Effect of *Opuntia ficus-indica* flowers maceration on quality and on heat stability of olive oil

Imène Ammar¹ · Amal BenAmira¹ · Ibtihel Khemakem¹ · Hamadi Attia¹ ·
Monia Ennouri^{1,2}

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Abstract This study was focused on the evaluation of the quality and the oxidative stability of olive oil added with *Opuntia ficus-indica* flowers. Two different amounts of *O. ficus-indica* flowers were considered 5 and 15% (w/w). The olive oils were evaluated towards their quality, fatty acids profile, total phenol contents and thermal properties by differential scanning calorimetry. The oxidative stability was also monitored by employing the Rancimat and the oven test based on accelerating the oxidation process during storage. The addition of *O. ficus-indica* flowers induced an increase in free acidity values and a variation in fatty acids profile of olive oils but values remained under the limits required for an extra-virgin olive oil. The obtained olive oils were nutritionally enriched due to the increase in their phenols content. The oxidative stability was generally improved, mainly in olive oil enriched with 5% *Opuntia ficus-indica* flowers. These findings proved that this enriched olive oil could be considered as a product with a greater added value.

Keywords *Opuntia ficus-indica* · Olive oil quality · Oxidative stability · Food improvement

Introduction

The olive oil is one of the most important food of the Mediterranean diet because it establishes the main source of fats. It is used for cooking as well as for seasoning salads. Its composition in fatty acids and in phenolic compounds contribute to its high nutritional quality (Paola et al. 2010). The importance of olive oil is related certainly to its particular aroma and to its health benefits. Currently, it is important to diversify the range of olive oils products as a way to increase its uses all over the world. The flavouring of oil is one of the parts of this process of diversification. Therefore, various studies have previously shown the beneficial effects of the addition of aromatic plants (thyme, rosemary, lavender, basil) (Ayadi et al. 2009), herbs and spices (Gambacorta et al. 2007) on the stability and sensorial characteristics of olive oil. Moldao-Martins et al. (2004) have studied the effect of essential oils extracted from *Mentha × Pipertia* and *Thymus mastichia* L. on the quality of olive oil. Bouaziz et al. (2008) have also tested the stabilisation of olive oil by adding natural antioxidants from olive leaves. Thus, incorporation of such plant secondary metabolites into olive oil might significantly contribute both to the health benefit of consumers and to the stabilisation of the product itself.

The addition of *Opuntia ficus-indica* flowers to virgin olive oils could be particularly interesting, since these flowers are an excellent candidate as a source of phytochemicals. These flowers have been reported to have a wide range of bioactive compounds, especially flavonoids (Ammar et al. 2012; De Leo et al. 2010). Besides, the *O. ficus-indica* flowers have a significant antioxidant activity, ascribed to their high contents of phenolics and polysaccharids (Alimi et al. 2011; Ammar et al. 2012). These flowers have also attracted much attention of their health benefits thanks to

✉ Imène Ammar
imene_ammam@yahoo.fr

¹ Laboratoire Analyse, Valorisation et Sécurité des Aliments, Université de Sfax, Ecole Nationale d'Ingénieurs de Sfax (ENIS), BP 1173-3038, Sfax, Tunisia

² Higher Institute of Applied Sciences and Technology of Mahdia, Sidi Messaoud 5111, Mahdia, Tunisia

their antimicrobial, antifungal and anti-inflammatory properties (Ammar et al. 2012; Benayad et al. 2014). Furthermore, among the most recognized popular uses of *O. ficus-indica* flowers include the flavoring of olive oil. However, the addition of these flowers in olive oil as a way to improve olive oil's stability has never been tested. Accordingly, the aim of this study was to evaluate the effect of the addition of *O. ficus-indica* flowers to olive oil. In this way the physico-chemical parameters of olive oils with and without flowers and their ability to stabilise the oxidative reactions in the olive oils were studied.

Materials and methods

Plant material

An extra virgin olive oil produced in the year 2012/2013 by a local production unit was used for the preparation of the flavored oils. The oil samples were from Tunisian “Chemlali” cultivar olives, collected in the region of Sfax (Tunisia). The oils were extracted by three phase's centrifugation system from mid-ripe olive fruit.

Opuntia ficus-indica flowers growing wild in the region of Sfax, Tunisia, were collected in one batch within the same sampling area during post-flowering stage in May–June 2013. The moisture content of flowers samples was determined by drying at 105 °C to constant weight (AOAC 1995) and it was found to be 20.3%. After that, flowers were cleaned and crushed to obtain a fine powder.

Preparation of oil with *Opuntia ficus-indica* maceration

Opuntia flowers-oil mixtures were prepared at a rate of 5% (O1) and 15% (O2) (w/w) using 1 L of olive oil for each sample. The mixtures were left upon constant agitation for 2 h at room temperature. After that the preparation were kept in the dark, in closed stainless bowls during 20 days in a fresh place to avoid oxidation phenomena. After this maceration step, the plant material was removed by filtration and the oil samples were placed in a series of obscure glass bottles having each a volume of 30 mL to assess the storage stability. The same procedure was followed for the control oil sample.

Physico-chemical analyses

Free fatty acids, peroxide and specific extinction values determination

The quality parameters analysed were free acidity (FA), peroxide value (PV) and specific coefficients of extinction

K_{232} and K_{270} . All the mentioned quality parameters were determined according to the European Official Method of Analysis (EEC Regulation 1348/2013 2013).

Chlorophylls, carotenoids and total 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 cyclo-hexane. The chlorophylls 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:

$$\text{Chlorophylls} = \frac{\text{Abs}_{670} \times 10^6}{613 \times 100 \times \text{density}} \text{ (mg/kg)}$$

$$\text{Carotenoids} = \frac{\text{Abs}_{470} \times 10^6}{2000 \times 100 \times \text{density}} \text{ (mg/kg)}$$

Total phenols contents (TPC) were measured in the polar fraction extracted from the olive oil samples using a methanol/water extraction according to the method described by Satue et al. (1995). Briefly, 5 g of oil sample was dissolved in 5 mL of hexane and its polar fraction was then extracted three times with 5 mL methanol: water (60:40, v/v). The mixture was vortexed and then centrifuged at 3500 rpm for 10 min. Total phenols contents were determined colorimetrically at 765 nm using the Folin–Ciocalteu reagent and results were expressed as gallic acid equivalents (mg GAE/kg of oil) (Gutfinger 1981).

Colour measurement

CieLab coordinates (L^* , a^* and b^*) has been directly read with a spectrophotocolourimeter (Trintometre, Lovibond PFX 195 V 3.2, Amesbury, UK). Illuminant D 65 was selected, along with observer CIE 64. The following color coordinates were measured: lightness (L^*) ranges from 0 (black) to 100 (white), redness (a^* , red-green) ranges from −100 (greenness) to +100 (redness) and yellowness (b^* , yellow-blue) ranges from −100 (blueness) to +100 (yellowness).

Fatty acid composition

Fatty acid composition was identified by gas chromatography after cold alkaline transesterification with methanolic potassium hydroxide solution (Annexes II and IX in European Community Regulation EEC/2568/91) and extraction with *n*-heptane. A chromatographic analysis was performed 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 $\times$ 0.25 mm of internal diameter and 0.25 μ m of film thickness). The flame ionization detection temperature 280 °C; injector temperature 250 °C. The oven temperature range was programmed from 180 to 250 °C. Nitrogen was used as the carrier gas at a flow rate of 1 mL/min. The peak identification was carried out by comparing the peak retention time with those of the standard fatty acids injected under the same operating conditions. Results were expressed as the relative percentage of the total peak areas.

Differential scanning calorimetry (DSC)

Thermodynamic characterization of olive oil samples was determined with a differential scanning calorimeter (DSC 2920 Modulated DSC-TA Instruments, Newcastle, DE, USA). Samples (2 ± 0.1 mg) were sealed in hermetic pans (SFI—aluminium, TA Instrument T11024). The reference chamber contained an empty pan. DSC was used to determine the phase transition temperature during the cooling and heating of samples. The samples were cooled at a rate of 15 °C per minute to -50 °C. The scan was completed over a range of -50 to 90 °C. A heat-cool cycle was performed in triplicate per sample with 15 min resting time at the extremes of each cycle and the DSC thermographs were recorded during the second melting. The DSC was calibrated for temperature and heat flow using ecosane ($T_p = 36.8$ °C, $H = 247.7$ J/g) and dodecane ($T_p = -9.65$ °C, $H = 216.73$ J/g).

Crystallization onset temperatures were determined by the intersection of the baseline with the absolute highest tangent of the crystallization curve. The DSC profiles were analyzed with the universal analysis software version 4.7A (TA Instruments, New Castle, USA) to obtain enthalpy of crystallization (J/g), Peak temperature (T_p), onset (T_{on}) and offset temperature (T_{off}). The range of transitions was calculated as the temperature difference between T_{on} and T_{off} .

Assessment of oxidative stability

Two methods were used to evaluate the oxidative stability for the oils fraction: Oven test and Rancimat.

Oxidative stability by Rancimat

The oxidative stability was assessed by measuring the induction time, in a Rancimat apparatus (Metrohm AG Series 679, Herison, Switzerland according to ISO 6886 2006). A sample of olive oil (3 g) was heated to 120 ± 2 °C and subjected to an air-flow at 20 L/h. The resulting volatile compounds were collected in water, and the

increasing water conductivity (μ S/cm) was continuously measured. The induction time (hours) is determined from the inflection point of the conductivity curve. All of the samples were analyzed in triplicate.

Thermal stability tests

Oil samples (70 g) were stored in equal portions in obscure flasks (30 mL capacity, 30 mm diameter and 70 mm height) in an oven (Binder, No: 970465, Tuttlinger, Germany) at 60 °C.

One bottle was taken from the incubator for analysis at predetermined time intervals. The heating at 60 °C aims to accelerate the storage at ambient temperature. The stability to the oxidation was evaluated by determining the peroxide value (PV), the specific absorptivity at 232 and 270 nm, the chlorophylls, carotenoids and total phenols contents.

Statistical analysis

The chemical analyses were carried out in triplicate and the results were expressed as mean value $\pm$ SD.

Data were analyzed using SPSS statistical software, version 16.0 (Chicago, IL, USA). SPSS was used to perform one one-way-analysis of variance (ANOVA) using Duncan's multiple range post hoc test at a 95% confidence level ($p < 0.05$) to identify differences among samples for the chemical composition averages before heating storage and the quality parameters at each storage time.

Results and discussion

Physico-chemical characteristics of olive oils

In order to evaluate the effect of *O. ficus-indica* flowers addition on the olive oil quality, different physico-chemical parameters were assessed. The results regarding the quality parameters of O1 and O2 olive oils (with 5 and 15% of *O. ficus-indica* flowers, respectively) and the control olive oil (untreated) are presented in Table 1. FA values showed a significant increase ($p < 0.05$) from 0.4% for the control olive oil to 0.6 and 0.7% for O1 and O2, respectively. In this context, previous studies also reported that FA values increased when Tunisian aromatic plants and garlic were added to extra virgin olive oils (Ayadi et al. 2009; Sousa et al. 2015). Meanwhile, as can be observed in Table 1, the FA values remained below the maximum permitted value for their classification as extra virgin oils ($\leq 2\%$) according to the EU Regulation 2568/91. The registered rise in the FA values could be

Table 1 Effect of the addition of *Opuntia ficus-indica* flowers to olive oil on the quality parameters, fatty acids composition and total phenol contents

Macerated olive oils	Control	O1	O2
FA (% oleic acid)	0.42 ± 0.02 ^a	0.62 ± 0.06 ^b	0.71 ± 0.02 ^b
Density (g/cm ³)	928.6 ± 3.21 ^a	958.3 ± 2.88 ^b	967 ± 3.00 ^c
Total phenols (mg/kg)	254.5 ± 0.14 ^a	258.8 ± 8.48 ^a	262 ± 2.82 ^b
Colour parameters			
<i>L</i> *	90.4 ± 0.59 ^a	72.8 ± 4.20 ^b	72.5 ± 2.84 ^b
<i>a</i> *	−12.4 ± 0.24 ^a	−7.5 ± 0.87 ^b	−6.7 ± 0.54 ^b
<i>b</i> *	48.1 ± 0.29 ^a	41.9 ± 1.54 ^b	43.5 ± 0.83 ^b
Fatty acid composition (%)			
C16:0	12.9 ± 1.41 ^a	13.81 ± 1.10 ^b	13.49 ± 0.38 ^b
C16:1 w7	4.75 ± 1.72 ^a	4.06 ± 1.07 ^b	3.78 ± 1.52 ^c
C18:0	1.47 ± 0.0019 ^a	1.28 ± 0.0025 ^a	2.36 ± 1.362 ^b
C18:1 w9	59.54 ± 1.41 ^a	61.66 ± 1.57 ^b	60.14 ± 0.85 ^b
C18:2 w6	19.14 ± 1.09 ^a	18.02 ± 1.28 ^b	18.34 ± 1.64 ^b
C18:3 w3	1.6 ± 0.73 ^a	1.17 ± 0.34 ^b	1.22 ± 0.58 ^b
SAFA	14.41 ± 1.40 ^a	15.09 ± 1.10 ^b	15.86 ± 1.75 ^c
MUFA	64.28 ± 0.30 ^a	65.72 ± 0.50 ^b	63.91 ± 0.69 ^c
PUFA	20.81 ± 1.80 ^a	21.18 ± 1.60 ^b	20.23 ± 1.08 ^a

Values given are the mean of three replicates ± standard deviation. Means with the same letter in the same line are not significantly different at $p > 0.05$. O1: olive oil sample macerated with 5% of *Opuntia ficus-indica* flowers and O2 olive oil sample macerated with 15% of *Opuntia ficus-indica* flowers

related to the co-extraction of undesirable compounds that promotes degradation reactions in the olive oil. Table 1 also illustrates a significant increase ($p < 0.05$) in density of olive oils from 928 g/cm³ for the control olive oil to 967 g/cm³ for the oil with *O. ficus-indica* flowers. This increase could be ascribed to the diffusion of particulate compounds (phenolic compounds, pigments, waxes) from *O. ficus-indica* flowers to olive oil during maceration process. This was in agreement with results of Ayadi et al. (2009) who demonstrated that the addition of lemon, lavender and menthe led to a significant increase ($p < 0.05$) in density of olive oils.

Phenolic compounds are naturally present in olive oils and are the major compounds responsible of the olive oil stability during storage and heating. The total phenol contents (TPC) show no significant difference between the control and O1. The addition of 15% *O. ficus-indica* flowers enriched the phenolic fraction of olive oils in the order of 3.4%. This was expected since it was showed previously, that the same plant material of *O. ficus-indica* flowers used in this study contain a high TPC of about 270 mg of gallic acid equivalent/g of methanolic extract (Ammar et al. 2015). Similar results were reported by Ayadi et al. (2009) when studying the aromatization of Tunisian olive oils and revealed that the addition of basil increased significantly TPC. The incorporation of *O. ficus-indica* flowers, may have incorporated phenolic compounds to the olive oil matrix. However, while studying the aromatization of olive oil, Sousa et al. (2015) observed that

the incorporation of different herbs and spices was not beneficial to the olive oils phenolic composition.

Colour parameters showed that the maceration caused a slight decrease of *L** and *b**, leading to darkening of olive oils. Values of *a** increased and *b** decreased compared to control oil. This may be explained by the presence of pigment in the macerated oil which was migrated from flowers to the oil during maceration.

Fatty acid composition

The fatty acids profile was assessed for control olive oil, O1 and O2 and their detailed composition is reported in Table 1. In all samples, oleic acid (C18:1) was the most abundant fatty acid, followed by linoleic acid (C18:2) and palmitic acid (C16:0). The distribution of fatty acid covered the normal range expected for olive oils (Ben-moussa et al. 2016). Oleic acid content increased significantly ($p < 0.05$) with the addition of *O. ficus-indica* flowers. Its values ranged from 59.5% for control olive oil to 60.14 and 61.66% for O1 and O2, respectively. The incorporation of the flowers to the olive oil decreased significantly ($p < 0.05$) C18:2. Control olive oils showed 19.14% of C18:2, while the macerated ones presented values equal or below to 18.34% (Table 1). In the case of C16:0 olive oils with *O. ficus-indica* flowers were those with the highest content. O1 had the highest of C16:0 (13.81%) and the lowest of C18:2 (18.02%) contents. Among the individual fatty acids, the addition of *O. ficus-*

indica flowers decreased significantly ($p < 0.05$) the amounts of palmitoleic acid (C16:1) and linolenic acid (C18:3).

The results obtained for the different fatty acid fractions revealed that the addition of *O. ficus-indica* flowers increase significantly the SAFA content, an increase that varied between 0.68% in O1 and 1.45% in O2. This could be probably due to the interactions between olive oil and the components of *O. ficus-indica* flowers during the maceration, being responsible of the partial hydrolysis of the PUFA double bonds and subsequently increasing of SAFA.

The addition of 5% of flowers increased significantly MUFA and PUFA contents by 1.44 and 0.37%, respectively.

Thermal behavior of olive oils

The differential scanning calorimetry (DSC) was used to assess thermal characteristics of control, O1 and O2 olive oils. Heating curves of the studied oils had similar, but not identical, thermograms. Indeed, they exhibited a simple thermogram after melting in the DSC (Fig. 1).

Higher T_{on} and T_p in comparison with control oil were found for O1 and O2, with both a high content of palmitic (C16:0) and linoleic acids (C18:1) (as shown in Table 1). This confirmed previous study where high statistical correlations were obtained among the thermal properties of extra virgin olive oils and amounts of these compounds (Chiavaro et al. 2010). In addition, O2 showed significant ($p < 0.05$) changes in thermal properties characterised mainly by a higher T_{on} and a lower melting enthalpy and range of transitions. These changes may be related to the formation of lipid oxidation products that can disrupt reorganization of different polymorphic forms. In this respect, the oxidation may have lead to a rearrangement of different polymorphs in oil and required lower melting enthalpy (Cuvelier et al. 2012). In fact, previous studies have shown that the decrease in melting enthalpy was mainly due to the formation of fat degradation products that have lower melting range than the scan temperature (Besbes et al. 2005; Gloria and Aguilera 1998). From the DSC results, it was evident that O1 did not show distinct changes and has better stability than O2. Thus, the *O. ficus-indica* flowers added at 5% preserved the quality of the olive oil.

Fig. 1 Melting thermograms and thermal parameters of control, O1 (sample with 5% *O. ficus-indica* flowers) and O2 (sample with 15% *O. ficus-indica* flowers)

Parameter	Oil sample		
	Control	O1	O2
Onset temperature (°C)	-24.9±0.43 ^a	-25.43±0.96 ^b	-26.05±0.6 ^c
Peak temperature (°C)	-5.86±0.18 ^a	-5.99±0.42 ^a	-6.6±0.49 ^b
Melting enthalpy (J/g)	61.05±0.09 ^a	63.37±0.33 ^b	60.76±0.34 ^c
Range of transition (°C)	-13.97±0.29 ^a	-13.1±0.28 ^a	-16.32±0.42 ^b

All values given are means of three determinations

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

Table 2 Induction time of the control, 5 and 15% enriched olive oils

Temperature (°C)	Sample	Induction time
120 °C	Control	2.83 h $\pm$ 0.2 ^a
	O1	2.73 h $\pm$ 0.5 ^a
	O2	2.42 h $\pm$ 0.15 ^b

Values given are the mean of three replicates $\pm$ standard deviation. Means with the same letter in the same column are not significantly different at $p > 0.05$. O1: olive oil sample macerated with 5% of *Opuntia ficus-indica* flowers and O2 olive oil sample macerated with 15% of *Opuntia ficus-indica* flowers

Thermo-oxidation effects on olive oils

Accelerated oxidation by Rancimat test

The oxidative stability of control and macerated olive oils with *O. ficus-indica* flowers was studied using Rancimat. The induction time obtained for O1 and O2 were 2.73 ± 0.5 h and 2.42 ± 0.15 h, respectively, while for control oil, the induction time was 2.83 ± 0.24 h (Table 2). It appears that the induction time increased remains insignificant ($p > 0.05$) for O1. Meanwhile, a significant ($p < 0.05$) increase in the induction time was

observed in O2, which reflects a tendency to a higher oxidation when compared to control olive oil.

This data suggested that during the maceration of olive oil with *O. ficus-indica* flowers, there is a diffusion of undesirable compounds different from phenolics that could have synergic reactions in the olive oil and negatively affect the oxidative stability. The increased oxidation obtained for O2 could be due to the accentuated effect of these compounds diffused from flowers to olive oils at higher rate than O1.

Accelerated oxidation of oil by oven-test

The oxidative stability study was also carried out, under accelerated oxidation conditions at 60 °C. The oxidation development by heating at 60 °C during 60 days was followed through PV, K_{232} and K_{270} . The measurements are illustrated in Fig. 2. Peroxide value (PV) is a measure of the peroxides and hydroperoxides concentration formed in the initial stages of lipid oxidation. With the evaluation of the specific extinction coefficients (K_{232} and K_{270}) it is also possible to study the progress of oxidation, complementing the observations for the PV.

Before heating at 60 °C, the control olive oil had a PV of 7.2 (mEq. O₂/kg), while O1 and O2 had 8.3 and 9.5

Fig. 2 Change in peroxide value (a) and conjugated diene [K_{232} (b) and K_{270} (c)] values of control olive oil, O1 (olive oil with 5% *O. ficus-indica* flowers) and O2 (olive oil with 15% *O. ficus-indica* flowers) during heating at 60 °C

(mEq. O₂/kg), respectively. The addition of both amounts of *O. ficus-indica* flowers appears to induce a slight increase in the formation of peroxides, but these values can be regarded as similar when compared with the legal limit for extra virgin olive oil, of 20 mEq.O₂/kg (EC Reg. 1989/2003). Initially, the PV for control, O1 and O2 increased slowly for 20 days at 60 °C. However, after that, the PV increased considerably, indicating a high level of peroxide formation. The evolution of the PV can provide information about the induction time. When the PV reached the value of 20 meq O₂/kg, this was considered as induction time, as this was the upper PV value established by the EU regulation (European Union Commission 1991) for the virgin olive oil category. This limit was reached after 18, 20, and 21 days in O2, O1 and control oil, respectively. This was in accordance with the induction time obtained by Rancimat (Table 2).

In this respect, the PV evolution with heating time revealed different patterns of oxidation. Infact, O2 showed higher PV than the control indicating less thermal stability. This behavior could be explained by formation of higher hydroperoxides than O1 and control olive oils. Meanwhile, O1 showed greater oxidative stability, as indicated by the PV evolution, at the end of the storage period.

The K₂₃₂ allows the evaluation of the presence of primary oxidation products (conjugated diene). The results obtained for K₂₃₂ (Fig. 2b) showed slow increase rate initially; a fact that has been found by Tsimidou et al. (1995) where the native polyphenolic contents in olive oil could protect it against the initial stage of auto-oxidation. After 15 days of heating at 60 °C, there was a considerable increase with values higher than 2.5 for control, O1 and O2 olive oils. These observations indicated an accelerated oxidation process and confirmed the results obtained for PV evolution.

As expected, the secondary products of oxidation (carbonyl conjugated and conjugated triene compounds), evaluated through K₂₇₀, increased in all the samples during storage (Fig. 2c). In particular, K₂₇₀ increased from about 0.11 for the control to 0.16 for O2 to reach at the end of the storage time 0.63 and 0.68 for the control and O2, respectively. In general, a tendency to a higher oxidation was observed for O2 as indicated by the high PV, K₂₃₂ and K₂₇₀ values. Thus, the quality and the oxidative stability of the O2 oil, could be negatively affected with the addition of 15% *O. ficus-indica* flowers, since the incorporation of flowers at this amount would seem to have a pro-oxidant effect. The results are in agreement with those of Issaoui et al. (2011) who reported that lemon and thyme at high concentrations (80 g/kg of oil) weren't efficient to protect the olive oils from thermo-oxidative processes. Moreover, pro-oxidant action of product used at high concentrations

to enrich olive oil, was also previously reported by Sousa et al. (2015) and Gambacorta et al. (2007).

On the other hand, a clear effect of *O. ficus-indica* flowers on O1 olive oil upto 30 days of assay for PV and K₂₃₂ and 15 day of assay for K₂₇₀ when a significant differences ($p < 0.05$) between O1 and control sample was observed. As a result, it was considered that improvement in oxidative stability for O1 could be observed only in a longer examined period of incubation. This was in agreement with the study of Bouaziz et al. (2008) which proved that the improvement in stability of oils enriched with olive leaf extracts could not be revealed before 50 days of incubation at 50 °C. As a consequence, O1 showed a better oxidative stability. This suggests the efficacy of *O. ficus-indica* flowers in the amount of 5% which apparently acted as inhibitor of oxidation product to gave the best oxidative stability.

Chlorophyll, carotenoid and phenol contents

In addition to polyphenols, chlorophylls and carotenoids play an important role in olive oil stability. Chlorophyll content of control olive oil, O1 and O2 measured during thermal oxidation at 60 °C is presented in Fig. 3a. The concentration of chlorophyll, initially was 4.2, 5.6 and 5.9 mg/kg for control, O1 and O2, respectively. A noticeable degradation was observed for all olive oils and it was even more marked after 25 days of storage at 60 °C for the control (78%), followed by O1 (54%) and O2 (37%). These results were in agreement with those of Ayadi et al. (2009) who reported that the chlorophyll fraction concentration decreased continually during heating time.

The decrease of carotenoids amounts during storage at 60 °C was similar to that of chlorophylls, as shown in Fig. 3b. Thus, the concentration of carotenoids tended to decrease with heat treatment in a range of 2.05–1.2 mg/kg for the control oil (about 45%). Concerning the decrease of carotenoids of O1 and O2, it was less accentuated (about 32 and 36%) within 55 days of treatment at 60 °C. These results suggest that O1 and O2 samples were more stable against degradation of chlorophyll and carotenoid pigments.

The influence of heating on total phenolic content (TPC) was also assessed and the results obtained are represented in Table 3. TPC decreased in all samples during storage. The phenolic loss was of about 70% for the control olive oil, 65% for O1 and 53% for O2. The most prominent decrease was observed for control.

Even oil with 15% *O. ficus-indica* flowers had the higher stability for chlorophyll, carotenoid and total phenols; it is the lowest effective against thermal oxidation as observed in the PV, K₂₃₂ and K₂₇₀ value. This result show that these compounds were unable to fully protect the oils during the

Fig. 3 Change in carotenoids (a) and chlorophyll (b) contents control olive oil, O1 (olive oil with 5% *O. ficus-indica* flowers) and O2 (olive oil with 15% *O. ficus-indica* flowers) during heating at 60 °C

Table 3 Total phenols contents of control and macerated olive oils during storage at 60 °C for 60 days

Total phenols contents (mg/kg)	0	15 days	30 days	45 days	60 days
Control	254.27 ± 12.11 ^a	129.34 ± 2.65 ^a	104.28 ± 5.43 ^a	82.95 ± 2.94 ^a	75.55 ± 2.98 ^a
O1	258.5 ± 11.65 ^a	135.98 ± 4.93 ^b	124.78 ^b ± 5.36 ^b	97.72 ± 10.48 ^b	85.3 ± 5.6 ^b
O2	262.5 ± 16.2 ^b	141.85 ± 7.16 ^c	132.35 ± 12.3 ^c	124.46 ± 5.7 ^c	122 ± 7.3 ^c

Values given are the mean of three replicates ± standard deviation. Means with the same letter in the same colon are not significantly different at $p > 0.05$. O1: olive oil sample macerated with 5% of *Opuntia ficus-indica* flowers and O2 olive oil sample macerated with 15% of *Opuntia ficus-indica* flowers

heating at 60 °C, and they acted antagonistically as pro-oxidants to contribute in the thermal oxidative degradation. The pro-oxidant activity of pigments in the olive oil has been well documented by other works (Gutierrez et al. 1992; Malheiro et al. 2012).

Conclusion

The addition of *O. ficus-indica* flowers to the olive oil did not considerably change its quality characteristics. On the contrary, oil got enriched with polyphenols which were diffused from flowers. Physicochemical changes were attributed to the diffusion of polyphenol and pigment compounds from flowers to the olive oil during the maceration process.

After storage at 60 °C, the olive oils with and without *O. ficus-indica* flowers undergoes changes leading to a reduction in oxidative stability, chlorophylls, carotenoids and total phenols content. In a general way, the behavior shown by the oils is definitely dissimilar. The olive oils with 15% *O. ficus-indica* flowers did not succeed in inhibiting oxidation and showed of oil worsed than that of control, by increasing PV, K_{232} and K_{270} values which reflected an increase in both primary and secondary oxidation products. This indicated that the addition of 15% flowers to olive oil promoted the diffusion of compounds which apparently acted as pro-oxidant. On the contrary, a

significant resistance to oxidation was observed in olive oil by addition of 5% flowers, mainly after 15 and 30 days of storage at 60 °C. Thus, the incorporation of these flowers at this level might significantly contributed to the stabilization of the olive oils and add value to this product.

Further studies are needed to select best conditions for the maceration of *O. ficus-indica* flowers with olive oil in order to avoid the pro-oxidant effect and maximize its oxidative stability.

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