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Oxidative stability of olive oil enriched with oleaster leaves under accelerated storage conditions

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Summary

Olive oil is rich in natural antioxidants that conserve its quality under storage conditions. However, there is a growing need to improve the quality of olive oil under storage conditions using phenol-enriched olive oil. In the present study, olive oil from the Chemlali cultivar was enriched with wild olive tree leaves or oleaster. The oil composition was analyzed before and after accelerated storage conditions using a Schaal test. Standard oil parameters, including free acidity; peroxide value; iodine value; specific extinction K232 and K270; fatty acid profile, and polyphenolic, chlorophyll, and carotenoid content, were evaluated for the control olive oil (COO) and the enriched olive oil (EOO). Polyphenolic compounds were identified for COO, EOO, and oleaster leaf extracts (OLE) using high-performance liquid chromatography. Antioxidant activity was analyzed using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2' azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) tests. The results showed that enriching the olive oil quantitatively and qualitatively improved the polyphenolic composition, pigment contents, and the antioxidant activity. The EOO was more resistant to oxidation under accelerated storage conditions. The addition of wild olive leaves also significantly improved the resistance of the olive oil to oxidation and can, therefore, be used as a source of natural antioxidants to improve the oxidative stability of edible oils.

Key-words: olive oil, oleaster leaves, oxidative stability, polyphenolic compounds, fatty acids

Introduction

Olive oil is an important component of the Mediterranean diet and is known to provide protection against cardiovascular diseases and cancer due to its fatty acid profile and minor components, such as polyphenolic constituents (Gharby et al., 2011). Lipid oxidation is the most important cause of olive oil quality deterioration during storage. The primary lipid oxidation products are hydroperoxides. Hydroperoxides are highly unstable and react to form secondary products, such as hydrocarbons and alcohols, which are oxidized to carboxylic acids (Bešter et al., 2008). Recent studies have suggested that the polyphenolic compounds that are naturally present in virgin olive oil improve its resistance to oxidative deterioration (Frankel, 1998). Polyphenolic antioxidants interrupt the initiation and propagation stages of the oxidative chain reaction by reacting with lipid radicals to form more stable products (Gordon et al., 2001). The oxidation process can also be delayed using endogenous antioxidants that enhance oxidative stability; in addition, synthetic antioxidants can be used to prolong the stability of oils under storage. However, toxicologists and nutritionists have sought to ascertain the noxious effects associated with the use of certain synthetic antioxidants, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) (Barlow, 1990). In recent times, interest in sources of natural antioxidants that can be used to enrich oils to reduce lipid oxidation has increased. For example, olive oil has been enriched with polyphenolic compounds obtained from olive cake (Suárez et al., 2010). The addition of olive leaves prior to the extraction process has been shown to improve the organoleptic quality and stability of olive oil (Di Giovacchino et al., 1995). Olive leaves also provide a rich source of natural antioxidants (Salah et al., 2012; Hannachi et al., 2019), including a diversity of polyphenolic compounds that may act as an effective defense system against free radical attacks (Bouaziz et al., 2008).

The aim of this study is to study the evolution of the oxidation process of olive oil enriched with oleaster leaves under accelerated storage conditions. Standard olive oil parameters, including the free acidity (FA), peroxide value (PV), iodine value (IV), specific extinction K232 and K270, fatty acid profile, polyphenolic profile, and pigment contents, were evaluated for the control olive oil (COO) and the enriched olive oil (EOO) before and after accelerated storage conditions. Antioxidant activity was determined for both the COO and EOO using the ABTS and DPPH radical scavenging activity.

Materials and methods

Materials

Olive oil of the Chemlali cultivar (*Olea europaea* L. var. *europaea*) was obtained from oil mills located in the Limaoua area of the Gabes region in southern Tunisia and was kept in amber-colored glass bottles in the dark at an ambient temperature. The leaves of wild olive trees or oleasters (*Olea europaea* L. var. *sylvestris*) were collected from Tounine (a natural ecosystem) from Gabes in southern Tunisia. The collected oleaster leaves were washed, air dried at room temperature, powdered using a laboratory mill (type FW135), and stored in a dry and dark location.

Methods

Enrichment of olive oil by oleaster leaves

The enrichment procedure involved immersing the oleaster leaf powder in olive oil. The maceration process was conducted with 100 mL olive oil and 10 g powdered oleaster leaves under agitation at 25 °C for 24 h. After maceration, the samples were filtered and kept in glass bottles in the dark at an ambient temperature. Accelerated oxidation stability testing was conducted on the EOO and the COO to evaluate their resistance to oxidation.

Accelerated oxidation experiment

The effect of storage on the oxidative stability of the oils was conducted using the Schaal oven method as simplest accelerated test. The samples were stored in the dark at a temperature of 65 °C in closed glass bottles for 24 days (Yang et al., 2013).

The oxidative stability testing was conducted by evaluating standard olive oil parameters every three days. The FA, PV, IV, and specific extinction coefficients at 232 and 270 nm (K232 and K270) were

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determined according to the methods described in the commission of the European communities (CEC, 2003). All parameters were evaluated for the EOO and the COO.

Extraction of polyphenolic compounds

Extraction of polyphenolic compounds from olive oil

Oil samples of 7.5 g were mixed with 5 mL methanol/water (80:20 v/v) solution and vigorously homogenized with a vortex. The two phases were separated by centrifugation at 3800 rpm for 15 minutes, and the hydroalcoholic phase was collected. The centrifuge (Labofuge 200) was equipped with an integrated angle rotor (12 × 15 cm), and 3030 × g was used as the relative centrifugal force (sedimentation coefficient). The extraction was repeated with 5 mL methanol/water (80:20 v/v) solution three times. The hydroalcoholic extracts were then combined and conserved at 4 °C in the dark (SERVILI at al., 1999).

Extraction of polyphenolic compounds from oleaster leaves

The extraction process of the oleaster leaf powder was performed in an ultrasonic bath (CLEAN 120 HD) at 20 °C for 40 minutes. Powdered oleaster leaves (5 g) were sonicated in 50 mL methanol HPLC grade. The oleaster leaf extract was subsequently filtered and centrifuged at 3800 rpm for 20 minutes. The obtained extracts were kept at 4 °C in the dark for analysis.

Determination of total polyphenolic content

The total polyphenolic content was determined using the Folin-Ciocalteau method (BENAVENTE-GARCIA et al., 2000). A total of 0.5 mL Folin-Ciocalteau (Prolabo) reagent was added to 0.1 mL polyphenolic extract obtained from olive oil and the diluted oleaster leaf extract. After 5 minutes, 4 mL sodium carbonate solution (1 M) was added. After 90 minutes of incubation at room temperature in the dark, the absorbance was measured at 760 nm using a T60 UV-visible spectrophotometer. Gallic acid was used to produce a calibration curve. The total polyphenolic content was expressed as mg gallic acid equivalent per kilogram of oil (ppm) and as mg GAE/kg dried matter (ppm) for the olive oil and oleaster leaf extract, respectively.

HPLC analysis of polyphenolic compounds

Olive oil (20 µL) and oleaster leaf methanol extracts were injected into an HPLC unit Ajilent technologies 1200 series equipped with a reverse phase dC18 column (5 µm, 4.6 × 250 mm) and a UV detector at 280 nm and integrator. The flow rate was 0.9 mL/min using water/acetic acid (V/V) as solvent A and acetonitrile as solvent B at 0.9 mL/min. Elution proceeded for around 40 minutes. The peaks of the polyphenolic compounds were identified by matching the retention time and the UV spectra of the standard peaks (BENAVENTE-GARCIA et al., 2000). Quantification of the polyphenolic compounds was conducted by calibration curves relative to external standards. The levels of polyphenolic compounds detected were expressed as ppm.

Standard olive oil parameters

The FA, PV, K232, K270 and fatty acid composition were determined according to official methods described in the commission of the European communities (CEC, 2003). The IV and other parameters were based on international standards or reports in the literature.

FA was expressed as oleic acid percentage and was determined by acid-base titration with a 0.177 N sodium hydroxide solution of free fatty acids in the olive oil samples previously dissolved in neutralized ethanol using phenolphthalein as an indicator. The FA was calculated as follows:

$$\text{FA} = \frac{(V \times N \times M)}{m \times 10}$$

V: volume (mL) of potassium hydroxide solution used in titration
N: concentration (0.1775 mole/L) of potassium hydroxide solution used in titration
M: molar weight (g/mole) of oleic acid (282.47 g/mole)
m: weight in grams of the sample.

The PV was determined by dissolving one gram of the olive oil samples in 15 mL acetic acid and 10 mL chloroform, to which 1 mL potassium iodide solution was subsequently added. The obtained solution was kept in the dark for five minutes at 25 °C to react with the saturated potassium iodine solution. A total of 75 mL distilled water was added. The liberated iodine was titrated with a 0.01 N sodium thiosulfate ($Na_2S_2O_3$) standard solution using starch solution as an indicator. White titration (white test) was conducted under the same conditions. The PV was expressed as milliequivalents of active oxygen per kilogram of olive oil (meq O_2/kg oil):

$$IP\ (meqO_2 / Kg = \frac{(V - V_0) \times 1000 \times \text{T}}{W}$$

V: volume (mL) of standardized sodium thiosulphate solution used for sample titration
V_0: volume (mL) of standardized sodium thiosulphate solution used for the white titration
T: sodium thiosulfate ($Na_2S_2O_3$) normality
W: olive oil weight (g).

The specific extinction coefficients K232 and K270 at 232 and 270 nm were measured using a T60 UV-visible spectrophotometer for 0.1 g olive oil samples dissolved in 10 mL cyclohexane using a quartz cuvette with a path length of 1 cm.

$$\text{K}_\lambda = \frac{\text{E}_\lambda}{(\text{C} \times \text{S})}$$

Kλ: specific extinction at wavelength λ (232 and 270 nm)
Eλ: extinction measured at wavelength λ (232 and 270 nm)
C: concentration of olive oil solution (g/100 mL)
S: path length of the quartz cell (1 cm)

The IV was assessed according to the analytical method described by the Association of Official Analytical Chemists (AOAC, 1980) by titration with 0.1 N $Na_2S_2O_3$ of 0.3 g olive oil sample previously dissolved in 10 mL chloroform in the presence of the WIJS reactive and 10% KI. The IV was expressed as g iodine per 100 g olive oil (gI/100g):

$$IV\ (gI / 100\ \text{g}) = \frac{(V_0 - V) \times 1.27}{W}$$

V_0: volume (mL) of standardized sodium thiosulphate solution used for the white titration
V: volume (mL) of standardized sodium thiosulphate solution used for the sample titration
W: olive oil weight (g)

Pigment contents

Pigment contents were determined by dissolving 7.5 g olive oil samples in hexane (25 mL), and the absorbance at 470 and 670 nm was measured to determine the carotenoid and chlorophyll contents, respectively (MINGUEZ-MOSQUERA, 1991). The chlorophyll and carotenoid contents were expressed as mg of pheophytin "a" and lutein per kg of oil (ppm), respectively.

$$Chlorophylls\ (ppm) = \frac{(A_{670} \times 10^6)}{(613 \times 100 \times d)}$$

$$Carotenoids\ (ppm) = \frac{(A_{470} \times 10^6)}{(2000 \times 100 \times d)}$$

A_{670}: absorbance at 670 nm
A_{470}: absorbance at 470 nm
d: optical pathway (1 cm).

Fatty acid composition using GC/MS

Fatty acid composition was carried out following their transformation on fatty acid methyl-ester (FAMEs) after cold alkaline transesterification with a methanolic potassium hydroxide solution. FAMEs were prepared by dissolving 0.2 g olive oil in 3 mL of hexane and adding 0.4 mL 2 N methanolic potassium hydroxide solution. After vigorous shaking for 30 seconds, the solution was leaved to stratify until the upper solution became clear. The upper layer containing the methyl esters was subsequently decanted (CEC, 2003). The obtained FAMEs (1 µL) were analyzed on an Agilent HP series GC 6890 coupled with an HP 5973 MS detector fitted with a CPSIL-88 column (30 m × 0.25 mm i.d., film thickness 0.20 µm). Helium was used as a carrier gas at 1.2 mL/min. The oven temperature was held at 60 °C for 1 minute, increased to 220 °C at 10 °C/min for 10 minutes, then to 220 °C at 15 °C/min for 15 minutes. The injector and flame ionization detector temperatures were maintained at 250 °C. The methyl ester peaks of the samples were identification via a comparison with those of the methyl esters reference mixtures.

Antioxidant activity

DPPH radical scavenging activity

A total of 20 µL olive oil extract was mixed with 200 µL 2,2-diphenyl-1-picrylhydrazyl (DPPH) methanolic solution (0.2 mM). The mixture was shaken and left for 30 minutes in the dark at 25 °C. The absorbance of the solution was measured at 517 nm to determine the concentration of the remaining DPPH.

The radical scavenging activity on DPPH radicals was determined by the following formula:

$$\text{Absorbance reduction at 517 nm} = \frac{Abs_{t0} - Abs_{ti}}{Abs_{t0}} \times 100$$

With $Abs_{t0:}$ control absorbance in presence of DPPH and Abs_{ti}: absorbance measured in the presence of a related concentration of Trolox with DPPH.

The curve absorbance reduction at a 517 nm function of the Trolox concentration (mM) was used to determine the DPPH radical scavenging activity of the samples. The radical scavenging activity was expressed as mg Trolox equivalent antioxidant capacity per kilogram of oil (mg TEAC/kg oil) for the olive oil extract and as mg TEAC/kg dry weight for the oleaster leaf extract.

$ABTS^+$ radical scavenging activity

The $ABTS^{\cdot+}$ radical was generated by mixing 7 mM 2,20-azinobis-3-ethylbenzothiazoline-6-sulphonate ABTS solution with 2.45 mM potassium persulfate ($K_2S_2O_8$). The mixture was kept in the dark at room temperature for 16 h before use. Following this, the $ABTS^{\cdot+}$ solution was diluted with ethanol to produce an absorbance of 0.700 ± 0.02 at 734 nm. A quantity of 20 µl extract was added to 180 µL ABTS+ solution. The absorbance was measured at 734 nm after 5 minutes of incubation (Djeridiane et al., 2006).

The radical scavenging activity on the ABTS radical was determined by the following formula:

$$\text{Absorbance reduction at 734 nm} = \frac{Abs_{t0} - Abs_{ti}}{Abs_{t0}} \times 100$$

With $Abs_{t0:}$ control absorbance in presence of ABTS and Abs_{ti}: absorbance measured in the presence of a related concentration of Trolox with ABTS.

The curve absorbance reduction at a 734 nm function of the Trolox concentration (mM) was used to determine the ABTS radical scavenging activity of the samples. The radical scavenging activity was expressed as mg Trolox equivalent antioxidant capacity per kilogram of oil (mg TEAC/kg oil) for the olive oil extract and as mg TEAC/kg dry weight for the oleaster leaf extract.

Statistical analysis

All experiments were performed in triplicate on three independent samples, and the results were presented as mean ± standard deviation. An analysis of variance (ANOVA) and a Duncan's multiple range test were performed using Xlstat software (www.xlstat.com) to evaluate the significance of the difference between the control and enriched olive oil at $p < 0.05$. The freely available Heatmapper web server (http://www.heatmapper.ca; Babicki et al., 2016) was used to generate a heat map to interactively visualize the data (i.e., the polyphenolic compounds, antioxidant activity, fatty acids, standard olive oil parameters) of the COO and EOO.

Results

Composition of EOO

The values of the studied parameters of the COO and EOO are provided in Tab. 1 and 2. The quality parameters were in accordance with extra virgin olive oil quality standards established by the International Olive Council (IOC, 2019). The FA, PV, specific extinctions, and IV did not change significantly after enrichment of the olive oil with oleaster leaves. The enrichment process did not change the fatty acid composition, which showed a high percentage of oleic acid (75.91 for COO and 77.28 for EOO). However, the enrichment process improved the pigment and polyphenolic contents, and the antioxidant capacity differed significantly between the COO and EOO. The oleaster leaves increased the level of chlorophyll from 1.35 ± 0.04 ppm in the COO to 7.37 ± 0.12 ppm in the EOO. The carotenoid content showed an average of 1.75 ± 0.03 ppm in the EOO and 0.58 ± 0.02 ppm in the COO. The total polyphenolic content was 123.80 ± 3.03 ppm in the COO and 441.61 ± 5.28 ppm in the EOO. Therefore, the process of enriching the olive oil with oleaster leaves improved the antioxidant activity of the EOO. The DDPH test showed that the antioxidant activity increased from 76.53 (COO) to 500.00 (EOO) mg TEAC/kg oil (EOO). The ABTS test showed that the antioxidant capacity of the EOO (928.88 mg TEAC/kg oil) improved compared with the COO (160.00 mg TEAC/kg oil; see Tab. 2).

Tab. 3 provides a list of the main phenolic acids and flavonoid compounds found in the EOO, COO, and OLE. Luteolin-7-o-glucoside (2979 ± 18.66 ppm) was the major compound in the OLE, followed by rutin (2684.05 ± 21.89) and quercetin3-o-rhamonoside (1541 ± 39.18 ppm). Meanwhile, acacetin, apigenin, p-coumaric acid, naringenin, and kaempferol were the major compounds in the COO. The enrichment process increased the levels of polyphenolic compounds detected in the EOO. For example, the level of rutin in the COO was 0.31 ± 0.03 ppm, while 21.78 ± 0.57 ppm was recorded in the EOO. The level of p-coumaric acid increased from 0.28 ± 0.03 (COO) to 0.89 ± 0.02 ppm (EOO). 4-O-caffeoylquinic and caffeic acids were only detected in OLE and EOO. Based on the polyphenolic compounds and antioxidant activities visualized by the heatmap (see Fig. 1), the EOO was distinguished by its polyphenolic richness and high antioxidant activity compared with the COO.

Tab. 1: Standard quality indices and fatty acid composition of studied olive oils.

	COO	EOO	Norma of extra virgin olive oil (IOC, 2019)
FA	1.20 ± 0.15 [a]	1.22 ± 0.17 [a]	< 2.0
PV (meq O_2/kg)	6.98 ± 1.20 [a]	7.01 ± 1.17 [a]	< 20
IV (gI/100g)	88.87 ± 3.12 [a]	87.56 ± 3.21 [a]	-
K232	2.49 ± 0.52 [a]	2.47 ± 0.90 [a]	< 2.50
K270	0.20 ± 0.05 [a]	0.23 ± 0.09 [a]	< 0.22
C16:0 (%)	11.44 ± 1.92 [a]	10.24 ± 1.00 [a]	7.5 - 20.00
C16:1(%)	0.90 ± 0.08 [a]	1.08 ± 0.05 [a]	0.30 - 3.50
C18:0 (%)	2.61 ± 0.05 [a]	2.97 ± 0.04 [a]	0.5 - 5.00
C18:1 (%)	75.91 ± 5.76 [a]	77.28 ± 4.59 [a]	55.00 - 83.00
C18:2 (%)	7.86 ± 1.12 [a]	6.71 ± 1.10 [a]	2.50 - 21.00
C20:0 (%)	0.27 ± 0.02 [a]	0.25 ± 0.02 [a]	≤ 0.60
C20:1 (%)	0.23 ± 0.06 [a]	0.20 ± 0.05 [a]	≤ 0.40

Each value is the mean ± standard deviation; FA: Free Acidity; PV: Peroxide Value; IV: Iodine Value; K232: specific extinction at 232nm; K270: specific extinction at 270 nm; COO: control olive oil; EOO: enriched olive oil with oleaster leaves; Means in the same line with different letters differ significantly ($p < 0.05$).

Tab. 2: Pigments, polyphenols, and antioxidant activity of oleaster leaves and studied olive oils extracts.

	OLE	COO	EOO
Chlorophylls (ppm)	8.76 ± 0.74	1.35 ± 0.04 [b]	7.37 ± 0.12 [a]
Carotenoids (ppm)	2.46 ± 0.62	0.58 ± 0.02 [b]	1.75 ± 0.03 [a]
Polyphenols (ppm)	689.80 ± 30.75	123.80 ± 3.03 [b]	441.61 ± 5.28 [a]
DPPH (mg TEAC/kg)	781.18 ± 2.71	76.53 ± 2.71 [b]	500.00 ± 9.54 [a]
ABTS (mg TEAC/kg)	1160.43 ± 4.63	160.00 ± 4.63 [b]	928.88 ± 11.03 [a]

Each value is the mean ± Standard deviation; TEAC: trolox equivalent antioxidant capacity; COO: control olive oil; EOO: enriched olive oil with oleaster leaves; OLE: oleaster leaves extract. mg TEAC/kg dry weight for OLE and mg TEAC/kg oil for COO and EOO; Means in the same line of studied olive oils (COO and EOO) with different letters differ significantly ($p < 0.05$).

Tab. 3: Polyphenolic compounds of olive oils and oleaster leaf extracts.

Polyphenolic compounds	**Brut formula**	**[M-H] - m/z**	**RT (min)**	**OLE**	**COO**	**EOO**
Quinic acid	$C_7H_{12}O_6$	191	2.032	645.43 ± 10.87	0.2 ± 0.01 [b]	7.00 ± 0.05 [a]
4-O-caffeoylquinic acid	$C_{16}H_{18}O_9$	353	12.483	29.32 ± 0.94	nd	0.30 ± 0.00
Chlorogenic acid	$C_{16}H_{18}O_9$	353	9.779	27.68 ± 0.14	nd	nd
Caffeic acid	$C_9H_8O_4$	197	14.445	4.65 ± 1.81	nd	0.10 ± 0.00
4,5-di-O-caffeoylquinic acid	$C_{25}H_{24}O_{12}$	515	26.839	142.38 ± 1.54	0.06 ± 0.00 [b]	1.34 ± 0.01 [a]
p-coumaric acid	$C_9H_8O_3$	163	20.873	25.29 ± 0.97	0.28 ± 0.03 [b]	0.89 ± 0.02 [a]
Rutin	$C_{27}H_{30}O_{16}$	609	23.804	2684.05 ± 21.89	0.31 ± 0.00 [b]	21.78 ± 0.57 [a]
Luteolin-7-o-glucoside	$C_{21}H_{20}O_{11}$	447	24.449	2979.38 ± 18.66	0.50 ± 0.02 [b]	23.93 ± 0.2 [a]
Quercetrin (quercetin-3-o-rhamnoside)	$C_{21}H_{20}O_{11}$	447	26.811	1541.16 ± 39.18	0.26 ± 0.00 [b]	12.46 ± 0.14 [a]
Naringin	$C_{27}H_{32}O_{14}$	579	25.849	95.86 ± 1.44	0.02 ± 0.00 [b]	0.76 ± 0.01 [a]
Apegenin-7-o-glucoside	$C_{15}H_{10}O_5$	431	26.733	123.29 ± 0.57	0.04 ± 0.00 [b]	1.08 ± 0.01 [a]
Quercetin (quercetin-3-o-rhamonoside)	$C_{15}H_{10}O_7$	447	26.802	153.7 ± 0.24	0.07 ± 0.00 [b]	1.23 ± 0.01 [a]
Kaempferol	$C_{15}H_{10}O_6$	285	31.768	55.85 ± 0.31	0.32 ± 0.00 [b]	0.79 ± 0.01 [a]
Naringenin	$C_{15}H_{12}O_5$	271	33.777	2.79 ± 0.03	0.12 ± 0.00 [a]	0.15 ± 0.00 [a]
Apigenin	$C_{15}H_{10}O_5$	269	34.353	6.55 ± 0.07	0.24 ± 0.00 [a]	0.29 ± 0.01 [a]
Acacetin	$C_{16}H_{12}O_5$	283	40.087	nd	0.01 ± 0.00 [a]	0.01 ± 0.00 [a]

Each value is the mean ± standard deviation; COO: control olive oil; EOO: enriched olive oil with oleaster leaves; OLE: oleaster leaves extract; nd: not determined. Means in the same line of studied olive oils (COO and EOO) with different letters differ significantly ($p < 0.05$).

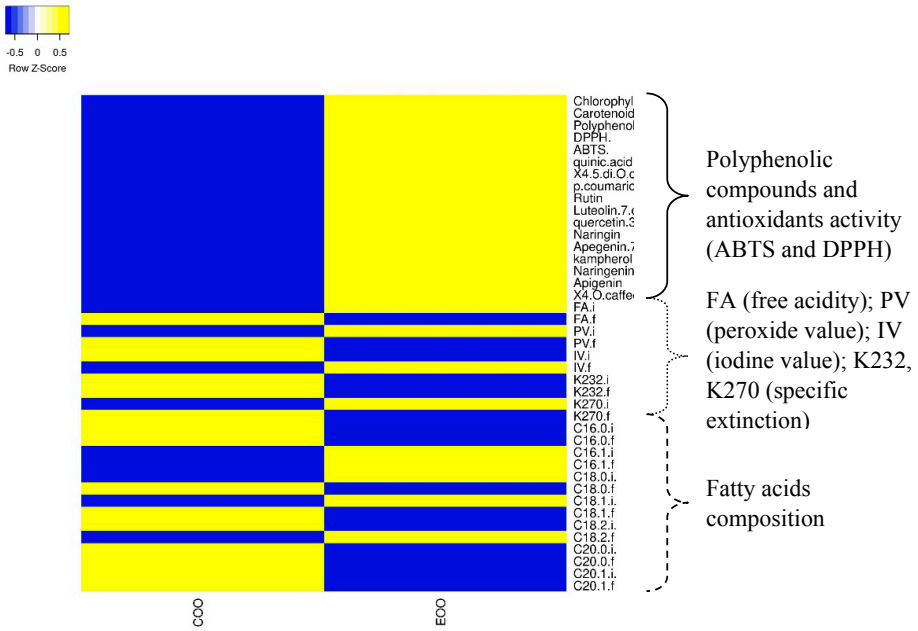

Fig. 1: Heatmap of olive oil parameters, polyphenolic compounds profile, and antioxidants activities (DPPH and ABTS) of the COO and EOO. The mean values refer to colors from the minimum displayed in blue to the maximum represented with yellow (i: initial value before accelerate storage condition; f: final value after accelerate storage condition).

Olive oil criteria under accelerated storage conditions

Standard oil parameters

The FA increased from 1.20 ± 0.15 to 2.81 ± 0.20 for the COO and to 2.40 ± 02.0 for the EOO. Therefore, the enrichment of olive oil with oleaster leaves appeared to improve its oxidative stability (see Fig. 2a).

The initial PV value was 6.98 ± 1.20 and 7.01 ± 1.17 meq O2/kg for the COO and EOO, respectively. After 24 days under accelerated storage conditions, the PV of the COO and EOO increased to 29.17 ± 2.15 and 21.74 ± 2.48 meq O2/kg, respectively. During the first six days, the peroxide values showed a slight increase. After 15 days under accelerated storage conditions, a substantial increase in the PV to a level of 11.98 ± 2.18 meq (O2)/kg in the EOO and 19.94 ± 2.11 meq (O2)/kg COO was noted (see Fig. 2b).

Under accelerated storage conditions, the IV decreased for the both olive oils. After 12 days, there was a clear difference in the IV of the COO (71.30 ± 3.23 gI/100 g) and the EOO (77.30 ± 2.17 gI/100 g). A lower value indicates a lower degree of unsaturation, which suggested that the double bonds in the EOO were more protected against autoxidation during accelerated storage conditions than the COO (see Fig. 2c).

Specific extinctions provided information on the oil quality under accelerated storage conditions. The K232 and K270 of the both the COO and EOO increased. The increase of K232 of COO and EOO was greater after nine days (see Fig. 3a). However, the increase in the K270 values was greater after 12 days (see Fig. 3b) for the both oils. A heatmap analysis showed that after accelerate storage conditions, the EOO was characterized by a lower FA and PV and high IV, suggesting that the EOO was more resistant to oxidation (see Fig. 1).

Pigments contents

The enrichment process increased the pigment contents (see Fig. 1, Tab. 2). Under accelerated storage conditions, the pigments contents decreased in the COO and EOO. After 24 days of accelerated storage condition, the EOO was richer in chlorophylls (3.10 ± 0.51 ppm) and carotenoids (0.26 ± 0.09 ppm) than the COO, with an amount of 0.24 ± 0.04 and 0.08 ± 0.01 ppm detected for chlorophylls and carotenoids, respectively (see Fig. 4).

Fatty acids composition

The results showed that, under accelerated storage conditions, a slight modification was noted in the fatty acid profiles of the COO and EOO (see Tab. 4). The reduction in the linoleic acid content was higher compared with the other fatty acids in both the EOO and COO. After 24 days under accelerated storage conditions, the decrease in linoleic acid in the COO was higher compared with the EOO. The EOO had a higher content of active antioxidant compounds, which protect unsaturated fatty acids against deterioration under accelerated storage conditions (see Fig. 1).

Discussion

The OLE demonstrated a higher polyphenolic content (689.80 ± 30.75 ppm) than the COO (123.80 ± 3.03 ppm), which agrees with the results of previous studies (Dabbou et al., 2011; Djenane et al., 2019; Hannachi et al., 2019, 2020). The variation in the polyphenolic content can be explained by factors such as climatic conditions, extraction method, and genotype. The level of polyphenols has been shown to positively correlate with biotic and abiotic stress in plants (Boscaiu et al., 2010). The oleaster trees utilized in the present study originated from a natural ecosystem and did not benefit from the same cultivation techniques as cultivated olive trees. As such, the oleaster trees had higher exposure to environmental stress than cultivated olive trees (Hannachi et al., 2008).

The use of synthetic food additives, such as BHT and BHA, has decreased because of their potential risks to human health demonstrated in many experimental animals (Farag et al., 2006). Therefore, identifying natural sources of antioxidants that can substitute for these synthetic compounds is of great interest. Olive leaves represent a potential source of natural antioxidants. In recent times, olive leaves have been used as additives to olives (2-3%) before processing to produce oils with an improved flavor and higher resistance to oxi-

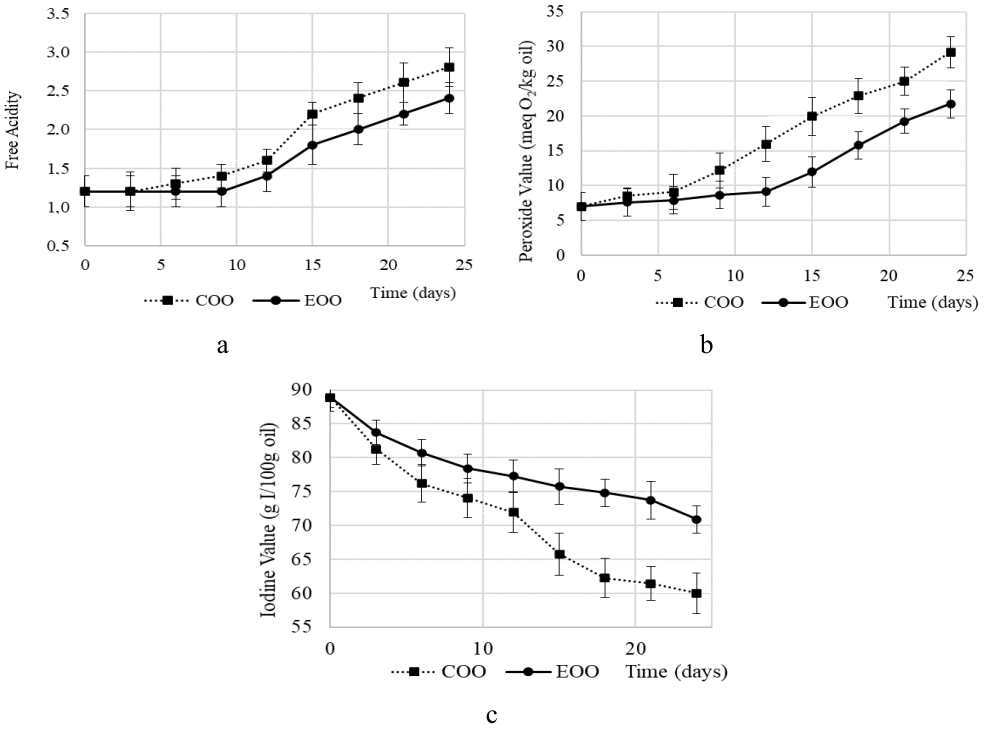

Fig. 2: FA (a), PV (b) and IV (c) of the COO and EOO under accelerated storage.

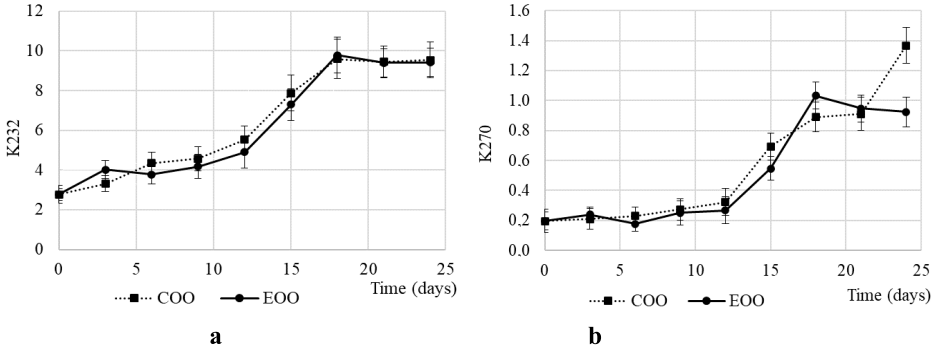

Fig. 3: Specific extinctions K232 (a) et K270 (b) of the COO and EOO under accelerated storage.

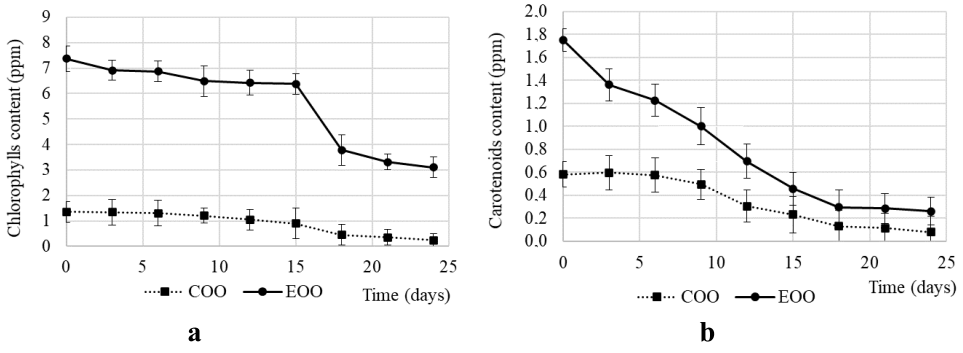

Fig. 4: Chlorophylls (a) and carotenoids (b) contents of the COO and EOO under accelerated storage.

dation (RANALLI et al., 2006). It has been reported that the addition of olive leaves during the olive oil extraction process can improve the quality of the oil (MALHEIRO et al., 2013; HANNACHI and ELFALLEH, 2020). Polyphenols extracted from olive leaves have been used to increase the nutritional value of table olives (LALAS et al., 2011). In addition, the enrichment of various edible vegetable oils with olive leaf extracts has been found to improve their oxidative stability (BOUAZIZ et al., 2008). Olive leaf extracts exhibit high antioxidant activity re-

Tab. 4: Fatty acid composition of the COO and EOO before and after accelerated storage.

	COO		EOO	
	Initial	Final	Initial	Final
	accelerated storage			
C16:0	11.44 ± 1.92 [a]	12.35 ± 0.94 [a]	10.24 ± 1.00 [a]	10.85 ± 0.57 [a]
C16:1	0.90 ± 0.08 [a]	0.99 ± 0.01 [a]	1.08 ± 0.05 [a]	1.03 ± 0.01 [a]
C18:0	2.61 ± 0.05 [a]	4.20 ± 1.39 [b]	2.97 ± 0.04 [a]	3.01 ± 0.43 [b]
C18:1	75.91 ± 5.76 [a]	73.62 ± 0.77 [a]	77.28 ± 4.59 [a]	73.06 ± 2.81 [a]
C18:2	7.86 ± 1.12 [a]	1.43 ± 0.11 [b]	6.71 ± 1.10 [a]	3.37 ± 0.17 [b]
C20:0	0.27 ± 0.02 [a]	0.36 ± 0.03 [b]	0.25 ± 0.02 [a]	0.29 ± 0.05 [b]
C20:1	0.23 ± 0.06 [a]	0.24 ± 0.06 [a]	0.20 ± 0.05 [a]	0.18 ± 0.01 [a]

Each value is the mean ± standard deviation; initial: before accelerated storage condition; final: after accelerated storage condition; Means in the same line of studied olive oils (COO and EOO) with different letters differ significantly ($p < 0.05$).

lating to the synergism of their compounds and may be more suitable as a functional food ingredient than olive fruit extracts (Hannachi et al., 2020). The number and location of the hydroxyl group in the polyphenolic compounds influences their antioxidant activity against various radicals (Xie et al., 2015).

The present study noted an improvement in the oxidative stability of olive oil enriched with oleaster leaves. This enrichment quantitatively and qualitatively improved the polyphenolic composition, which explained the improvement in the oxidative stability of the EOO under accelerated storage conditions. It has been reported that polyphenolic compounds are responsible for approximately 50% of the stability of olive oil (Gutiérrez et al., 2001) and show higher antioxidant activity than tocopherols in oils when exposed to high temperatures (Jiménez et al., 2017). Polyphenols show antioxidant potential through their ability to donate hydrogen atoms to lipid radicals, thereby producing lipid derivatives and antioxidant radicals (Shahidi and Ambigaipalan, 2015). Paiva-Martins (2007) reported a better score in taste quality after enriching oil with a polyphenol leaf extract and found insignificant differences between the flavor of oil before and after enrichment. Elsewhere, the potential bioaccesibility of the polyphenol components of enriched oils compared with a control has been demonstrated based on an *in vitro* gastrointestinal digestion model (gastric and duodenal steps; Suárez et al., 2010). Various other studies have focused on the enrichment of edible oils with olive leaves, such olive, palm, and sunflower oils (Bouaziz et al., 2008; Japon-Lujan et al., 2008; Hannachi and Elfalleh, 2020). Oleaster leaves have been used as a food supplement added to raw minced beef as a means of enhancing safety and prolonging shelf life (Djenane et al., 2019).

The results of the oil standard parameters, such the PV, which measures the level of hydroperoxide products formed in the initial stages of lipid oxidation and is an indicator of the primary oxidation state of oils, showed that the EOO was more stable against lipid oxidation. The PV is one of the most frequently evaluated quality parameters during olive oil production, storage, and marketing (Nouros et al., 1999). The absorptions at 232 and 270 nm are of special importance and provide information on the oxidative stability of virgin olive oil (CEC, 2003). A conjugated dienic system constitutes around 90% of the hydroperoxides formed by lipoperoxidation that are absorbed in the UV range (232 nm). A conjugated triene system absorbed in the UV range of 270 nm reflects the extent of oxidation (Laguerre et al., 2007). The increase of absorption at 232 nm and 270 nm due to the formation of conjugated dienes and trienes is proportional to the uptake of oxygen and the formation of peroxides during the early stages of oxidation. It has been shown that the polyphenolic compounds of hydrolysate olive leaf extract decreased the formation of lipid hydroperoxides and conjugated dienes and trienes formation in refined oils during pan-frying (Bouaziz et al., 2008), confirming the results of the present study, which found that the improvement of the EOO could be explained by its higher content of active antioxidant compounds afforded by the enrichment process. Hence, the enrichment of olive oil with oleaster leaves may retard the formation of oxidation products. These results agree with the results noted by Aydeniz and Yilmaz (2012), who observed the effect of polyphenolic compounds on the acidity of frying oils.

The chlorophyll and carotenoid content of olive oil interfere with its oxidative stability and can act as antioxidants in the dark and as prooxidants in light (Gutierrez et al., 1992). In the present study, enrichment increased the pigment content in EOO, which explained the improvement in oxidative stability. All samples showed reduced chlorophyll contents at the end of storage, which indicated that the compounds acted as antioxidants, as the accelerated storage condition test was conducted in the dark. Chlorophylls and carotenoids provide a more intense green pigmentation and confer a higher nutritional quality (Malheiro et al., 2013).

A significant decrease in linoleic acid C18:2 of both the COO and EOO from 7.86 ± 1.12 to 1.43 ± 0.11 (COO) and 3.37 ± 0.17% (EOO) was noted in the present study. Linoleic and palmitic acids are usually used as indicators of the extent of fat deterioration because linoleic acid is more susceptible to oxidation than palmitic acid, which is more stable (De Marco et al., 2007). Unsaturated fatty acids are more susceptible to oxidation and are the major cause of the deterioration of lipids (Shahidi and Zhong, 2010).

Conclusion

Olive oil enriched with oleaster leaves showed improved polyphenolic and chlorophyll contents, which contributed to the increase in radical scavenging activity. The application of accelerated storage conditions based on a Schaal test affected the quality of both the COO and EOO, though the COO was more affected than the EOO. Standard oil parameters and fatty acid composition showed that the olive oil enriched with oleaster leaves was more resistant to oxidation. The oleaster leaves protected the oil against oxidative deterioration and, therefore, constitute a natural source of antioxidants that can be used to improve the shelf life of foods.

Conflict of interest

No potential conflict of interest was reported by the authors.

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