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Enhanced Stability of Refined Soybean Oil Enriched with Phenolic Compounds of Olive Leaves

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SOYBEAN oil is a common vegetable oil, which is used for edible purposes. However, this oil is rich in unsaturated fatty acids that enhance oil deterioration by their oxidation. Such deterioration can be reduced using suitable antioxidants, preferably natural antioxidants. This work was made to assess the suitability of phenolic compounds that can be extracted from olive leaves to improve soybean oil stability against oxidation. Olive leaves extracts were tested for their content of phenolic compounds and have been then added to soybean oil which was subjected to accelerated oxidation. The oxidation stability was monitored following several technical methods commonly used for this purposes such as peroxide value, *p*-Anisidine value, as well as induction time by Rancimat method. The results have proved that the extracts of olive leaves can be used efficiently as a safe natural antioxidant in edible oils. However, the dose to be added should be adjusted as to achieve the activity of synthetic antioxidants.

Keywords: Olive leaves extract, Bioactive compounds, Antioxidant activity, Refined Soybean oil, Oxidation stability

Introduction

Vegetable oil is one of the major components of our food. Examples of vegetable oils are those extracted from oil seeds such as sunflower, sesame and cottonseed while some others are extracted from beans such as peanut and soybean. Soybean production in the world represents more than 50 % of total production of all oilseeds [1]. Linoleic fatty acids are the major fatty acid in soybean oil, which ranges from 38 to 60 %, followed by oleic acid in range of 20 to 50 % [1]. This fatty acid composition makes soybean oil very susceptible to oxidation [2].

Oil oxidation not only produces unpleasant flavor and rancid odors, but also reduces essential fatty acid contents and produces toxic compounds. These effects greatly reduce the quality and safety of the oil for edible purposes. Moreover, free radicals produced via the oxidation reaction chain causes ageing, mutagenesis, carcinogenesis and heart diseases. Hence, the addition of antioxidants as radical scavengers to edible oils seems to be essential. Much research work has been recently devoted to replace synthetic antioxidants by natural antioxidants to avoid possible adverse effects of synthetic ones on human health [3-6].

Olive leaves extracts have been proved to be a good source of natural antioxidants [7]. Such extracts have been already used with olive, sunflower and palm oils [8-10].

This work was proposed to assess the potential of extracts of olive leaves as antioxidants in soybean oil in place of synthetic antioxidants.

Material and Methods

Materials

Fresh green olive leaves were collected by hand from olive trees in gardens of Tarom, Iran.

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Refined, bleached and deodorized (RBD) soybean oil (SBO) was donated by Ardabil Oil Factory (Ardabil, Iran). All chemicals and reagents were purchased from Merck (Darmstadt, Germany) and TBHQ powder was obtained from Sigma Chemical Co. (St. Louis, MO, USA).

Methods

The experimental work was devoted to prepare the olive leaves extract and identify its contents of phenolic compounds qualitatively and quantitatively and to assess the olive leaves extract (OLE) as antioxidants.

Preparation of the Extract

Olive leaves were dried in a ventilated oven (Memmert GmbH Co., Büchenbach, Germany) at 40 ºC to reach a moisture content about 1 %. Then, the dried leaves were ground to pass through sieves (1 mm opening). Hundred g of powder was extracted immediately using methanol/water at ratio 80:20, respectively, for 12 h under agitation in dark place at room temperature. Methanol was selected for the extraction of phenolic compounds in according to the recommendations of other researchers in this field who proved that it is the most suitable one [11, 12]. The solution was then filtered through Whatman 0.45 µ filter paper, and the solvent was recovered in a rotary evaporator (Heidolf, Schwabach, Germany) at a temperature of 35 °C. The concentrated extract (CE) was stored in a dark brown glass vial at -20 °C for further analysis [13].

Chromatographic identification of phenolic compounds by HPLC

An Agilent 1200 HPLC system (Agilent Technologies, Waldbronn, Germany) equipped with a binary pump, vacuum degasser, auto-sampler, and a UV–VIS detector was used for the chromatographic determination. By means of an Agilent Eclipse Plus C18 column (4.6 × 150mm, 1.8 µm particle size) protected by a guard cartridge of the same packing, operating at 25 °C and a flow rate of 0.8 mL min^{-1}, phenolic compounds were separated individually. The mobile phases used, as elution solvents, were water with acetic acid (0.5 %) (Phase A) and acetonitrile (Phase B). The solvent gradient was adjusted according to the linear gradient described by Abaza *et al.* [14]; the samples (10 µL) were injected. The HPLC system was coupled to a Bruker Daltonik micro TOF mass spectrometer (Bruker Daltonik, Bremen, Germany) equipped with an ESI interface. Parameters for analysis were set using negative ion mode with spectra acquired over a mass range from m/z 100–2500. The optimum values of the ESI/TOF/MS parameters were: capillary voltage, +4.5 kV; drying gas temperature, 190 °C; drying gas flow, 9 L min^{-1}; and nebulizing gas pressure, two bar.

Total phenolic content (TPC)

Total phenol content of olive leaves extract was determined by using the Folin-Ciocalteu method with a modification of Zahran *et al.* [15]. The extract was dissolved in dimethyl sulfoxide in a ratio of 1 g extract in 20 mL Dimethyl sulfoxide. Olive leaf extract (500 µL) and/or standard (gallic acid) solutions were mixed with 2.5 mL Folin-Ciocalteu reagent (1:10 dilution with deionized water) and left to stand 3 min at room temperature and then 2 mL of sodium carbonate solution (7.5 % in deionized water) were added. After incubation for 1 h at room temperature in a dark place, the absorbance was measured at 725 nm by UV spectrophotometer (Shimadzu UV-2550; Shimadzu, Kyoto, Japan). Results were expressed as mg of gallic acid equivalents (GAE) per g of extract. The extracted phenolic compounds were then assessed as antioxidants.

Antioxidant activity by DPPH

Antioxidant activities of the extracts as well as standard sample of ascorbic acid were evaluated through using 1,1-diphenyl-2-picrylhydrazyl ($DPPH^{\cdot}$) radical assay as reported by Zahran and Soliman [16]. Two mL of 0.1 mM $DPPH^{\cdot}$ of the methanolic solution was added into 20, 40, 60, 80 and 100 µg of the extracts and then 1 mL methanol was added. The mixture was thoroughly mixed and kept in a dark place for 30 min. The control was prepared by mixing 1.5 mL of $DPPH^{\cdot}$ and 1mL methanol. Ascorbic acid was considered as standard. The absorbance of the mixture was recorded at 517 nm using spectrophotometer (Shimadzu UV-2550; Shimadzu, Kyoto, Japan) and percentage inhibition was calculated from the following equation:

$$\%DPPH^{\cdot} = [(Abs._{control} - Abs._{sample}) / Abs._{control}] \times 100 \quad (1)$$

The antioxidant activity of the extracts expressed as IC_{50} values, which is calculated from the inhibition percent versus concentration plot. The IC_{50} value indicates concentration (in µg mL^{-1}) of the extract, which is required to scavenge 50 % of $DPPH^{\cdot}$ free radicals.

Addition of OLE to refined SBO

The OLE was dissolved in an appropriate volume of 1,2-propanediol (% 20 w/w) and was then added into the refined SBO at different concentrations (w/w), followed by sonication (4 cycles) at a power between 50 % and 60 % for 2 min., using Ultrasonic Probe Sonicator (Athena Technology, Mumbai, India) to dissolve the OLE in oil. The oil samples were then examined for their oxidative stability using Rancimat method.

Oxidative stability (Rancimat method)

The Rancimat test was performed to determine the best concentration of the extract for increasing soybean oil stability. The oil samples were prepared by the addition of the extracts at levels of 200, 500 and 1000 ppm (dried extract). Other two oil samples have been similarly tested including a sample of SBO as control and another sample of the same oil containing TBHQ (200 ppm). The oil samples were heated in the Rancimat equipment 743 (Metrohm, Herisau, Switzerland) at 110 ºC with an airflow rate of 20 L h^{-1} passing through the samples. The time from the beginning until oxidation of oil (water conductivity rise, caused by the adsorption of volatiles derived from oil oxidation), was recorded as the induction time in hours [8].

Accelerated oxidation test (Schaal Oven test)

Accelerated oxidation test was made using four oil samples, 200 g each. These four oil samples included SBO as control, and SBO to which TBHQ was added at 200 ppm [17], while the other two samples were oils to which 1000 and 1500 ppm of dried OLE were added. All of the oil samples were kept at 60 ºC for 21 days. Samples were withdrawn from each of the four samples every seven days. The withdrawn samples were tested for peroxide value (PV) and *p*-Anisidine value (*p*-AV) [18, 19].

Fatty acid composition

Fatty acid composition of the oil was determined following the modified method of Zahran and Tawfeuk [20]. One mL of *n*-hexane was added to 15 mg of oil samples and vortexed for 30 sec. followed by 1 mL of sodium methoxide (0.4 mol). The mixtures were vortexed for 30 seconds and were allowed to settle for 15 minutes. The upper phase contained the fatty acids methyl esters (FAMEs) was recovered and analyzed by gas chromatography (GC-FID). The FAMEs were separated with an HP 6890 plus gas chromatography Hewlett Packard, Alexsandria, Mason, VA, USA), using a capillary column Supelco™ SP- 2380 capillary column (30 m×0.25 mm×0.20 µm) (Sigma-Aldrich, St. Louis, MO, USA), 30 m length, diameter 0.25 mm and film thickness 0.25 µm. Detector (FID) and the injection temperature was 260 °C. The column temperature was 50 °C (3 min) to 225 °C (17.5 min) at 10 °C/min. and hold at 225 °C (10 min). The carrier gas was helium at flow rate 1.2 mL min^{-1}. FAMEs were identified by comparing their relative and absolute retention times to those of authentic standards of FAMEs (from C4:0 to C24:0). A built-in data-handling program provided by the manufacturer of the gas chromatograph (HP) did all of the quantifications. The fatty acid composition was reported as a relative percentage of the total peak area.

Oil degradation percent (ODP)

Changes in the content of fatty acids in the samples, defined as a loss of $C_{18:2}$ and $C_{18:3}$ ($L_{18:x}$) in relation to the initial content of these fatty acids in control oils, were calculated according to the equation developed in a previous work by Aniołowska *et al.* [21] as follow:

$$L_{18:x} = [(C_{18:x})_0 - (C_{18:x})_t / (C_{18:x})_0] * 100 \quad (2)$$

Where: $C_{18:x} = C_{18:2}$ and $C_{18:3\ or}$ ($C_{18:2} + C_{18:3}$); 0, initial oil and t, thermally treated oil.

Statistical Analysis

Data collected were analyzed using SAS statistical version 9.1 software (SAS Co., North Carolina, USA, 2003). All experiments were performed with two replications, and One-way ANOVA followed by Duncan's test was applied to evaluate differences between mean values ($P \leq 0.05$).

Results and Discussion

Properties of refined SBO and OLE which are relevant to their quality

Soybean oil was analyzed to determine the main properties in terms of acid, peroxide, and *p*-Anisidine values. Free fatty acids (FFA %) can stimulate oxidative degeneration of oils by enzymatic and/or chemical hydrolysis to form off volatile components [22]. The obtained results showed that the acid value of refined SBO was 0.34 ±0.01 mg g^{-1}, and the PV was 0.53 ±0.05 mEq.O_2 kg^{-1}. These values agree with the acceptable levels reported by the Codex Alimentarius Commission [23]. The *p*-Anisidine value, which refers to the development of secondary oxidation products [24], was found to be 5.90 ±0.10, (Tab. 1).

TABLE 1: Quality properties of soybean oil and olive leaves extract.

Item	Value
Acid value (mg g^{-1})	0.34 ± 0.01
Peroxide value (mEq. O_2 Kg^{-1})	0.53 ±0.05
p-Anisidin value	5.90 ±0.10
Antioxidant capacity (IC_{50}) of SBO (µg mL^{-1})	0.618 ±0.11
Total phenolic contents of OLE (mg GAE g^{-1})	89.47 ±2.37
Antioxidant capacity (IC_{50}) of OLE (µg mL^{-1})	33.31 ±2.55
Antioxidant capacity (IC_{50}) of ascorbic acid (µg mL^{-1})	5.53 ±0.42

Values = mean ± standard division

According to the results of previous research work in this scope, methanol was found to be the best solvent for extracting phenolic compounds from plant leaves [11]. As shown in Table (1), TPC of the methanolic olive leaves extract was 89.47 ±2.37 mg GAE g^{-1} of dried extract. This content is lower than that of described by Skrget *et al.* [12], who showed that TPC of olive trees extracted with methanol was 144 g GAE kg^{-1} of dried extract. The differences between this report and the obtained result could be due to differences in variety, plant parts, climate and processing conditions [25].

According to the results listed in Table (1), the antioxidant capacity of olive leaves extracts, IC_{50} was 33.31±2.55 µg mL^{-1} which was higher than that of ascorbic acid, IC_{50} being 5.53 ±0.42 µg mL^{-1}. This means that OLE is much stronger antioxidants than ascorbic acid, which agree with previous findings [26]. These results also agreed with the data reports by Hayes *et al.* [27], who reported the IC_{50} of olive leaves extract was 35-µg mL^{-1}, which was also attributed to the synergistic characteristic among polyphenol compounds present in olive leaves extracts.

Chromatographic profile and identification of phenolic compounds

The chromatographic profile of OLE was determined by HPLC. The chromatograph of individual phenolic compounds was shown in Figure (1) and assuming of the content of various components are listed in Table (2). Four main phenolic groups can be clearly distinguished: simple phenols or cinnamic acids (tyrosol), flavonoids (rutin, luteolin glucoside, luteolin rutinoside, apigenin-7-*o*-glucoside, chrysoeriol-7-*o*-glucoside, and apigenin with different isomeric forms), various secoiridoids (mainly oleuropein, oleuropein glucoside, oleoside, and *10*-hydroxy-oleuropein with different isomeric forms) and the phenylpropanoid glycosides (verbascoside). Tyrosol was present at low concentration (0.33 mg g^{-1} CE) for the simple phenols. Flavonoids and secoiridoids are the two main represented groups of phenols followed by the phenylpropanoid glycosides class (verbascoside), (Tab. 2). Flavonoids amount was the highest (38.51 mg g^{-1} CE) compared to other classes, and are essentially represented by rutin, luteolin glucoside, luteolin rutinoside, apigenin-7-*o*-glucoside, chrysoeriol-7-*o*-glucoside, and apigenin with different isomeric forms (Tab. 2). On the other hand, the secoiridoid amount was ordered after the flavonoids, which was presented in 18.30 mg g^{-1} CE, and represented by oleuropein, oleuropein glucoside, oleoside and *10*-hydroxy-oleuropein with different isomeric forms (Tab. 2). However, the last phenolic compounds found in low concentration (0.81 mg g^{-1} CE) was verbascoside wich belongs to phenylpropanoid glycosides family (Tab. 2). The levels of phenolic compounds classes found in olive and leaves in agreement with those found in the literature [28, 29, 14].

TABLE 2: HPLC-ESI-TOF/IT-MS Identification and quantification of individual phenolic compounds in the OLE

No. of Peak	RT* (min)	Compounds	Formula	Measured m/z	Concentration (mg/g)**
1	5.17	Tyrosol	$C_8H_{10}O_2$	137	0.33 ± 0.02
2	5.47	Luteolin rutinoside	$C_{27}H_{29}O_{15}$	593	0.34 ±0.03
3	5.89	Rutin	$C_{27}H_{29}O_{16}$	609	1.12 ±0.08
4	6.81	Rutin isomer1	$C_{27}H_{29}O_{16}$	609	0.12 ±0.1
5	7.21	Rutin isomer2	$C_{27}H_{29}O_{16}$	609	1.72 ±0.15
6	7.44	Luteolin glucoside	$C_{21}H_{19}O_{11}$	447	0.11 ±0.01
7	7.59	10-hydroxy-oleuropein	$C_{25}H_{32}O_{14}$	555	17.13 ±1.04
8	7.81	Luteolin rutinoside isomer1	$C_{27}H_{29}O_{15}$	593	0.74 ±0.06
9	8.22	Verbascoside	$C_{29}H_{36}O_{15}$	623	0.81 ±0.12
10	8.58	Luteolin glucoside isomer1	$C_{21}H_{19}O_{11}$	447	0.13 ±0.01
11	8.74	Luteolin rutinoside isomer2	$C_{27}H_{29}O_{15}$	593	2.01 ±0.27
12	8.89	Apigenin-7-*O*-glucoside	$C_{21}H_{19}O_{10}$	431	6.54 ±0.84
13	9.07	Luteolin glucoside isomer2	$C_{21}H_{19}O_{11}$	447	0.13 ±0.01
14	9.42	Chrysoeriol-7-*O*-glucoside	$C_{22}H_{21}O_{11}$	461	2.21 ±0.32
15	9.51	Oleuropein glucoside	$C_{31}H_{41}O_{18}$	701	0.63 ±0.04
16	10.25	Oleuropein	$C_{25}H_{31}O_{13}$	539	0.22 ±0.02
17	11.37	Oleuropein isomer1	$C_{25}H_{31}O_{13}$	539	0.15 ±0.03
18	11.71	Oleoside	$C_{16}H_{21}O_{11}$	389	0.17 ±0.05
19	12.87	Apigenin	$C_{15}H_9O_5$	269	23.34 ±1.46

** RT, retention time; ** results are presented as mean values ± standard deviation expressed as mg g^{-1} of concentrated extract.*

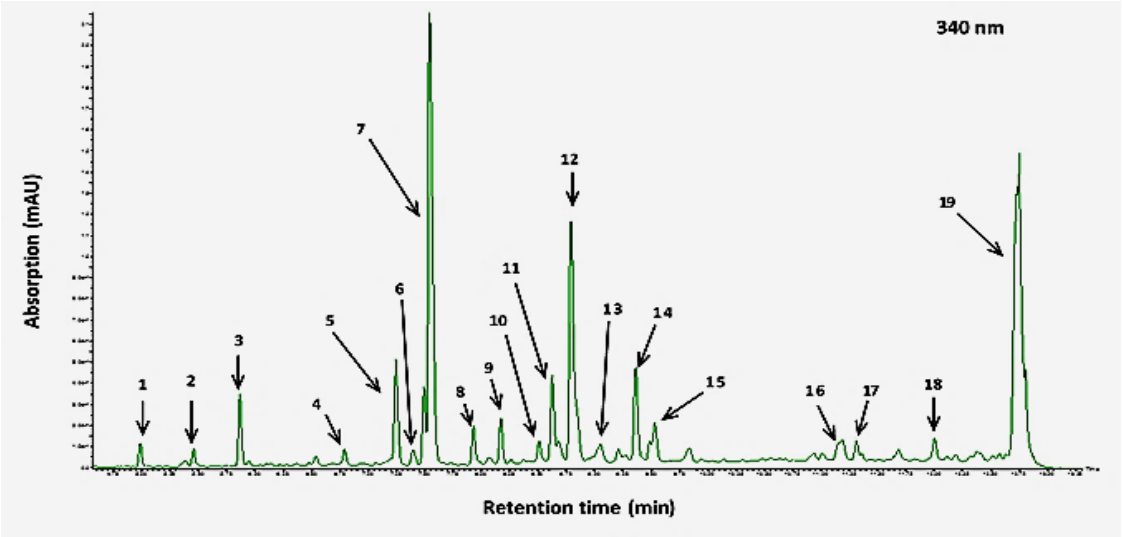


Fig. 1: HPLC chromatograph of olive leaves extract. Picks: (1) Tyrosol, (2) Luteolin rutinoside, (3) Rutin, (4) Rutin isomer1, (5) Rutin isomer2, (6) 10-hydroxy-oleuropein, (7) Luteolin glucoside, (8) Luteolin rutinoside isomer1, (9) Verbascoside, (10) Luteolin glucoside isomer1, (11) Luteolin rutinoside isomer2, (12) Apigenin-7-o-glucoside, (13) Luteolin glucoside isomer2, (14) Chrysoeriol-7-o-glucoside, (15) Oleuropein glucoside, (16) Oleuropein, (17) Oleuropein isomer1, (18) Oleoside, (19) Apigenin.

Oxidative stability of refined SBO to which OLE is added

Results of Rancimat test

Results of Rancimat test shown in Figure (2) indicate that the induction periods of refined soybean oil (as control) and SBO samples containing 200, 500 and 1000 ppm of OLE, and reference sample containing 200 ppm TBHQ were 6.87, 8.39, 9.92, 10.97 and 14.24 h, respectively. It seems that OLE is effective antioxidants and its effect increases by increasing the dose added to the oil as shown in Figure (3). These results were supported by those of Jimenez *et al.* [13], Bouaziz *et al.* [10] and Kiritsakis *et al.* [30].

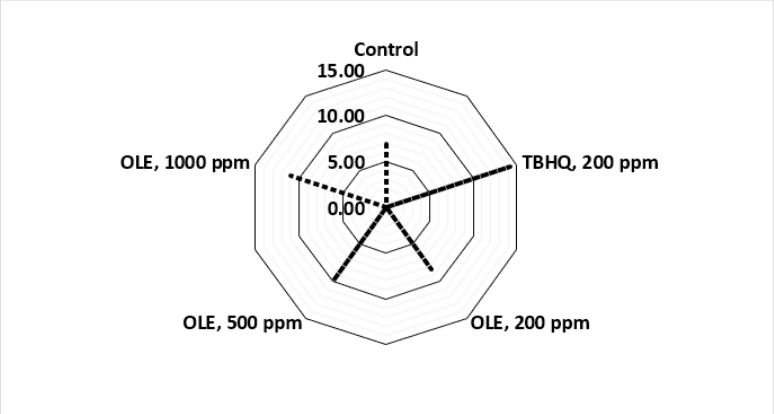


Fig. 2: Induction time of SBO enriched with three different levels of OLE (200, 500 and 1000 ppm) compared with reference sample (TBHQ) and control

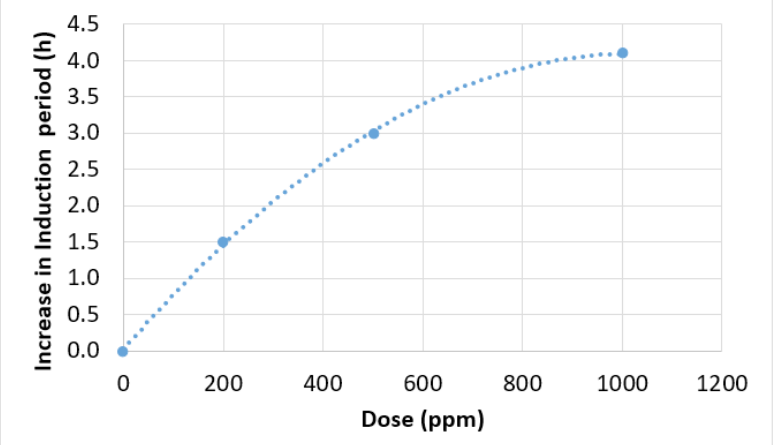


Fig. 3: Effect of the dose of OLE added to soybean oil on the increase in induction period (h) using Rancimat

Results of oxidative stability test via accelerated oxidation at 60 ºC

The increase in peroxide value and that in *p*-Anisidine value during accelerated oxidation are presented in Figures (4) and (5), respectively. Peroxide value (PV) is the most commonly used method to assess the amounts of primary oxidation products mainly peroxides in oils [31, 32]. Figure (4) shows the PV progression during the incubation of the prepared oil samples at 60 ºC for 21 days. In all samples, the PV generally increased from the beginning of the incubation period to the last day. The contro l sample, without any additive, showed a maximum PV (94.42 mEq.O_2 kg^{-1}) after 21 days of incubation. A significant difference (p<0.05) was observed among the PVs of the control and the oil samples contain 1000 and 1500 ppm of OLE and 200 ppm TBHQ. The oil samples contain the extracts exhibited lower PVs as compared with the control. However, these samples showed higher PVs compared to the sample containing 200 ppm of TBHQ. Similar results were previously obtained by Bouaziz *et al.* [10, 31], who reported that lipid peroxides formation were significantly (p<0.05) reduced by the addition of 400 ppm of olive leaves phenolic compounds to refined olive oils compared to control.

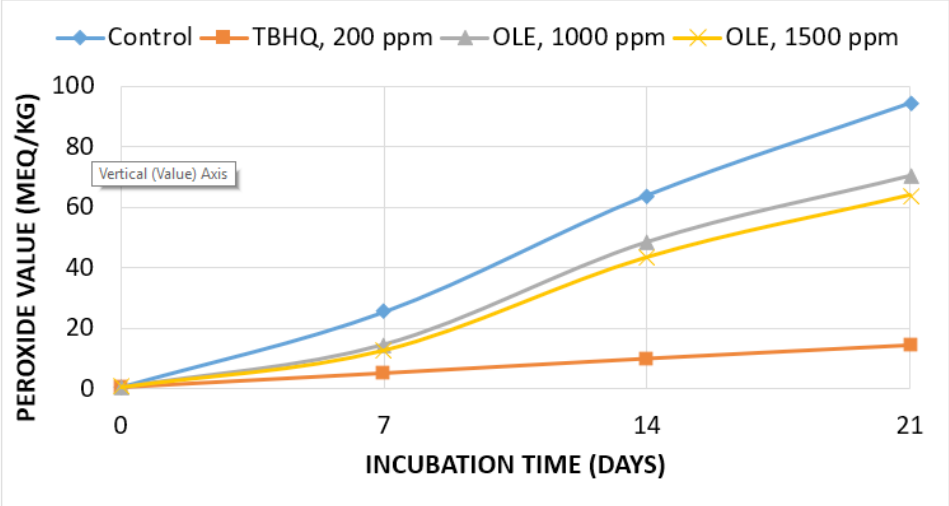


Fig. 4: Peroxide values of SBO enriched with two levels of OLE (1000 and 1500 ppm) compared with reference sample (TBHQ) and control, during 21 days of incubation at 60 °C

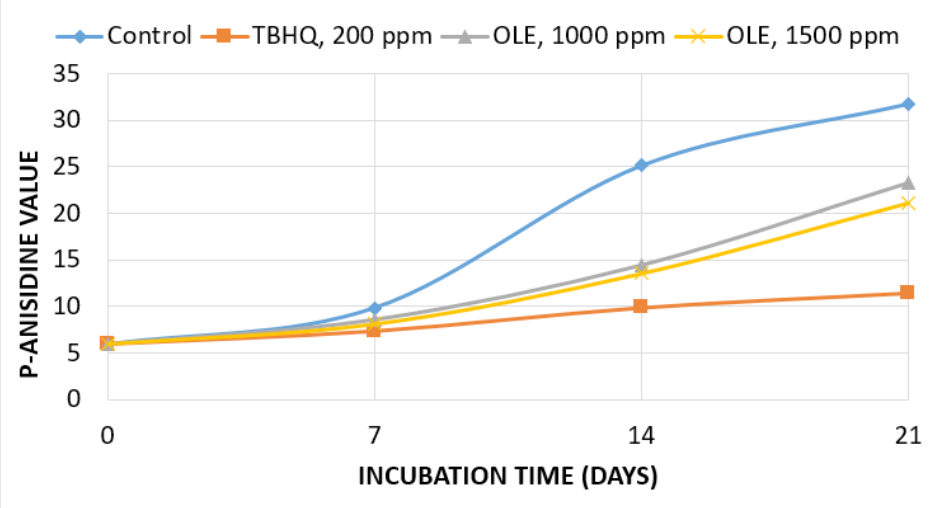


Fig. 5: *P*-Anisidine value of SBO enriched with two levels of OLE (1000 and 1500 ppm) compared with reference sample (TBHQ) and control, during 21 days of incubation at 60 °C

In order to ensure better monitoring of lipid oxidation process, the simultaneous detection of primary and secondary lipid oxidation products (aliphatic aldehydes, ketones, alcohols, acids and hydrocarbons) is necessary. *p*-AV is a reliable measurement of the amount of secondary oxidation products [33]. The results of *p*-AVs obtained during 21 days at 60 ºC are shown in Figure (5). These results demonstrated that the formation of secondary oxidation products increases during incubation. The *p*-AV of the control reached a maximum level of 31.77 ±0.89 from an initial value of 5.93 ±0.02 after 21 days of incubation. The oil samples contain different amounts of extract (1000 and 1500 ppm) showed a significantly ($p<0.05$) lower level of secondary products (approximately 23 at the end of storage) compared with the control. However, olive leaves extract was less effective in the inhibition of secondary products formation than TBHQ. This result is in agreement with that reported by Zhang *et al.* [17].

Effect of accelerated oxidation on fatty acid composition

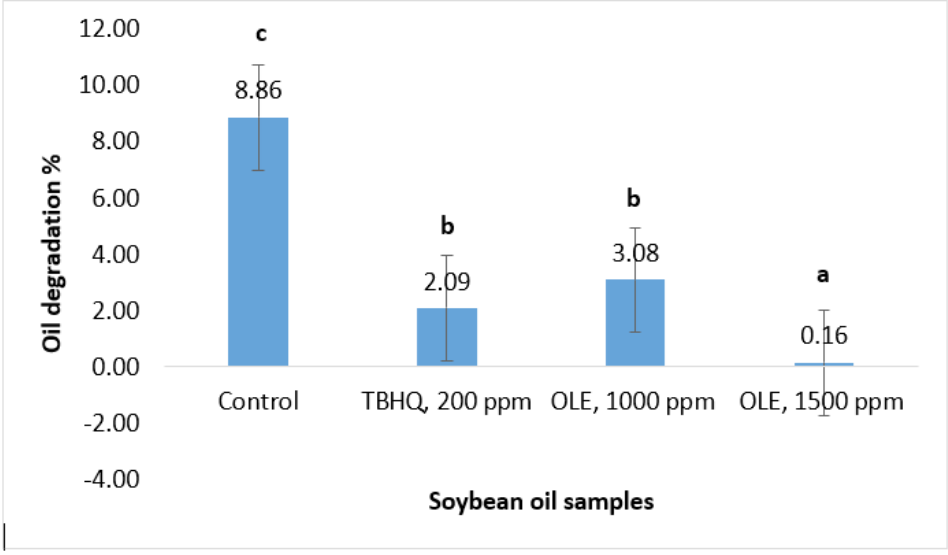
The changes in the fatty acid composition of oil samples are shown in Table (3). The listed data showed that oxidative acceleration at 60 ºC for 21 days caused changes in fatty acid profiles especially in control sample, whereas total saturated fatty acids (SFA) increased from 14.39 to 19.06 % while total UFA decreased from 85.62 to

80.94 %. So that, the oil degradation was affected by addition of OLE at different concentrations as well as synthetic antioxidants, whereas ODP of oil samples treated with 1000 and 1500 ppm OLE were 3.08 and 0.16%, respectively. However, ODP of oil sample contains TBHQ at 200 ppm was 2.09 % compared with the control sample (8.86 %), this means that ODP was affected significantly by the addition of OLE (Fig 6). Zahran [34] and Zaher *et al.* [35], concluded that the relative percentage of different fatty acid groups of soybean oil affected by frying at 180 ±5 °C in compared with control samples.

TABLE 3: Changes in fatty acid profile of treated soybean oil samples and control after 21 days of incubation at 60 °C

Oil samples	Fatty acids							
	$C_{16:0}$	$C_{18:0}$	$C_{18:1}$	$C_{18:1,\ trans}$	$C_{18:2}$	$C_{18:3}$	∑ SFA	∑ USFA
Control (zero time)	10.13 ±0.34	4.26 ±0.08	24.35 ±0.14	ND*	53.06 ±0.84	8.21 ±0.13	14.39	85.62
Control (21 days)	13.21 ±0.11	5.85 ±0.11	23.25 ±0.38	1.85 ±0.03	49.33 ±0.37	6.51 ±0.11	19.06	80.94
TBHQ (21 days)	10.18 ±0.27	5.25 ±0.22	22.92 ±0.15	1.65 ±0.10	51.79 ±0.55	8.2 ±0.05	15.43	84.56
OLE**, 1000 ppm (21 days)	11.35 ±0.12	6.10 ±0.31	23.17 ±0.23	ND	51.11 ±0.16	8.27 ±0.64	17.45	82.55
OLE, 1500 ppm (21 days)	9.71 ±0.84	5.16 ±0.17	22.41 ±0.13	1.56 ±0.11	53.09 ±0.25	8.08 ±0.11	14.87	85.14

**ND = not detected; **OLE, olive leaves extract; results are presented as mean values ± standard deviation*



Values i each column accompanied by different letters (a, b and c) are significantly different (P<0.05); results are presented as mean values ± standard error means

Fig. 6: Oil degradation percent of SBO enriched with two levels of OLE (1000 and 1500 ppm) compared with reference sample (TBHQ) and control after 21 days of incubation at 60 °C

Conclusion

The results have proved that the extracts of olive leaves can be used efficiently as a safe natural antioxidant in edible oils. However, the dose to be added should be adjusted as to achieve the activity of synthetic antioxidants. Further work seems to be necessary to identify such doses in comparison to the dose of synthetic antioxidants usually used for this purposes.

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References

1. El-Hamidi M. and Zaher F. A., Production of vegetable oils in the world and in Egypt: an overview. *Bulletin of the NRC*., **42**(1), 19, (2018).

2. Yanishlieva N. V. and Marinova E. M., Stabilisation of edible oils with natural antioxidants. *Euro J. of Lipid Sci. and Tech*., **103**(11), 752-767, (2001).

3. Gunstone F. D., Bailey's Industrial Oil and Fat Products, *Edible Oil and Fat Products: General Applications* (5th Edn.). *Trends in Food Sci. and Tech.,* **11**(7), 379-380, (1996).

4. Farag R. S., Badei A. Z., Hewedi F. M. and El-Baroty G. S., Antioxidant activity of some spice essential oils on linoleic acid oxidation in aqueous media. *J. of the Amer. Oil Chem. Soc.,* **66**(6), 792-799, (1989).

5. Esmat A. Y., Hassan R. E., Abo-ElWafa G. A., Abou-ElSoud M. M. and Megahed M. G., Physicochemical characteristics and oxidative stability of Egyptian corn germ oil produced by aqueous enzymatic extraction. *Grasas y Aceites,* **69**(4), 275, (2018).

6. Naeem M. A., Zahran H. A. and Hassanein M. M., Evaluation of green extraction methods on the chemical and nutritional aspects of roselle seed (*Hibiscus sabdariffa* L.) oil. *OCL*., **26**, 33, (2019).

7. Briante R., Patumi M., Terenziani S., Bismuto E., Febbraio F. and Nucci R., *Olea europaea* L. leaf extract and derivatives: antioxidant properties. *J. of Agric. and Food Chem.,* **50**(17), 4934-4940, (2002).

8. Salta F. N., Mylona A., Chiou A., Boskou G. and Andrikopoulos N. K., Oxidative stability of edible vegetable oils enriched in polyphenols with olive leaf extract. *Food Sci. and Tech. Inter.*, **13**(6), 413-421, (2007).

9. De Leonardis A., Aretini A., Alfano G., Macciola V. and Ranalli G., Isolation of a hydroxytyrosol-rich extract from olive leaves (*Olea Europaea* L.) and evaluation of its antioxidant properties and bioactivity. *Euro Food Res. and Tech.*, **226**(4), 653-659, (2008).

10. Bouaziz M., Feki I., Ayadi M., Jemai H. and Sayadi S., Stability of refined olive oil and olive-pomace oil added by phenolic compounds from olive leaves. *Euro J. of Lipid Sci. and Tech*., **112**(8), 894-905, (2010).

11. Mariod A. A., Ibrahim R. M., Ismail M. and Ismail N. Antioxidant activity of the phenolic leaf extracts from Monechma ciliatum in stabilization of corn oil. *J. of the Amer. Oil Chem. Soc.,* **87**(1), 35-43, (2010).

12. Škerget M., Kotnik P., Hadolin M., Hraš A. R., Simonič M. and Knez Ž. Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities. *Food Chem*., **89**(2), 191-198, (2005).

13. Jimenez P., Masson L., Barriga A., Chávez J. and Robert P., Oxidative stability of oils containing olive leaf extracts obtained by pressure, supercritical and solvent-extraction. *Euro J. of Lipid Sci. and Tech.,* **113**(4), 497-505, (2011).

14. Abaza L., Taamalli A., Arráez-Román D., Segura-Carretero A., Fernández-Gutierrérez A., Zarrouk M. and Youssef N. B.,. Changes in phenolic composition in olive tree parts according to development stage. *Food Res. Inter.,* **100**, 454-461, (2017).

15. Zahran H. A., El-Kalyoubi M. H., Khallaf M. M. and Abdel-Razek A. G., Improving oils stability using natural leaves. *Middle East J. of Appl. Sci.,* **5**(1), 26-38, (2015).

16. Zahran H. A. and Soliman H. M., UPLC-Q-TOF/MS Screening of Bio-Active Compounds Extracted from Olive Mill Solid Wastes and their Effect on Oxidative Stability of Purslane Seed Oil. *Current Sci. Int.,* **7**(2), 307-319, (2018).

17. Zhang Y., Yang L., Zu Y., Chen X., Wang F. and Liu F., Oxidative stability of sunflower oil supplemented with carnosic acid compared with synthetic antioxidants during accelerated storage. *Food Chem*., **118**(3), 656-662, (2010).

18. AOCS, Official and Tentative Methods of the American Oil Chemists' Society, AOCS Press,

Champaign, (1990).

19. AOCS. *Official and tentative methods of the American Oil Chemists' Society*, AOCS Press, Champaign, USA., (1998).

20. Zahran H. A. and Tawfeuk H. Z., Physicochemical properties of new peanut (*Arachis hypogaea* L.) varieties. *OCL*., **26**, 19, (2019).

21. Aniołowska M., Zahran H. and Kita A., The effect of pan frying on thermooxidative stability of refined rapeseed oil and professional blend. *J. of Food Sci. and Tech.,* **53**(1), 712-720, (2016).

22. Lise-Halvorsen B. and Blomhoff R., Determination of lipid oxidation products in vegetable oils and marine omega-3 supplements. *Food and Nut. Res*., **55**(1), 5792, (2011).

23. Alimentarius C. *Codex standard for named vegetable oils*, *Codex Stan.,* **210**, 1–13, (1999).

24. Sun T. and Ho C. T., Antioxidant activities of buckwheat extracts. *Food Chem.*, **90**(4), 743-749, (2005).

25. Mehta U. and Swinburn B. A., Review of factors affecting fat absorption in hot chips. *Critical Rev. in Food Sci. and Nut.,* **41**(2), 133-154, (2001).

26. Abas F., Lajis N. H., Israf D. A., Khozirah S. and Kalsom Y.U., Antioxidant and nitric oxide inhibition activities of selected Malay traditional vegetables. *Food Chem*., **95**(4), 566-573, (2006).

27. Hayes J. E., Stepanyan V., Allen P., O'grady M. N. and Kerry J. P., Effect of lutein, sesamol, ellagic acid and olive leaf extract on the quality and shelf-life stability of packaged raw minced beef patties. *Material Sci*., **84**(4), 613-620, (2010).

28. Tayoub G., Sulaiman H., Hassan A. H. and Alorfi M., Determination of oleuropein in leaves and fruits of some Syrian olive varieties. *Int. J. Med. Aromatic Plants,* **2**(3), 428-433, (2012).

29. Taamalli A., Abaza L., Arráez Román D., Segura Carretero A., Fernández Gutiérrez A., Zarrouk M. and Ben-Youssef N., Characterisation of phenolic compounds by HPLC–TOF/IT/MS in buds and open flowers of 'Chemlali'olive cultivar. *Phytochemical Anal*., **24**(5), 504-512, (2013).

30. Kiritsakis K., Kontominas M. G., Kontogiorgis C., Hadjipavlou-Litina D., Moustakas A. and Kiritsakis A., Composition and antioxidant activity of olive leaf extracts from Greek olive cultivars. *J. of the Amer. Oil Chem. Soc.,* **87**(4), 369-376, (2010).

31. Bouaziz M., Fki I., Jemai H., Ayadi M. and Sayadi S., Effect of storage on refined and husk olive oils composition: Stabilization by addition of natural antioxidants from Chemlali olive leaves. *Food Chem.,* **108**(1), 253-262, (2008).

32. Elabd E. M., Zahran H. A. and Abdalla A. M., A comparative study of the effects of three Moringa species on obesity-induced oxidative stress state in liver tissue. *Inter J. Pharma and Bio Sci*., **8**, 572-584, (2017).

33. Benavente-Garcıa O., Castillo J., Lorente J., Ortuno A. and Del Rio J. A., Antioxidant activity of phenolics extracted from *Olea europaea* L. leaves. *Food Chem*., **68**(4), 457-462, (2000).

34. Zahran H. A. *Maximizing the Utilization of Olive Oil Processing Wastes* (Doctoral dissertation, Ain Shams University), (2015).

35. Zaher F., Gad M. S., Aly S. M., Hamed S. F., Abo-Elwafa G. A., and Zahran H. A., Catalytic cracking of vegetable oils for producing biofuel. *Egy J. Chem*., **60**(2), 291-300, (2017).