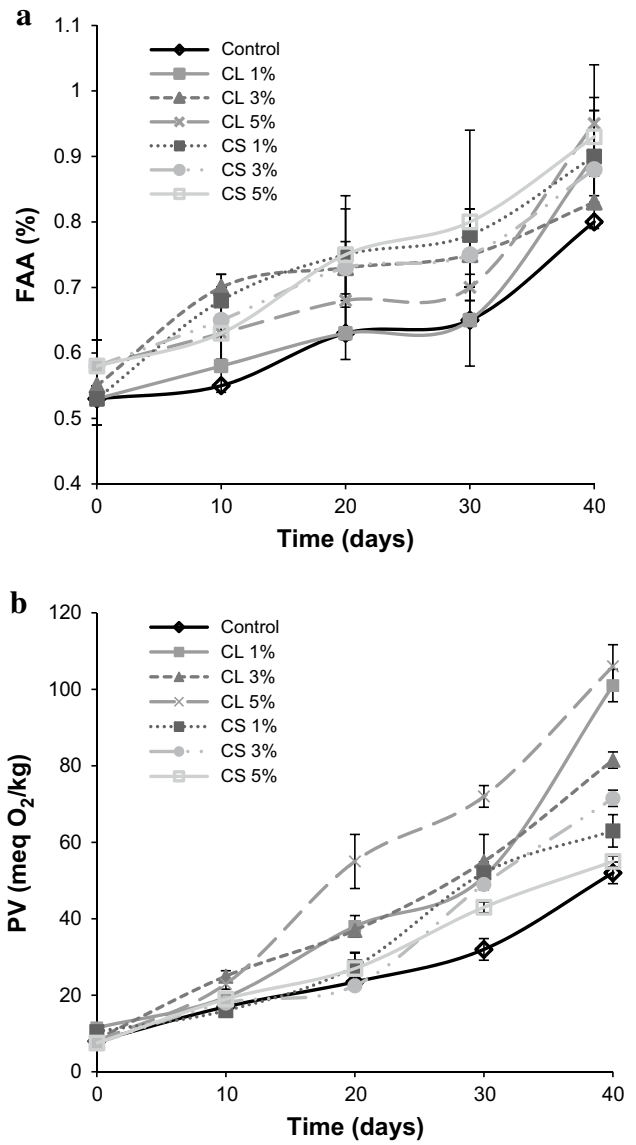


Fig. 1 Change of FAA values (**a**) and PV values (**b**) of control and flavored olive oils during thermal oxidation at 60 °C

the FOO and the control during heat treatment is given in Fig. 1b. Initially, olive oils oxidation was low, and then it increased drastically. The first period is called induction period (IP) or induction time (IT) [20]. The induction period for olive oils has been estimated as the necessary time for a sample to reach a peroxide value of 20 mequiv O_2/kg (the maximum permitted limit to losing the classification of the virgin olive oil category) [1]. Figure 1b shows that after 40 days of storage at 60 °C, the control and FOO with CS 5 % had the lowest PV. According to Table 3, FOO with sweet orange peels were more resistant to oxidation than those flavored with sweet lemon peels. Indeed, the IT of FOO with CL 3 % was 7 days while it was 16 days for that flavored with CS 3 %. This result may be related to the fact that CS zests contained less water than the CL zests. The control and FOO with CS 3 % were the most stable oils with the longest IT.

Table 3 Induction time of control and flavored olive oils

Samples	Induction time (days)
Control	16
CL 1 %	10
CL 3 %	7
CL 5 %	9
CS 1 %	15
CS 3 %	16
CS 5 %	12

Oxidative Stability by Rancimat

The evolution of oxidative stability of FOO and the control, measured by the Rancimat equipment is shown in Table 4. The oxidative stability of all samples decreased significantly during storage at 60 °C. Therefore, during heat treatment, there was an alteration in fatty acids which liberated secondary oxidation products (peroxides, alcohols, aldehydes and carboxylic acids). This result is entirely consistent with those reported by Rodrigues *et al*. and Bouaziz *et al*. [17, 21]. In this study, results showed that the control sample had a higher oxidative stability in all the exposure times tested comparative to the FOO samples. The presence of water in zests might induce the oxidation process. During storage at 60 °C, FOO with CS exhibited an oxidative stability higher than FOO with CS. This result was entirely consistent with the PV and the reducing power assay. In fact, FOO with CS reported lower PV and higher reducing power values comparing to FOO with CL. This finding may be caused by the saturation of FOO by CL with bioactive compounds which led to the auto-oxidation of these substances and then the reduction in their oxidative stability.

After 40 days, FOO with CL and CS at the concentrations 1 and 3 % were no longer resistant to oxidation which means that these oils were saturated with secondary oxidation products.

Changes in Chlorophylls, Carotenoids and Total Phenols Contents

Chlorophylls and carotenoids are not only responsible for the color of the oil but they also play an important role in its oxidative activity due to their antioxidant nature in the dark and pro-oxidant activity in the light [1]. Chlorophylls are responsible for the greenish coloration of certain olive oils. The change of chlorophyll content in FOO and control during thermal oxidation at 60 °C is presented in Fig. 2a.

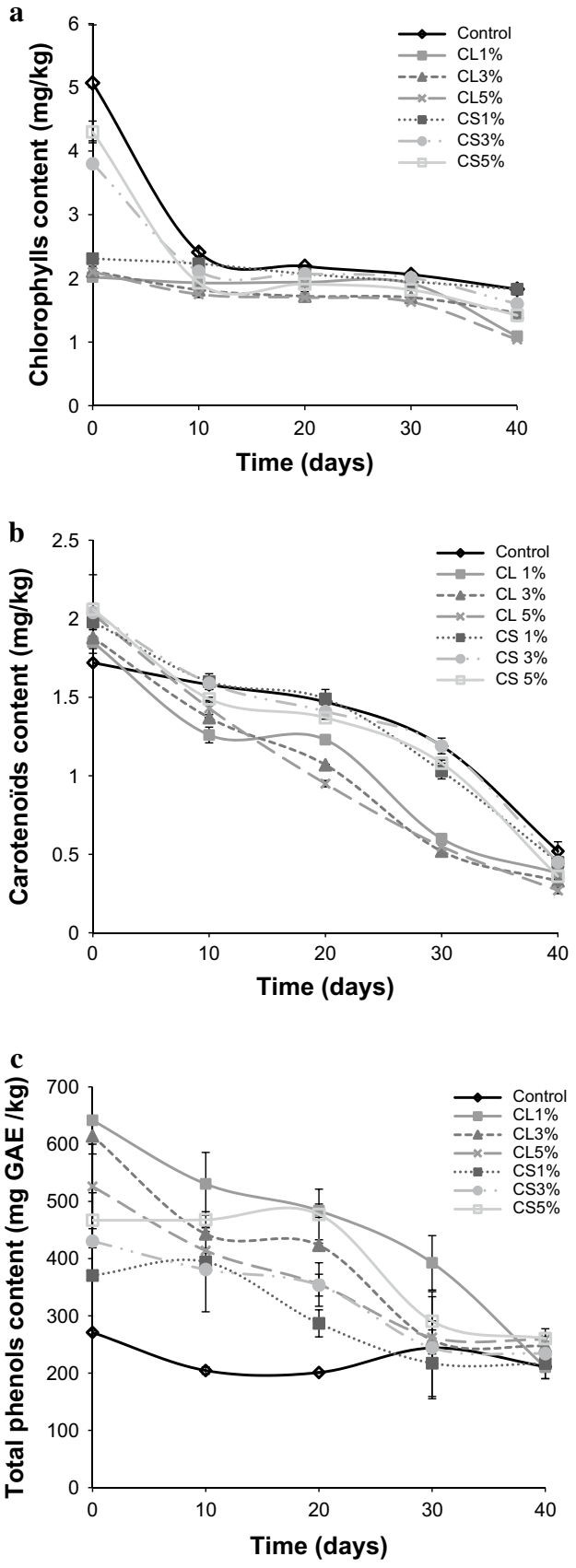
Table 4 Changes in the oxidative stability (h) of control and olive oils flavored by sweet orange and sweet lime zests during storage at 60 °C

Oxidative stability (h)	Time (days)				
Samples	0	10	20	30	40
Control	4.24 ± 0.20	2.26 ± 0.10	2.49 ± 0.17	1.12 ± 0.03	0.92 ± 0.09
CL 1 %	3.95 ± 0.05	2.08 ± 0.09	2.04 ± 0.09	0.00 ± 0.00	0.00 ± 0.00
CL 3 %	3.93 ± 0.10	1.77 ± 0.03	1.51 ± 0.03	0.30 ± 0.00	0.00 ± 0.00
CL 5 %	3.99 ± 0.02	1.88 ± 0.02	1.62 ± 0.03	0.96 ± 0.01	0.01 ± 0.00
CS 1 %	3.99 ± 0.08	2.42 ± 0.21	2.15 ± 0.10	0.38 ± 0.01	0.00 ± 0.00
CS 3 %	4.03 ± 0.11	2.45 ± 0.15	2.25 ± 0.06	0.37 ± 0.02	0.00 ± 0.00
CS 5 %	3.96 ± 0.04	2.01 ± 0.02	1.99 ± 0.13	0.95 ± 0.04	0.72 ± 0.05

Before treatment, FOO had less chlorophylls content than control except the oil flavored with CS 5 %. This decrease may be due to the degradation of chlorophylls caused by water or chlorophyllases coming from peels. After heat treatment, chlorophylls content decreased significantly during the first 10 days for the control and the FOO and then it remained practically constant. This result is in disagreement with that of Ayadi *et al*. [1] who reported that the chlorophylls fraction concentration decreased continually during heating time. Carotenoids are the pigment responsible for the yellow coloration of olive oils [1]. Figure 2b shows the change of carotenoids content in FOO and control during thermal oxidation at 60 °C. At the beginning (time zero), FOO contained more carotenoids than olive oil. This result agrees with that of Ayadi *et al*. [1] who demonstrated that the addition of lemon zests increased carotenoids content. In fact, the level of carotenoids in the unheated oils was respectively, 1.72, 1.85, 1.88, 2.03, 1.98, 2.04, and 2.06 mg/kg for the control and the FOO with CL 1 %, CL 3 %, CL 5 %, CS 1 %, CS 3 % and CS 5 %. These results may be due to the richness of citrus zest in carotenoids. In addition, FOO with sweet orange peels have higher content of carotenoids than that flavored with sweet lemon peels. During heating at 60 °C, carotenoids content decreased drastically. This content decreased by about 70, 79, 82, 86, 77, 78 and 82 %, respectively for the control and the FOO with CL 1 %, CL 3 % CL 5 % CS 1 %, CS 3 %, CS 5 % after 40 days. The degradation of carotenoids pigments seems to be caused by the high temperature and the presence of oxygen. Criado *et al*. [22] reported that the presence of oxygen and free radicals coming from thermal oxidation could accelerate the rate of carotenoids degradation. Besides, carotenoids destruction was pronounced for FOO by the increase in citrus zests content. This result can be explained by the presence of water in zests which increased the degradation rate of carotenoids. Figure 2b shows too that the decrease in carotenoids content was more obvious for FOO with sweet lemon zests. This result agrees perfectly with PV profile. According to Fig. 2a, b, chlorophylls and carotenoids profiles were different with heating time. In fact, chlorophylls contents decreased drastically after a short period of heating to reach an asymptotic value, however, carotenoids contents decreased gradually. Indeed, chlorophylls compounds seemed to be more sensitive to the temperature than carotenoids. Phenolic compounds are among the most important components because of their nutritional properties and their conservative activities. As shown in Fig. 2c, before heat treatment, olive oil was significantly affected by the addition of citrus zests. In fact, the addition of zests increased the polyphenols content. For FOO, this content ranged from 370.6 to 641.6 mg GAE/kg; however, it reached 271 mg GAE/kg for the control. Moreover, FOO with CL zests contained more phenolic compounds than that flavored with CS zests. For FOO with CL, polyphenols content decreased as zests concentration increased. This result may be caused by the saturation of oils with polyphenols after the addition of 1 % of CL zests, which led to the auto-degradation of polyphenols. Total phenols content of all samples decreased after the storage at 60 °C. These results are in agreement with the findings of Ayadi *et al*. and Ben Youssef *et al*. [1, 19] who showed that the polyphenols content decreased during the storage of olive oils. The degradation was more pronounced for FOO. In fact, after 40 days, the degradation rate was about 22, 61, 60, 51, 41, 45 and 44 %, for the control and FOO with CL 1 %, CL 3 % CL 5 %, CS 1 %, CS 3 %, CS 5 %, respectively. The decrease in phenols content may be due to their degradation caused by the presence of the oxygen and the high temperature. The degradation of polyphenols was pronounced in the case of FOO with CL zests. This result was in agreement with PV, carotenoid and chlorophyll profiles.

Conclusion

Results revealed that there was no change in the FAA value for flavored oils. The incorporation of zests at concentrations of 3 and 5 % had no significant effect on olive oil. In addition, the aromatization by citrus zests contributed



◀ **Fig. 2** Change of chlorophylls contents (**a**), carotenoids contents (**b**) and total phenols contents (**c**) of control and flavored olive oils during thermal oxidation at 60 °C

to increases in polyphenols content. It also led to the rise in carotenoids content especially for FOO with CS zests. Physicochemical changes seemed to be attributed to the migration of polyphenols, carotenoids and other specific compounds from zests to the olive oil during the maceration process. Besides, FOO with citrus peels exhibit good antioxidant properties which increase with the rise in aroma content. This may be due to the richness of citrus peels with natural antioxidants which were transferred to the olive oil. Through this study, we have demonstrated that after storage at 60 °C, the degradation rate of bioactive compounds was more noticeable for FOO. The presence of water in zests might speed up the degradation of polyphenols, chlorophylls and carotenoids.

Acknowledgments The authors would like to extend their thanks to Mr. Kamel Maaloul, English teacher at the Faculty of Science of Sfax, for having proofread this paper.

References

1. Ayadi MA, Grati-Kammoun N, Attia H (2009) Physico-chemical change and heat stability of extra virgin olive oils flavoured by selected Tunisian aromatic plants. Food Chem Toxicol 47:2613–2619
2. Veillet S, Tomao V, Chemat F (2010) Ultrasound assisted maceration: an original procedure for direct aromatization of olive oil with basil. Food Chem 123:905–911
3. Kratz M, Cullen P, Kannenberg F, Kassner A, Fobker M, Abuja PM, Assmann G, Wahrburg U (2002) Effect of dietary fatty acids on the composition and oxidizability of low density lipoprotein. Eur J Clin Nutr 56:72–81
4. Nevado JJB, Robledo VR, Callado CS-C (2012) Monitoring the enrichment of virgin olive oil with natural antioxidants by using a new capillary electrophoresis method. Food Chem 133:497–504
5. Messina V, Biolatto A, Descalzo A, Sancho A, Baby R, de Reca NW (2009) Effect of pan-frying in extra-virgin olive oil on odour profile, volatile compounds and vitamins. Int J Food Sci Tech 44:552–559
6. Matos LC, Pereira JA, Andrade PB, Seabra RM, Oliveira MBPP (2007) Evaluation of a numerical method to predict the polyphenols content in monovarietal olive oils. Food Chem 102:976–983
7. Moldão-Martins M, Beirão-da-Costa S, Neves C, Cavaleiro C, Salgueiro L, Beirão-da-Costa ML (2004) Olive oil flavoured by the essential oils of *Mentha* × *piperita* and *Thymus mastichina* L. Food Qual Prefer 15:447–452
8. Rehman Z-U (2006) Citrus peel extract—a natural source of antioxidant. Food Chem 99:450–454
9. Anagnostopoulou MA, Kefalas P, Papageorgiou VP, Assimopoulou AN, Boskou D (2006) Radical scavenging activity of various extracts and fractions of sweet orange peel (*Citrus sinensis*). Food Chem 94:19–25

10. AOAC (1999) Official methods of analysis, 16th edn. Association of Official Analytical Chemists, Washington, DC
11. Mosquera MML, Rejano NL, Gandul RB, Sánchez GA, Garrido FJ (1991) Colour pigment correlation in virgin olive oil. J Am Oil Chem Soc 68:332–336
12. Bouaziz M, Fki I, Sahnoun Z, Sayadi S (2005) Antioxidant phenolic compounds extracted from olive cultivar chemlali in hypercholesterolemic rats effect. Bio Org Med Chem 13:5362–5370
13. Bersuder P, Hole M, Smith G (1998) Antioxidant from a heated histidine-glucose model system. I. Investigation of the antioxidant role of histidine and isolation of antioxidants by high performance liquid chromatography. J Am Oil Chem Soc 75:181–187
14. Ben Taârit M, Msaada K, Hosni K, Marzouk B (2012) Fatty acids, phenolic changes and antioxidant activity of clary sage (*Salvia sclarea* L.) rosette leaves grown under saline conditions. Ind Crop Prod 38:58–63
15. Yildirim A, Mavi A, Kara AA (2001) Molecular mechanisms of butylated hydroxylanisole-induced toxicity: induction of apoptosis through direct release of cytochrome *c*. Mol Pharmacol 58:431–437
16. EEC Regulation 1348/2013 (2013) On the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis. Off J Eur Union L338:31–67
17. Rodrigues N, Malheiro R, Casal S, Asensio-S-Manzanera MC, Bento A, Pereira JA (2012) Influence of spike lavender (*Lavandula latifolia* Med.) essential oil in the quality, stability and composition of soybean oil during microwave heating. Food Chem Toxicol 50:2894–2901
18. Nogata Y, Sakamoto K, Shiratsuchi H, Ishii HT, Yano M, Ohta H (2006) Flavonoid composition of fruit tissues of citrus species. Biosci Biotech Bioch 70(1):178–192
19. Ben Youssef N, Ouni O, Dabbech N, Baccouri B, Abdelly C, Zarrouk M (2012) Effect of olive storage period at two different temperatures on oil quality of two Tunisian cultivars of *Olea* Europea, Chemlali and Chétoui. Afr J Biotechnol 11(4):888–895
20. Velasco J, Dobarganes C (2002) Oxidative stability of virgin olive oil. Eur J Lipid Sci Technol 104:661–676
21. Bouaziz M, Fki I, Jemai H, Ayadi M, Sayadi S (2008) Effect of storage on refined and husk olive oils composition: stabilization by addition of natural antioxidants from Chemlali olive leaves. Food Chem 108:253–262
22. Criado M-N, Romero M-P, Casanovas M, Motilva M-J (2008) Pigment profile and colour of monovarietal virgin olive oil from Arbequina cultivar obtained during two consecutive crop seasons. Food Chem 110(4):873–880