International Journal of Nutrition Sciences

Journal Home Page: ijns.sums.ac.ir

ORIGINAL ARTICLE

Antioxidant Activity of Methanolic Extract of Olive Leaf on Oxidative Stability of Sunflower Oil

Sattar Bahmaninia[1], Maryam Abbasvali[1,2*], Seyed Shahram Shekarforoush[3]

1. Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, Shahrekord University, Shahrekord, Iran
2. Department of Food Hygiene and Quality Control, School of Nutrition and Food Sciences, Shiraz University of Medical Sciences, Shiraz, Iran
3. Department of Food Hygiene and Public Health, School of Veterinary Medicine, Shiraz University, Shiraz, Iran

ARTICLE INFO

Keywords:
Antioxidant
Olive cultivars
Oxidative stability
Sunflower oil
Iran

**Corresponding author:*
Maryam Abbasvali, Ph.D;
Department of Food Hygiene and Quality Control,
School of Nutrition and Food Sciences, Shiraz University of Medical Sciences,
Shiraz, Iran.
Tel: +98-71-37251001
Email: abbasvali.m@gmail.com
Received: July 28, 2022
Revised: October 29, 2022
Accepted: November 8, 2022

ABSTRACT

Background: Approximately 90% of the fatty acids in sunflower oil are unsaturated. This study evaluated the antioxidant activity of leaf methanol extract of four Iranian olive cultivars on the oxidative stability of sunflower oil.

Methods: Leaf methanolic extracts of four Iranian olive cultivars (Zard, Roghani, Shiraz, and Dezfool) were prepared by microwave extraction method. Total phenolic content, diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, and reducing power of the extracts were determined. Peroxide and anisidine values of sunflower oil treated with the extracts were measured during 30 days of storage at 60°C.

Results: The concentration of methanolic extract of the Roghani cultivar (46.27±2.76 µg/mL) required to scavenge 50% of the initial DPPH radicals (IC_{50}) showed a significant difference ($p<0.05$) with the IC_{50} of butylated hydroxytoluene (BHT) (112.90±14.81 µg/mL), meaning that the percent inhibition of the DPPH radical of the methanol extract of the Roghani cultivar was higher than that of BHT ($p<0.05$). During the 30 days storage period, sunflower oil samples without antioxidants showed significantly higher anisidine and peroxide values than samples treated with olive leaf extracts ($p<0.05$). In all treated samples, as the concentration of the extracts increased, peroxide and anisidine values significantly decreased ($p<0.05$).

Conclusion: This study showed that the methanolic extracts of olive leaves had the ability to limit the oxidation of lipids and can be considered as a potential antioxidant source of natural origin. The methanolic extract of Roghani cultivar had the highest effect on the oxidative stability of sunflower oil.

Please cite this article as: Bahmaninia S, Abbasvali M, Shekarforoush SS. Antioxidant Activity of Methanolic Extract of Olive Leaf on Oxidative Stability of Sunflower Oil. Int J Nutr Sci. 2022;7(4):217-224. doi: 10.30476/ijns.2022.97385.1208..

Introduction

Approximately 90% of the fatty acids in sunflower oil are unsaturated, with oleic and linoleic acids accounting for the majority of the fatty acids. When compared to other vegetable oils, the high concentration of α-tocopherol is advantageous. Among its many health benefits, sunflower oil reduces plasma lipoprotein total cholesterol, thereby avoiding one of the primary causes of arteriosclerosis. Lipid oxidation is considered one of the most critical factors affecting the quality attributes of sunflower oil and other plant oils during

processing, with polyunsaturated fatty acids being the saturated fatty acids most sensitive to factors such as temperature and oxygenation. This type of deterioration shortens the oil's shelf life. Because of the undesirable changes in oil properties, this process is hazardous to humans (1).

During the oxidation of oil, low-molecular-weight off-flavor compounds are produced that make the oil less acceptable or unacceptable to consumers. Oxidation of oil produces toxic compounds and also destroys essential fatty acids. Oxidation of oil is very important in terms of palatability, nutritional quality, and toxicity of edible oils (2). There are some methods for preventing the oxidation of edible oils. The most common and best method for reducing lipid peroxidation is the addition of antioxidant agents to food formulations. The added antioxidants maintain the quality and increase the shelf life of many food products. Activities of antioxidants include scavenging free radicals, chelating metal catalysts, and reducing hydroperoxides into stable compounds (3). The origin of antioxidants can be natural or synthetic. Synthetic antioxidants have undesirable effects on human health, and their use is restricted in several countries. The most commonly used synthetic antioxidants, butylated hydroxyanisole (BHA), propyl galate (PG), butylated hydroxytoluene (BHT), and tertiary butyl hydroquinone (TBHQ) have been suspected to cause or promote negative health effects (4).

Today, there is an increasing tendency toward using natural antioxidants. Many plant extracts have been studied and examined for their valuable use as antioxidant agents. Natural antioxidants derived from plants are divided into three categories of phenolic compounds, vitamins, and carotenoids (5). Olive tree (*Olea europaea* L.) leaves have been widely used for years in ancient cultures in folk medicine (6), because of their therapeutic effect. Olive leaf contains natural bioactive compounds such as oleuropein, verbascoside, rutin, tyrosol, hydroxytyrosol, flavonoids (luteolin, apigenin, and quercetin), caffeic acid, and tannins (7), all of which have biologic activities such as antioxidant, antibacterial, and antiproliferative properties (8, 9).

Olive leaves contain large amounts of useful phenolic compounds (10), which are known as strong antioxidant agents (11). Phenolic compounds are classified as secondary metabolites found in a variety of plants, including roots, seeds, leaves, fruits, stems, and so on. These molecules are produced by the plant in order to defend itself or to promote growth in adverse conditions (12). Olive leaves are an abundant by-product of olive trees and a cheap raw material; this polyphenol profile suggests a potential use as a source of natural antioxidants, thus increasing the economic value of the olive leaf. The chemical composition of olive leaf varies according to many factors such as climatic conditions, olive cultivar, tree age (13), genetics, agricultural practices, extraction procedures, temperature, geographical location (14), and the biological cycle of the olive tree (15).

Microwave-assisted extraction (MAE) provides a rapid delivery of energy to a total volume of solvent and solid plant matrix, followed by efficient and homogeneous heating of the solvent and solid matrix. Because water within the plant matrix absorbs microwave energy, internal superheating promotes cell disruption, facilitating chemical desorption from the matrix and improving bioactive compound recovery. For the extraction of bioactive compounds from plants, MAE has been considered a potential alternative to traditional solid-liquid extraction. It has been used to extract bioactive compounds for several reasons, including shorter extraction times, less solvent usage, and higher extraction yield. Because of its ease of use and low cost, MAE is comparable to other modern extraction techniques such as supercritical fluid extraction. MAE is a powerful extraction technique for bioactive compounds when economic and practical considerations are taken into account (16). Furthermore, the functionality of bioactive compounds has not been degraded during this process (17).

Iran is one of the most significant olive producers in the world, and in recent years, olive cultivation has grown in Iran (18). Zard, Roghani, Dezfool, and Shiraz cultivars are cultivated more than others (19). The purpose of this study was to use the MAE method to extract bioactive compounds from the methanol extract of olive leaves of four famous Iranian cultivars and add them to sunflower oil to increase its antioxidant effect against heat and increase the nutritional value of the oil.

Materials and Methods

To prepare olive leave extract, fresh green leaves of four olive cultivars (Zard, Roghani, Shiraz, and Dezfool) were collected in September 2013 from a research olive garden near Shiraz, Iran. Leaves were dried in open air under shady conditions and then ground in an electrical grinder to obtain a fine powder that was then stored. Methanolic extracts of the olive leaf powder were prepared via the MAE method. For extraction, a microwave oven (Samsung, model ME3410W, Malaysia) equipped with a flask and a condenser was used. According to Pan *et al.* method (2003), one hundred grams of ground powdered leaf were mixed with 1000 mL of 80% v/v aqueous methanol and microwaved (200

W power level, 15 minutes) (20) with modifications. After the extraction time, the flask was allowed to cool at room temperature before being opened. Whatmann No. 1 filter paper was used to filter the extract. To obtain a concentrated extract, the solvent was removed using a rotary evaporator (IKA RV10D S99, Germany) at 40°C. The extract was lyophilized to a dry powder in a lyophilizer (Zirbus, Vaco 5, Germany) and stored at -21°C until use.

Total phenolic content of the olive leaf extracts was determined according to the Folin-Ciocalteu reagent (FCR) colorimetric method as described by Singleton and Rossi (1965) with slight modifications (21). Half a milliliter of properly diluted lyophilized leaf extract (0.05% w/v, 0.5 mg/mL) was added to 2.5 mL of Folin-Ciocalteu reagent (Sigma-Aldrich; St. Louis, MO) (10-fold diluted with distilled water) and 2 mL of sodium carbonate (Merck; Darmstadt, Germany) (7.5% w/v). Further, the contents were mixed using a vortex mixer and allowed to stand for 60 minutes at room temperature. The absorbance was measured at 765 nm using a spectrophotometer (UNICO 2100, Dayton, NJ). A mixture of distilled water and reagents were used as a blank. The concentration of total phenolic compounds in the extracts was determined by comparing the absorbance of the extract samples to that of the gallic acid standard solutions. All samples were determined in triplicate, and the results were averaged. Total phenolic content was expressed as mg gallic acid equivalents (GAE) per gram of dry olive leave extract.

To assess the antioxidant activity of the olive leaf extracts by 2, 2-diphenyl-1-picrylhydrazyl (DPPH, Sigma-Aldrich, St. Louis, MO) radical scavenging assay, half a milliliter of 100, 200, and 500 µg/mL of lyophilized olive leaf extracts were mixed with 2.5 mL of 0.004% DPPH in methanol. After shaking, the tubes were left in the dark for 60 minutes at room temperature, and the absorbance was measured at 517 nm. Methanol and 100, 200, and 500 µg/mL BHT (Sigma-Aldrich; St. Louis, MO) were used as control and standards, respectively. The percentage of inhibition activity was calculated as $[(A_0-A_1)/A_0]\times100$, where A_0 was the absorbance of the control and A_1 was the absorbance of the extract/BHT. The concentration of sample required to reduce 50% of DPPH radicals (IC_{50}) was calculated from linear regression analysis (22).

The reducing power was measured by a modification of the method used by Oyaizu (1986) (23). Half a milliliter of different concentrations of lyophilized olive leaf extracts (25, 50, and 75 µg/mL) were mixed with 0.5 mL of sodium phosphate buffer (0.2 M, pH 6.6) and 0.5 mL of potassium ferricyanide (10 mg/mL, Sigma-Aldrich, St. Louis, MO). The mixture was incubated at 50°C for 20 min. After cooling at room temperature, 0.5 mL of trichloroacetic acid (100 mg/mL, Sigma-Aldrich, St. Louis, MO), 0.5 mL of distilled water, and 0.1 mL of ferric chloride, were added and incubated for another 10 min at 50°C. The absorbance was measured at 700 nm against a blank of distilled water. BHT (25 and 50 µg/mL) was used as a standard antioxidant in the assay.

To evaluate the antioxidant activity of the olive leaf extract on oxidative stability of sunflower oil, freshly refined sunflower oil without any synthetic antioxidants was purchased from the Nahangol oil company (Broujen, Chaharmahal, and Bakhtiyari, Iran). Lyophilized olive leaf extracts with concentrations of 500 and 1000 µg/mL and TBHQ with a concentration of 75 µg/mL were added to the oil separately. Samples were subjected to accelerated oxidation in dark condition of an oven without air circulation at 60°C for 30 days. In each sampling day (0, 5, 10, 15, 20, 25, and 30), samples were removed from the oven and subjected to chemical analysis. Sunflower oil without any antioxidant was considered negative control. All samples were analyzed in triplicate.

For chemical analysis of sunflower oil samples, the peroxide value and anisidine value of treated oil samples were determined according to the procedure described by the American Oil Chemist's Society (24). The peroxide value was measured by treating a solution of the sunflower oil samples (5±0.05 g) in 30 mL acetic acid-chloroform with 0.5 mL of saturated potassium iodide solution and by titrating with 0.1 N sodium thiosulfate. The determination of anisidine value was done by reading the absorbance of a solution of the sunflower oil samples (0.5-4±0.001 g) in 25 mL isooctane, treated with 1 mL p-anisidine reagent at 350 nm, solvent with p-anisidine reagent was used as a blank in the reference cuvette.

All statistical analyses were performed in triplicate. To compare groups, an analysis of variance (ANOVA) was conducted, and means comparisons were done by Duncan's multiple range tests. P values less than 0.05 were considered statistically significant. Analysis was undertaken using the SPSS for Windows package (Version 22, SPSS Inc., Chicago, IL, USA).

Results

Table 1 presents the mean amount of total phenolic compounds in the methanolic extract of leaf from four Iranian olive cultivars. The total phenolic compound content varied between cultivars, but there was no significant difference (p>0.05) between them.

Table 1: Total phenolic content, radical-scavenging activity, and reducing power (at concentrations of 25, 50, and 75 µg/mL) of methanolic extracts of leaves of four cultivars of Iranian olive

Olive leaf cultivars	Total phenolic content (mg gallic acid equivalents/g)	Radical-scavenging activity IC_{50} (µg/mL)	Reducing power (Absorbance at 700 nm) at concentrations of		
			25 (µg/mL)	50 (µg/mL)	75 (µg/mL)
Dezfool	226.05±15.11^{a}	147.10±11.80^{a}	0.33±0.01^{bcA}	0.41±0.01^{abB}	0.56±0.01^{aC}
Shiraz	226.21±9.57^{a}	140.71±12.90^{a}	0.26±0.01^{aA}	0.38±0.04^{aB}	0.61±0.01^{abC}
Zard	232.08±14.57^{a}	69.39±30.05^{b}	0.32±0.01^{bA}	0.42±0.01^{abB}	0.59±0.01^{abC}
Roghani	231.92±11.27^{a}	46.27±2.76^{c}	0.36±0.01^{cA}	0.47±0.01^{bB}	0.63±0.01^{bC}
BHT	-	112.90±14.81^{a}	0.65±0.06^{dA}	1.02±0.21^{cB}	-

BHT: Butylated hydroxytoluene; Data are Mean±SD of three independent extracts. IC_{50}=The concentration of olive leaf extract needed to decrease 50% of 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) radicals; Different small letters indicate statistically significant differences in columns (p<0.05). Different capital letters indicate statistically significant differences in row (p<0.05).

The concentration of extracts required to reduce 50% of DPPH radicals (IC_{50}) was presented in Table 1, and the percent inhibition activity of a methanolic extract of four olive leaf cultivars and BHT was presented in Figure 1. According to the results shown in Table 1, the methanolic extract of the Roghani cultivar had the lowest IC_{50} (46.27±2.76 µg/mL) which was significantly ($p<0.05$) different from the IC_{50} of BHT (112.90±14.81 µg/mL). This result indicated the higher antioxidant activity of the Roghani cultivar. In the DPPH radical scavenging method, the percent inhibition activity in all extracts and BHT significantly increased as the concentration increased ($p<0.05$).

According to the results presented in Table 1, the reducing power of all extracts and BHT was significantly increased as the concentration increased (p<0.05), which was related to increasing total phenolic compound content (25). Methanolic extract of the Roghani cultivar with a concentration of 75 µg/mL had the highest reducing power among the extracts. At the same concentration, all olive leaf extracts had significantly lower reducing power than BHT (p<0.05).

The content of secondary oxidation products (anisidine value) in treated sunflower oil samples stored at 60°C for 30 days was shown in Figure 2. The anisidine value decreased significantly (p<0.05) during the storage period in all samples as the concentration of the extracts increased. Adding olive leaf extracts lowered the anisidine value of sunflower oil in comparison with the sample without any antioxidant (p<0.05). The oil sample containing 1000 µg/mL methanolic extract of the Roghani cultivar had a lower anisidine value than the other groups across all storage days (p<0.05). In all experiment days, samples without antioxidants showed a higher anisidine value. The anisidine values of a 1000 µg/mL methanol extract of the Roghani cultivar and the sample without antioxidant were 23.15 and 33.39 on day 30 of storage, respectively.

The oxidation degree of sunflower oil was

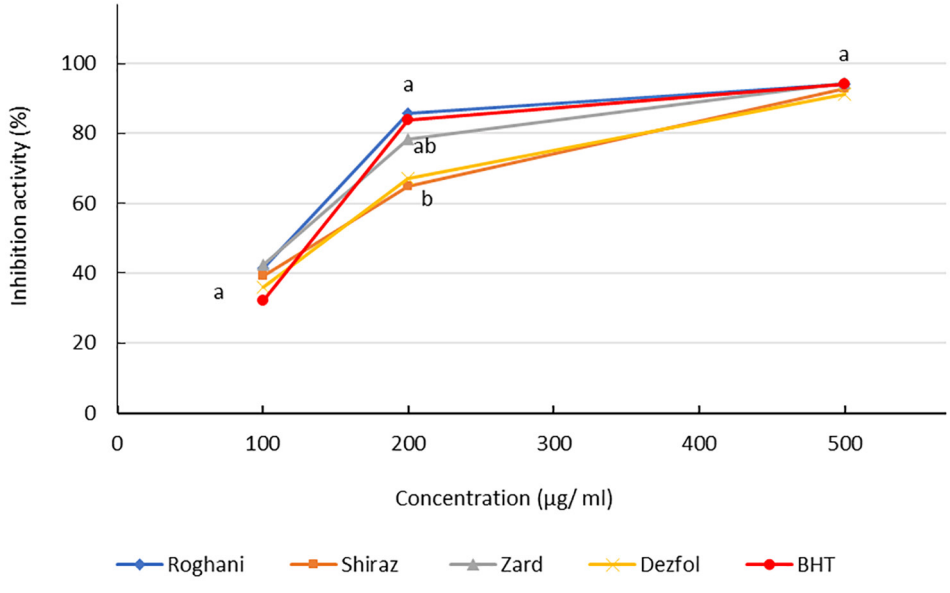

Figure 1: Inhibition activity of different concentrations of methanolic extracts of leaves of four cultivars of Iranian olive and synthetic antioxidant (butylated hydroxytoluene: BHT). Data are mean of three independent extracts. The different letters in the same concentration indicate significant differences ($p<0.05$).

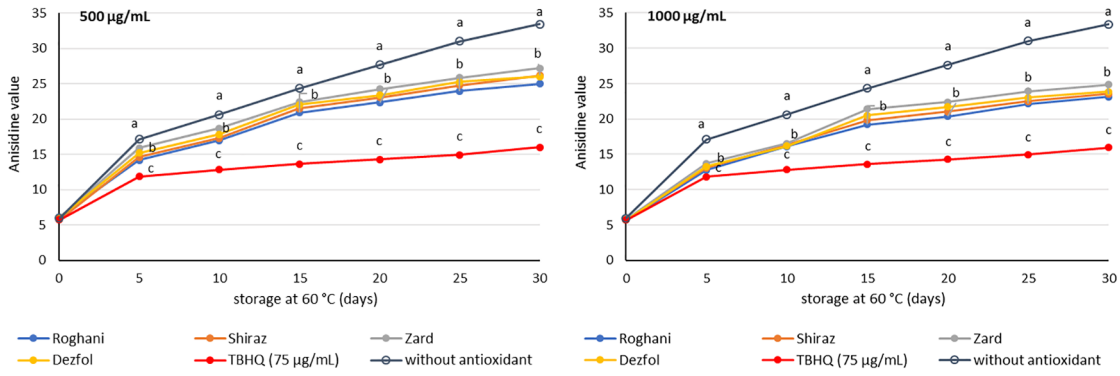

Figure 2: P-anisidine values of sunflower oil treated with 500 and 1000 µg/mL of methanolic extracts of leaves of four cultivars of Iranian olive and synthetic antioxidant (tertiary butyl hydroquinone: TBHQ) during storage at 60°C for 30 days. The different letters in the same sampling day indicate significant differences ($p<0.05$).

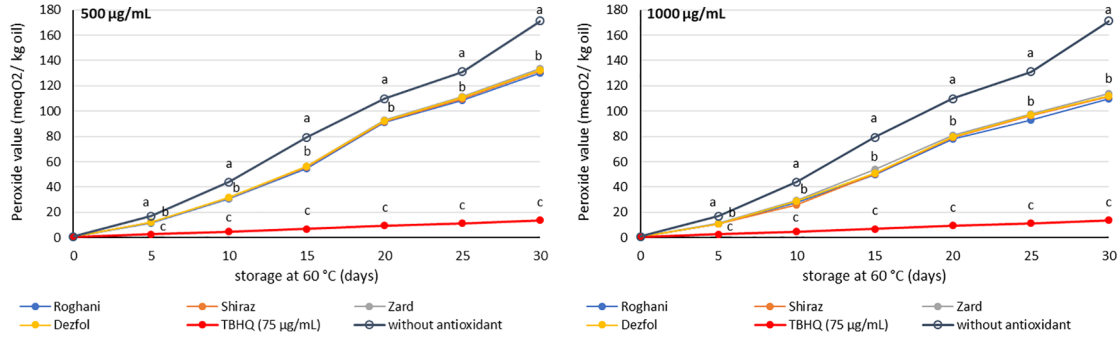

Figure 3: Peroxide value of sunflower oil treated with 500 and 1000 µg/mL of methanolic extracts of leaves of four cultivars of Iranian olive and synthetic antioxidant (tertiary butyl hydroquinone: TBHQ) during storage at 60°C for 30 days. The different letters in the same sampling day indicate significant differences ($p<0.05$).

determined by its peroxide value during storage for the antioxidant effect of olive leaf extracts at 500 and 1000 µg/mL concentrations and TBHQ at 75 g/mL concentration. The influence of methanol extracts on the peroxide value in sunflower oil samples was shown in Figure 3. As illustrated in Figure 3, the peroxide value of all samples increased with storage time. But this increased trend for samples with TBHQ was very slow compared to other groups. The peroxide value of the sunflower oil sample without any antioxidants reached a maximum value of 171.16 $meqO_2$/kg oil after 30 days of storage at 60°C (Figure 3), and in all days of the experiment, this group showed the higher peroxide value ($p<0.05$). During storage time, in all samples, as the concentration of the extracts increased, peroxide values significantly decreased ($p<0.05$).

Discussion

Sunflower oil enrichment has been studied using various natural antioxidant sources revealing that enriching sunflower oil with various natural antioxidants improves oil stability globally (26, 27). Phenolic compounds can chelate metal catalysts, scavenge free radicals, and retard the process of lipid oxidation. The antioxidant activity of olive leaf extract is directly affected by total phenols (28). Phenolic compound is one of the most important natural antioxidant components of plant origin. The total phenol content of olive leaf was determined in some researches. Nashwa *et al.* and Mohammadi *et al.* reported 90.48, and 206.81 mg GAE/g of sample, respectively, for total phenolic compounds in a methanol extract of olive leaf (29, 30). Rafiee *et al.* (2012) reported the total phenol content of the microwave-assisted methanol extract of olive leaf of the Roghani cultivar as 226.84 mg tannic acid equivalent (TAE)/g of extract (31). Differences in the total polyphenol composition of olive leaf extracts between diverse studies in the literature could be related to several parameters, such as the harvest season, olive leaf cultivar (32), geographical location, age of leaves, previous treatment of olive leaves (dried and ground), extraction procedures, and the final volume to which each extract was concentrated (33).

The reducing power of a compound might be a result of its hydrogen-donating capacity (34). The ability of the antioxidant compound to reduce Fe^{3+} to Fe^{2+} represents the reducing power of the antioxidant (35). Fe^{2+} can be monitored by measuring the formation of Perl's Prussian Blue at 700 nm (36). Reducing power will increase accordingly with the increase in absorbance. Our result is in agreement

with Rafiee *et al.* (31). Altemimi also reported that the percentage of reducing power was 143.3% and 183.8% for the methanol extract of the olive leaf and BHT, respectively (37). Nashwa and Abdel-Aziz and Altemimi measured the reducing power of the methanol extract of the olive leaves and found that there was a potential effect for this extract to be used as a reducing factor, and this result indicated the antioxidant activity of the methanolic extract of olive leaves (36, 37).

Peroxide value is one of the most widely used and important quality control measurements for food systems, especially edible oils, because it is a very sensitive indicator of the primary oxidation state of the oils (38). The application of the leaf extract of two cultivars (Coroneiki and Roghani) of olive plant in sunflower oil has been studied by Rafiee *et al.* (39). The results of the peroxide value and thiobarbituric acid index revealed that a methanol extract of Cronaiky at 1000 µg/mL could control the oxidation of sunflower oil in a manner comparable to BHA and BHT at 100 and 200 µg/mL levels.

Bouaziz *et al.* found the enrichment of olive oil with olive leaf extract resulted in an appreciable resistance to oxidative deterioration due to its phenolic antioxidant content (40). Salta *et al.* enriched commercially available oils (olive oil, sunflower oil, palm oil, and a vegetable shortening) with polyphenols by adding olive leaf extract (33). Results of their study showed that both antioxidant capacity and oxidative stability were substantially improved for all the oils studied after supplementation.

Conclusion

This study showed that the methanolic extracts of olive leaves had the ability to limit the oxidation of lipids and can be considered as a potential antioxidant source of natural origin. The methanolic extract of Roghani cultivar had the highest effect on the oxidative stability of sunflower oil.

Acknowledgment

This research was financially supported by Shahrekord University, which is gratefully acknowledged. The authors would like to thank Mr. Lotfipoor (an employee of the Nahangol oil company) for providing oil samples for this research and Mr. Khosravi for his technical assistance.

Conflict of Interest

None declared.

References

1 Jaski JM, Abrantes KK, Zanqui AB, et al. Simultaneous extraction of sunflower oil and active compounds from olive leaves using pressurized propane. *Curr Res Food Sci*. 2022;5:531-44. DOI: 10.1016/j.crfs.2022.03.002. PMID: 35295736.

2 Choe E, Min DB. Mechanisms and factors for edible oil oxidation. *Comprehen Rev Food Sci Food Safety*. 2006;5:169-86. DOI:10.1111/j.1541-4337.2006.00009.x.

3 Yanishlieva-Maslarova NV, in: Pokorny J, Yanishlieva N, Gordon M, editors. Antioxidants in Food: Practical Applications. Cambridge: Woodhead Publishing Ltd; 2001.p. 36-55.

4 Barlow SM. Toxicological aspects of antioxidants used as food additives. In: Food antioxidants. Dordrecht: Springer; 1990. p. 253-307.

5 Gharby S, Oubannin S, Ait Bouzid H, et al. An overview on the use of extracts from medicinal and aromatic plants to improve nutritional value and oxidative stability of vegetable oils. *Foods*. 2022;11:3258. DOI: 10.3390/foods11203258.

6 Somova LI, Shode FO, Ramnanan P, et al. Antihypertensive, antiatherosclerotic and antioxidant activity of triterpenoids isolated from Olea europaea, subspecies africana leaves. *J Ethnopharmacol*. 2003;84:299-305. DOI: 10.1016/s0378-8741(02)00332-x. PMID: 12648829

7 Samet I, Han J, Jlaiel L, et al. Olive (*Olea europaea*) leaf extract induces apoptosis and monocyte/macrophage differentiation in human chronic myelogenous leukemia K562 cells: insight into the underlying mechanism. *Oxid Med Cell Longev*. 2014;2014:927619. DOI: 10.1155/2014/927619. PMID: 24803988.

8 García-Villalba R, Larrosa M, Possemiers S, et al. Bioavailability of phenolics from an oleuropein-rich olive (*Olea europaea*) leaf extract and its acute effect on plasma antioxidant status: Comparison between pre-and postmenopausal women. *Eur J Nutr*. 2014;53:1015-27. DOI: 10.1007/s00394-013-0604-9. PMID: 24158653.

9 Gökmen M, Kara R, Akkaya L, et al. Evaluation of antimicrobial activity in olive (*Olea europaea*) leaf extract. *Am J Microbiol*. 2014;5:37-40. DOI: 10.3844/ajmsp.2014.37.40.

10 Brahmi F, Mechri B, Dabbou S, et al. The efficacy of phenolics compounds with different polarities as antioxidants from olive leaves depending on seasonal variations. *Industrial Crops Products*. 2012;38:146-152. DOI: 10.1016/j.indcrop.2012.01.023.

11 Erbay Z, Icier F. The importance and potential uses of olive leaves. *Food Rev Int.* 2010;26:319-334. DOI: 10.1080/87559129.2010.496021.

12 Gharby S, Oubannin S, Ait Bouzid H, et al. An overview on the use of extracts from medicinal

and aromatic plants to improve nutritional value and oxidative stability of vegetable oils. *Foods*. 2022;11:3258. DOI: 10.3390/foods11203258.

13 Niaounakis M, Halvadakis CP. Olive processing waste management: literature review and patent survey. 2nd ed. Elsevier; 2006.

14 Ranalli A, Contento S, Lucera L, et al. Factors affecting the contents of iridoid oleuropein in olive leaves (*Olea europaea* L.). *J Agric Food Chem*. 2006;54:434-40. DOI:10.1021/jf051647b. PMID: 16417301.

15 Brahmi F, Mechri B, Dhibi M, et al. Variations in phenolic compounds and antiradical scavenging activity of *Olea europaea* leaves and fruits extracts collected in two different seasons. *Industrial Crops and Products*. 2013;49:256-264. DOI: 10.1016/j.indcrop.2013.04.042.

16 Wang L, Weller CL. Recent advances in extraction of nutraceuticals from plants. *Trends Food Sci Technol*. 2006;17:300-12. DOI: 10.1016/j.tifs.2005.12.004.

17 Alvi T, Asif Z, Khan MK. Clean label extraction of bioactive compounds from food waste through microwave-assisted extraction technique-A review. *Food Bioscience*. 2022;29:101580. DOI: 10.1016/j.fbio.2022.101580.

18 Noormohammadi Z, Trujillo I, Belaj A, et al. Genetic structure of Iranian olive cultivars and their relationship with Mediterranean's cultivars revealed by SSR markers. *Scientia Horticulturae*. 2014;178:175-183. DOI: 10.1016/j.scienta.2014.08.002.

19 Abbasvali M, Esmaeili Koutamehr M, Moshtaghi H, et al. Effect of leaf extract of olive (Olea europaea) cultivars on Staphylococcus aureus. *Online J Vet Res*. 2015;19:519-529.

20 Pan X, Niu G, Liu H. Microwave-assisted extraction of tea polyphenols and tea caffeine from green tea leaves. *Chem Eng Process*. 2003;42:129-133. DOI: 10.1016/s0255-2701(02)00037-5.

21 Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am J Enol Viticul*. 1965;16:144-158.

22 Braca A, De Tommasi N, Di Bari L, et al. Antioxidant principles from bauhinia tarapotensis. *J Nat Products*. 2001;64:892-895. DOI: 10.1021/np0100845. PMID: 11473417.

23 Oyaizu, M. Studies on products of browning reaction. *Jap Nutr Diet*. 1986;44:307-315. DOI:10.5264/eiyogakuzashi.44.307.

24 American Oil Chemist's Society, Official methods and recommended practices of the American Oil Chemist's Society, 5th ed, American Oil Chemist's Society, S Champaign, IL (USA) 1998, Methods: Cd 8–53, Cd 18–90.

25 Afshari A, Sayyed-Alangi SZ. Antioxidant effect of leaf extracts from Cressa cretica against oxidation process in soybean oil. *Food Sci Nutr*. 2017;5:324-333. DOI: 10.1002/fsn3.396. PMID: 28265367.

26 Jiménez P, García P, Bustamante A, et al. Thermal stability of oils added with avocado (*Persea americana* cv. Hass) or olive (*Olea europaea* cv. Arbequina) leaf extracts during the French potatoes frying. *Food Chem*. 2017;221:123-129. DOI: 10.1016/j.foodchem.2016.10.051. PMID: 27979083

27 Turan S, Gunal Köroglu D, Kiralan M, et al. Enhancement of sunflower oil stability during deep-frying using extracts from olive oil by-products and soy lecithin. *Int Food Res J*. 2019;26:1269-1277.

28 Khemakhem I, Gargouri OD, Dhouib A, et al. Oleuropein rich extract from olive leaves by combining microfiltration, ultrafiltration and nanofiltration. *Separat Purificat Technol*. 2017;172:310-7. DOI: 10.1016/j.seppur.2016.08.003.

29 Nashwa MF, Abdel-Aziz ME. Efficiency of olive (*Olea europaea* L.) leaf extract as antioxidant and anticancer agents. *J Agroalim Process Technol*. 2014;20:46-53.

30 Mohammadi A, Jafari SM, Esfanjani AF, et al. Application of nano-encapsulated olive leaf extract in controlling the oxidative stability of soybean oil. *Food Chem*. 2016;190:513-519. DOI: 10.1016/j.foodchem.2015.05.115. PMID: 26213004.

31 Rafiee Z, Jafari SM, Alami M, et al. Antioxidant effect of microwave-assisted extracts of olive leaves on sunflower oil. *J Agric Sci Technol*. 2012;14:1497-1509.

32 Mitsopoulos G, Papageorgiou V, Komaitis M, et al. Total phenolic content and antioxidant activity in leaves and drupes of ten olive varieties. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*. 2016;44:155-161. DOI: 10.15835/nbha.44.1.10381.

33 Salta FN, Mylona A, Chiou A, et al. Oxidative stability of edible vegetable oils enriched in polyphenols with olive leaf extract. *Food Sci Technol Int*. 2007;13:413-421. DOI: 10.1177/1082013208089563.

34 Apak R, Güclü K, Özyürek M, et al. Mechanism of antioxidant capacity assays and the CUPRAC (cupric ion reducing antioxidant capacity) assay. *Microchimica Acta*. 2008;160:413-9. DOI: 10.1007/s00604-007-0777-0.

35 Abbasvali M, Ranaei A, Shekarforoush SS, et al. The effects of aqueous and alcoholic saffron

(*Crocus sativus*) tepal extracts on quality and shelf-life of pacific white shrimp (*Litopeneous vannamei*) during iced storage. *J Food Quality.* 2016;39:732-42. DOI: 10.1111/jfq.12225.

36 Mehrabani D, Nazempour M, Mehdinavaz-Aghdam R, et al. MRI tracking of human Wharton's jelly stem cells seeded onto acellular dermal matrix labeled with superparamagnetic iron oxide nanoparticles in burn wounds. *Burns Trauma.* 2022;10:tkac018. DOI:10.1093/burnst/tkac018. PMID:36380853.

37 ????

38 Zhang Y, Yang L, Zu Y, et al. Oxidative stability of sunflower oil supplemented with carnosic acid compared with synthetic antioxidants during accelerated storage. *Food Chem*. 2010;118:656-62. DOI: 10.1016/j.foodchem.2009.05.038.

39 Rafiei Z, Jafari SM, Alami M, et al. Antioxidant properties of olive leaf extract and its application in sunflower oil. *Res J Food*. 2011;21:11-23.

40 Bouaziz M, Fki I, Jemai H, et al. Effect of storage on refined and husk olive oils omposition: Stabilization by addition of natural antioxidants from Chemlali olive leaves. *Food Chem*. 2008;108:253-62. DOI: 10.1016/j.foodchem.2007.10.074.