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Using of olive leaves extract for increasing the stability of refined soybean oil

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Abstract

Olive leaves are considered a waste during olive oil extraction and appear as a good source of natural antioxidant because it contains a high percent of phenolic compounds. This work aims to evaluate the effect of olive leaves extract (OLE) on acceleration of lipid oxidation in refined soybean oil. The olive leaves extract was added to refined soybean oil with different concentrations (5, 10, 50, 100 and 200 ppm). The butylated hydroxytoluene (BHT) at concentration 200 ppm was used as a control. The refined soybean oil (SBO) samples treated with OLE and BHT which exposed to accelerated oxidation in the dark conditions in an oven at (70±3°C) for 72 hours. The olive leaves extract showed a high antioxidant activity like synthetic antioxidant (BHT) at 200 ppm by measuring the peroxide values (PV) and thiobarbituric acid value (TBA) during accelerated storage. The olive leaves extract approximately has the same strong antioxidant activity of the BHT when is used as natural antioxidant in soybean oil at 200 ppm.

Keywords: Soybean oil, accelerated storage, peroxide value and thiobarbituric acid value, Olive leaf, antioxidants, Lipid oxidation.

Introduction

Olive tree leaves (*Olea Europaea L.*) are normally utilized in the Mediterranean area as traditional medicine. Generally, the polyphenols are existed in extra virgin olive oil, or the fruit is lower than that detected in olive tree leaves. Olive leaves are considered as a cheap crude material which could be utilized as a better source of rich-added value bioactive compounds, even involving polyphenols (**Magrone et al., 2017**). Polyphenol concentration relied on the type of cultivar, geographical zone and tree age, whereas the polyphenol extract yield from olive leaves is intensity impacted by the extraction methods (**Pandey and Rizvi, 2009**).

The olive leaves extract (OLE) is effective antioxidants and its effect raised by increasing the dose added to the edible oil, the extracts of olive leaves could be utilized effectively as a safe natural antioxidant in edible oils. Nevertheless, the dose to be used must be modified as to obtain the high activity of synthetic antioxidants (**Zahran and Najafi, 2020**).

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Lipid oxidation is a complicated chain of reactions that take place during oil processing, storage, shipment and final preparation of food materials containing lipids. Oxidation mechanism begins occur instantly after oil extraction (**Mohammadi et al., 2016**). The oxidation could be altered the flavor and nutritional benefits of oil through the loss of beneficial matters and the formation of a new toxic compounds involving oxidized sterols, oxidized fatty acids and triacylglycerols (TAG) polymers, which could have an ability affected on human health and that make the olive oil less accepted or unacceptable to consumers (**Malvis et al., 2019**)

The synthetic antioxidants like butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are restricted by European Regulation as well as by Codex Alimentarius and FDA Food Additive Status List. The substitutional path is the utilize of natural antioxidants, like plant extracts, tocopherol and essential oils from spices and herbs. These natural compounds and mixtures have been utilized for formation antioxidant packaging materials **(Moudache et al., 2016)**.

The objectives of this study were investigation the effects of olive leaves extract on the oxidative stability of soybean oil in comparison with synthetic antioxidants.

Materials and Methods:

Olive leaves sample:

Olive leaves samples were collected during olive oil production by different extraction systems (Continuous Process Three-Phases (**CP3P**) and press process (**PP**)).

Reagents and Chemicals:

Sodium thiosulphate, potassium hydroxide, potassium iodide, iodine, thiobarbituric acid (TBA) were purchased from El-Gomhoria Company, Egypt.

All solvents used in this study (methanol, benzene, glacial acetic acid and chloroform) were obtained from El-Gomhoria Company, Egypt.

Preparation and extraction of the Olive leaves:

The olive leaves were dried by air drying at room temperature then ground into a fine powder in a mill. One hundred grams of the sample were initially extracted with hexane (three times) at room temperature. Ten grams of the above defatted residue was extracted with 200 mL methanol 80% for 30 min at 30°C in an ultrasonic bath (Soniclean, 220 V, 50 Hz and 250 W model 250HD, Soniclean, Pty Ltd, Thebarton, SA, Australia).

The extract was filtered, then the solvent removed by a rotary evaporator at temperature below 40°C. stock solution prepared by re-dissolved 2.5 gm of dried extract in 25 mL of methanol 80%. The stock solution stored at -20°C for further analysis.

Determination of total phenolics content:

The total phenolic content of extracts were measured by the Folin-Ciocalteu method according to (**Mohdaly *et al.*, 2011**). A 20 µL aliquot of extract solution was mixed with 1.16 mL distilled water and 100 µL of Folin-Ciocalteu reagent, followed by the addition of 300 µL of Na_2CO_3 solution (20%). Subsequently, the mixture was incubated in a shaking incubator at 40°C for 30 min and its absorbance at 760 nm was measured. Gallic acid was used as a standard for calibration curve. Total phenolic content expressed as gallic acid

equivalent (GAE) was calculated by using the following equation based on the calibration curve:

$$Y = 0.0248x + 0.237 \ (R^2 = 0.997)$$

Where x is the absorbance and Y is the concentration (mg GAE g^{-1} dry weight (DW)

Determination of peroxide value:

Peroxide value (Recommended Practice Ca 5a-40) of olive oil were determined according to **(AOCS, 2017)**.

Thiobarbituric acid (TBA) value:

Thiobarbituric acid (TBA) value as an index of fat oxidation was determined directly on the samples as described by **(Matsushita et al., 2010)**. Exactly 0.67g of thiobarbituric acid was dissolved in distilled water with the aid of heat from a steam bath. The thiobarbituric acid solution was transferred to 100ml volumetric flask. The TBA solution was cooled and complete to the volume with distilled water. The TBA solution was mixed with an equal volume of glacial acetic acid to prepare the TBA reagent.

The known weights of the oil sample (3.00g) was transfered into a glass stoppered maisel-Gerson tube then was dissolved in 10 ml benzene. Ten ml of TBA reagent were then pipetted into the Maisel-Gerson tube and shacked in a horizontal position for a period of 4 minutes at approximately 125 oscillations per minute. The contents were then transferred to a separatory funnel and the aqueous layer was withdrawn into a test tube. The tube was then placed in a boiling water bath for 30 min. The absorbance of the developed color was recorded at 530 nm against distilled water.

Determination oxidative stability of refined soybean oil (Schaal Oven test):

The stock samples of refined soybean oil (SBO) containing different concentrations of OLE (5, 10, 50, 100 and 200 ppm, rely on the dry extract weight) and BHT (200 ppm) were prepared then stirred by magnetic stirrer for 60 min. after that the antioxidant-treated oil samples were evaporated in a vacuum oven at temperature lower than 40°C to vaporize the solvent. A sequence of bottles was totally filled with treated SBO samples and closed. A control sample was prepared utilizing soybean oil exposed to the same condition of treatments. The SBO samples exposed to accelerated oxidation in the dark conditions in an oven at 70°C for 3 days. The samples were withdrawn regularly every 4, 8, 24, 32, 36, 48, 56, and 72 h for analysis. Directly after storage time, the withdrawn samples were tested for peroxide value (PV) and TBA.

Results and Discussion

Chemical composition of leaves:

The chemical composition of olive leaves is shown in **Table 1**. Olive leaves according to the results in **Table 1** could be considered as a rich source of minerals. Olive leaves need for more investigation to extract useful minerals and use these minerals in food fortification. The crude lipids content of olive leaves probably is referred to its high content of wax. These results are in agreement with that reported by (**Erbay and Icier, 2009**)

Table 1 : Chemical composition of olive leaves (g/100g on wet and dry weight basis).

parameters	OL	
	WW	DW
Moisture content	48.15± 0.41	
Protein	4.84± 0.65	9.34±0.01
Ash	5.42±0.05	10.45±0.02
Crude lipids	2.82±0.02	5.44±0.01
Nitrogen free extract	38.77±0.03	74.77±0.02

Each value represents mean ± S.D of three repeated.

Determination of total phenolic compounds of by-products extract:

Phenolic compounds in plants constitute a major class of secondary plant metabolites with bioactive potential attributed to antioxidant activity. These extracts are rich sources of a polyphenols with a large spectrum of biological activities. Therefore, valuable compounds could be obtained from those materials for the preparation of functional food ingredients and nutraceuticals. Polyphenols from olives may have significant health benefits such as antiatherogenic, antimicrobial, antitumoral, anti-inflammatory, cardioprotective and cytoprotective properties (**Munekata et al., 2020**).

Table 2 : The phenols concentration in the olive leaves and olive pomace from different methods extraction.

Sample	Total phenolic (Mg GAE g^{-1} DW)	Total phenolic (%, WW)
OPCM	96.78±0.23	3.95
OPPM	96.89±0.21	3.75
OLM	101.52±0.18	5.23

OPCM = pomace from CP3P, OPPM = pomace from PP, OLM = olive leaf For USAE and CE extraction systems. while (M) stands for a methanol solvent (80%). DW: based on dry weight, WW: based on wet weight.

The results of total polyphenol content showed in **Table 2** and **Fig 1**. The olive leaves extract contained the highest concentration of total polyphenols on wet weight it contained the lowest concentration from total phenols on dry weight basis in comparison with olive pomace produced from different extraction methods (101.52±0.18 mg GAE/g DW). Meanwhile, there are no marked differences between the two pomace samples where the total polyphenol content of olive pomace from CP3P method (**OPCM**) and olive pomace from PP method (**OPPM**) extracts were 96.78±0.23 and 96.89±0.21 respectively. These results are comparable to that reported by (**Ghasemi et al., 2018**).

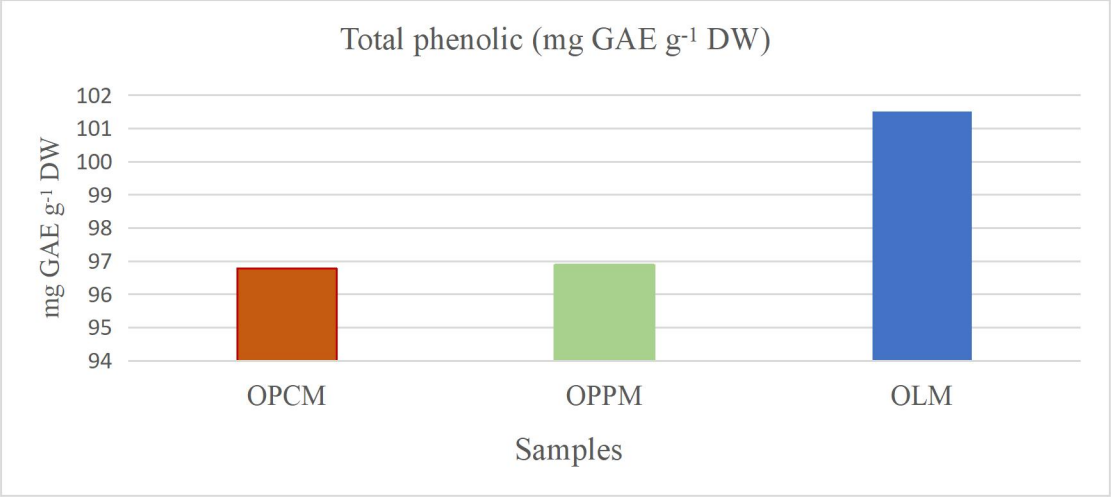

Fig. 1 . Shows the phenols concentration in the olive leaves and olive pomace.

Peroxide value under acceleration storage:

Peroxide value (PV) is the most widely used method to measure the amounts of peroxides and hydroperoxides produced in the primary stages of lipid oxidation. In this study, **Fig. 2** shows the PV developing during the incubation time of the soybean oil samples at 70ºC for 72 h in the absence and presence of the olive leaves extract and BHT. In all oil samples, the PV values increase for all treatments from zero time to the end of the incubation period.

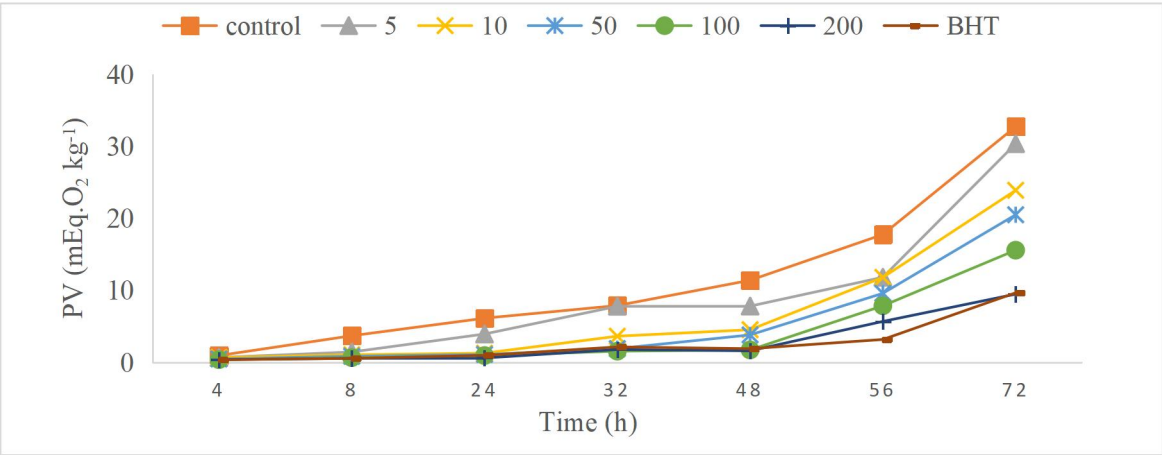

Fig. 2. Developing the peroxide values during the incubation period of oil samples under accelerated storage at 70°C.

The control sample, without any antioxidant additive demonstrated a maximum PV (32.73±5.41 mEq.O_2 kg^{-1}) after 72 hours of incubation period as shown in **Table 3 and Fig. 2**. There are significant differences ($p<0.05$) were noticed between the PVs of the control and all samples treated with OLE and BHT contain 5, 10, 50, 100 and 200 ppm of OLE and 200 ppm BHT. The PV values of control were higher than all treatments. Nevertheless, the samples contain 5, 10, 50 and 100 ppm from olive leaves extract showed higher PVs compared to the samples containing 200 ppm of olive leave extract and BHT. The samples containing 200 ppm of olive leaves extract and BHT nearly have the same PVs and that is mean 200 ppm of OLE and BHT have the same effect of antioxidant activity.

The same results were obtained by (**Bouaziz et al., 2010; Zahran and Najafi, 2020)**, they reported that lipid peroxides development were significant ($p<0.05$) decreasing with increasing the addition of olive leaves phenolic extracts to olive oils compared to control sample.

Thiobarbituric acid under acceleration storage:

The thiobarbituric acid (TBA) test measures malonaldehyde (MDA) produced due to the oxidation of fatty acids with three or more double bonds. TBA was found satisfactory to assess the quality of fats and oils, such as lard, cooking fat and soybean, sunflower and rapeseed oils in the early stages of rancidity **(Min Hu and Charlotte Jacobsen, 2016**).

Table 3 : Effect of addition olive leaves extracts and BHT on the PVs of soybean oil under accelerated storage (70 ±3°C).

Treatments	Olive leaves extract concentration (ppm)						
Incubation (h)	Control	5	10	50	100	200	BHT 200 ppm
4	0.96^{c}±0.20	0.69^{b}±0.13	0.69^{b}±0.13	0.49^{a}±0.13	0.39^{a}±0.00	0.39^{a}±0.00	0.38^{a}±0.01
8	3.71^{e}±0.16	1.46^{d}±0.08	1.07^{c}±0.13	0.87^{b}±0.12	0.67^{a}±0.40	0.58^{a}±0.27	0.58^{a}±0.28
24	6.14^{e}±0.26	3.95^{d}±0.13	1.29^{c}±0.02	1.11b^{c}±0.19	0.97^{b}±0.01	0.62^{a}±0.03	0.99^{b}±0.00
32	7.90^{e}±0.09	7.80^{e}±0.33	3.65^{d}±0.19	1.92b^{c}±0.01	1.39^{a}±0.02	1.79^{ab}±0.27	2.16^{c}±0.27
48	11.41^{e}±0.05	7.82^{d}±0.18	4.55^{c}±0.41	3.83^{b}±0.54	1.47^{a}±0.29	1.61^{a}±0.02	1.91^{a}±0.04
56	17.76^{e}±1.85	11.83^{d}±0.26	11.81^{d}±0.18	9.60^{c}±2.67	7.87^{c}±0.27	5.70^{b}±0.50	3.19^{a}±0.02
72	32.73^{d}±5.41	30.36^{d}±1.99	23.90^{c}±0.18	20.50^{c}±2.20	15.58^{b}±0.26	11.38^{a}±0.01	11.62^{ab}±0.12

Mean values in each value followed by a different higher-case-letter are significantly different by Duncan's test at $p \leq 0.05$.

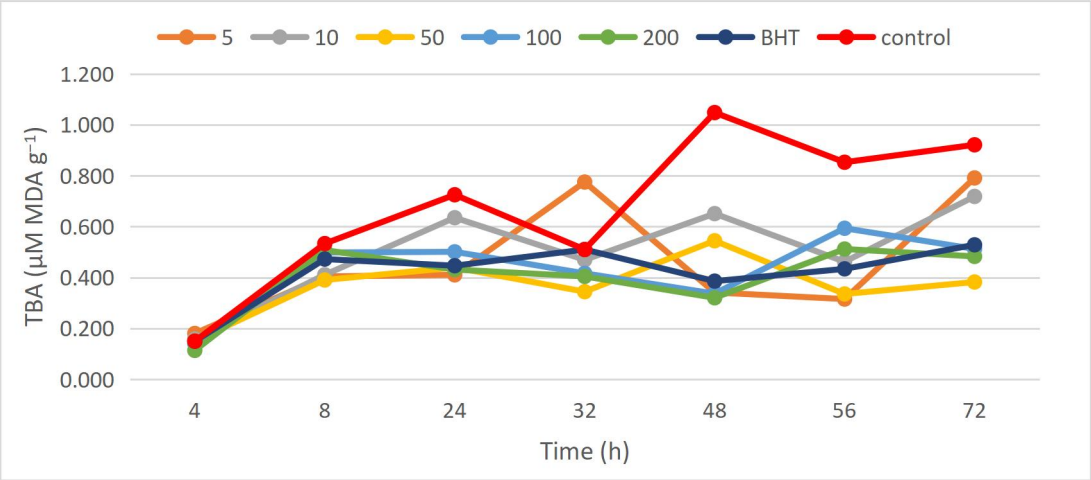

Fig. 3. Developing the TBA during the incubation period of oil samples under accelerated storage.

The results in **Table 4** and **Fig. 3** showed the TBA value for soybean oil samples treated with olive leaves extract, BHT and control. The TBA values for all treatments and control sample increase with increasing the incubation period until 24 hours. The TBA values in control are higher than all treatments until 24 hours. The TBA values for the control and treatments fluctuated between decreasing and increasing until 72 hours. After 72 hours the TBA value of treatments is lower than control that is mean olive leaves extract and BHT inhibited the oxidative rancidity. The fluctuation of TBA value for all samples and control may be due to aldehydes could be associated with the oxidation of secondary autoxidation fragments and formation of carboxylic acids so the value of thiobarbituric acid will decrease. The results are in agreement with that reported by (**Mohammadi et al., 2016 and Taghvaei et al.,2014**).

Table 4 : Effect of addition olive leaves extracts and BHT on the TBA values of soybean oil under accelerated storage (70 ±3°C).

Treatments	Olive leaves extract concentration (ppm)						
Incubation (h)	Control	5	10	50	100	200	BHT 200 ppm
4	0.150 ±0.01	0.181 ±0.00	0.159±0.00	0.151±0.00	0.141±0.00	0.114±0.00	0.150±0.00
8	0.533±0.00	0.405±0.00	0.411±0.001	0.391±0.00	0.499±0.00	0.508±0.001	0.473±0.00
24	0.725±0.00	0.411±0.00	0.635±0.20	0.440±0.00	0.501±0.00	0.432±0.00	0.447±0.00
32	0.510±0.001	0.775±0.00	0.470±0.00	0.345±0.00	0.417±0.00	0.405±0.00	0.510±0.001
48	1.048±0.004	0.342±0.016	0.651±0.003	0.545±0.017	0.337±0.004	0.321±0.005	0.386±0.00
56	0.853±0.004	0.316±0.018	0.462±0.003	0.336±0.002	0.594±0.001	0.512±0.099	0.434±0.001
72	0.921±0.001	0.791±0.003	0.719±0.001	0.383±0.003	0.513±0.001	0.483±0.002	0.529±0.002

Each value represents mean ± S.D of three repeated.

Conclusion

We can conclude that from obtained results that the olive leaves extract could be utilized as a safe natural antioxidant in edible oils in comparison with the synthetic antioxidant. Olive leaves extract have the same effect of synthetic antioxidant on retarding oxidative rancidity in edible oils. Further work should be necessary to evaluate the s

ensory characteristics after addition the olive leaves extract to the edible oils.

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إستخدام مستخلص أوراق الزيتون لزيادة ثبات فول الصويا المكرر

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تعتبر أوراق اشجار الزيتون مخلفات ناتجة أثناء إنتاج زيت الزيتون. وتعتبر مصدر جيد للحصول علي مضادات الاكسدة الطبيعية نظراً لارتفاع محتواها من المواد الفينولية ويهدف هذا البحث إلي تقييم المستخلص الكحولي لاوراق الزيتون في تأخير التزنخ الاكسيدي في زيت فول الصويا المكرر. وذلك بتقدير رقم البيروكسيد ورقم حمض الثيوبربتيوريك لعينات الزيوت المضاف لها مضاد الاكسدة الصناعي (BHT) فقط بتركيز 200 جزء في المليون وعينات مضاف لها مستخلص اوراق الزيتون بتركيزات مختلفة والتي تم الأسراع من عملية التزنخ بها بالتحضين علي درجة حرارة 70°م في الظلام وذلك لمده 72 ساعة وقد اظهرت النتائج ان إضافة المستخلص الكحولي لاوراق الزيتون بتركيز 200 جزء في المليون له نفس تأثير مضاد الاكسدة الصناعي بوتيلاتيد هيدروكسي تولوين(BHT بركيز 200 جزء في المليون. وتعتبر اوراق الزيتون مصدر جيد وامن للحصول علي مضادات الاكسدة الطبيعية والمستخلص الكحولي لها له نفس قوة ونشاط مضاد الاكسدة الصناعي والمعروف بالبوتيلاتيد هيدروكسي تولوين لتأخير التزنح الاكسيدي لزيت فول الصويا.