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Artemisia absinthium L. flower extract as an antioxidant in sesame oil

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Abstract:
In this study, we investigated the effect of adding *Artemisia absinthium* L. flower extract on the properties of sesame oil.
At first, *A. absinthium* essential oil was extracted and analyzed for free radical scavenging power (DPPH), total phenols, flavonoids, and the main constituent compounds by gas chromatography and high-performance liquid chromatography. Then, 5 sesame oil samples were prepared, namely a control sample (without *A. absinthium* extract), samples with 0.5, 1, and 1.5% ethanolic extract of *A. absinthium*), as well as a sample containing tert-butyl hydro quinone. The samples were kept in an incubator at 40°C for 35 days. They were analyzed on days 0, 7, 14, 21, 28, and 35 for the values of peroxide, acid degree, thiobarbituric acid-reactive substances, *p*-anisidine, total oxidation, conjugated dienoic acid, and oxidative stability (Rancimat method).
As the storage period progressed, physical and oxidative changes increased in all the samples. On day 35, the control sample demonstrated high peroxide value, acid degree value, thiobarbituric acid-reactive substances, *p*-anisidine value, total oxidation index, as well as conjugated dienoic acid. These results were significantly ($p < 0.05$) higher than those in the sample with 1.5% *A. absinthium* extract. The extract had nearly the same protective effects as synthetic antioxidant tert-butyl hydro quinone. Thus, *A. absinthium* extract at the concentration of 1.5% was more effective than the other samples in reducing the rate of lipid oxidation in sesame oil.
A. absinthium extract demonstrated good potential as an effective natural antioxidant that is able to extend the shelf life of sesame oil.

Keywords: *Artemisia absinthium*, essential oil, extract, sesame, oxidative stability, lipid oxidation, natural antioxidant

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INTRODUCTION

Nowadays, vegetable oils are more commonly included in the diet due to their beneficial effects such as reducing blood cholesterol [1]. Sesame (*Sesame indicum* L.), also known as till or gingelli, is one of the oldest oil plants cultivated by humans. Thanks to its delicious oil, sesame is called the queen of oil seeds. Sesame oil has high nutritional value and is therefore quite expensive. This oil is more resistant and durable than other edible oils, with a high percentage of unsaturated fatty acids [2] and more unsaponifiable matter (about 2%). Its unsaponifiable substances include sterols, triterpenes and triterpene alcohols, tocopherols, and sesame lignans [3].

Oxidation is the main degradation reaction that occurs in most edible oils. Lipid oxidation primarily depends on the composition of fatty acids and the presence of minor components. This phenomenon leads to the production of primary and secondary oxidative compounds that are harmful to health. Lipid oxidation breaks down fatty acids, causes a decrease in food quality, and results in undesirable color, taste, and toxic compounds, lowering the product's consumer acceptance [4]. Antioxidants are used to prevent the destruction of food due to oxidation [5]. They should be inexpensive, nontoxic, effective at low concentrations (0.02–0.001%), and stable during processing and storage. Antioxidants do not have adverse effects on the color, taste, smell, or other food characteristics [6].

Antioxidants are substances that prevent oil oxidation by inhibiting the formation of free radicals or by stopping

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the release of free radicals. They are classified into two categories: synthetic and natural antioxidants. Synthetic antioxidants such as butylated hydroxyl toluene, butylated hydroxyl anisole, and tert-butyl hydro quinone (TBHQ) are very effective and therefore widely used in edible oils. However, due to possible toxic and carcinogenic effects, synthetic antioxidants must be limited in animal studies [7]. Therefore, natural antioxidants from plant sources may be used as a synthetic substitute in food to improve oxidative stability [8, 9]. Today, consumers generally prefer natural antioxidants to synthetic ones. To improve and preserve the sensory properties of food, natural antioxidants from spices and certain plants are now commonly used and their usage is widely accepted by consumers [10].

Artemisia absinthium, popularly known as common wormwood, belongs to the family *Asteraceae* (*Compositae*). The genus *Artemisia* consists of over 500 different species and is distributed in almost all temperate zones. Many *Artemisia* species have been studied for their phytochemical profiles and pharmacological properties. *A. absinthium* has a long history as a medicinal plan. It is native to temperate regions of Eurasia and Northern Africa but is rather rare in Algeria. *A. absinthium* contains an essential oil that is characterized by a particular color ranging from green to dark blue. One of its distinct features is the presence of large amounts of thujone, an oxygenated monoterpene, which can account for 40–90% of the oil. This compound is normally present in two isomeric forms, α- and β-thujone, and the latter is more abundant. Other important constituents of *A. absinthium* are non-volatile sesquiterpene lactones (particularly absinthin) which are responsible for the bitter taste of its extracts [11]. *A. absinthium* exhibits several pharmacological activities, including antimicrobial, insecticidal, antiviral, hypoglycemic, hepatoprotective, and anti-inflammatory. It is also effective in healing wounds and treating cardiovascular diseases. In addition, it has shown a broad-spectrum of antioxidant and anticancerous effects [12].

Pollution, stress, and industrial food consumption have recently increased the production of free radicals and antioxidants are the most effective compounds against them. There has been a lot of research into antioxidant characteristics of different plants and their derivatives such as essential oil and extracts. Incorporating natural antioxidants in human daily diet, as well as their use in clinical practices, has plenty of health benefits [13].

As far as we know, no publications have featured *A. absinthium* to enhance the oxidative stability of edible oils. Therefore, we aimed to stabilize sesame oil during storage by using *A. absinthium* extract as a potential alternative to synthetic antioxidants. This natural antioxidant can significantly increase the shelf life of sesame oil, which makes it a promising compound in the food industry and medicine.

STUDY OBJECTS AND METHODS

Plant material. Flowers of the *Artemisia absinthium* L. plant were collected in April 2022 from the natural habitats in Ardabil (Ardabil, Azerbaijan Province, Iran). This plant species was identified by the Botanical Herbarium of the Fars Agricultural and Natural Resources Education Center using reliable botanical sources.

Oil extraction from sesame seed. Sesame oil was obtained from a local cold-pressing company in Jahrom town (Zolal Cold Pressing, Fars Province, Iran).

Required chemicals. The chemicals included DPPH (2,2-diphenyl-1-picrylhydrazyl), Folin-Ciocalteu's reagent, gallic acid, ethanol, and aluminum chloride obtained from Merck (Germany), as well as other chemical substances and reagents of analytical grade purchased from Merck (Germany) and Sigma Aldrich (USA).

Required tools. The equipment and tools included a GC-MS device, a spectrophotometry device, a colorimeter, a refrigerator, a clevenger, a basket heater, a desiccator, an oven, a Benmari bath, a watch glass, a microplate, a test tube with a cap, a capillary tube, a microsampler, a head sampler, a Besher graduated cylinder pipette, an Erlen balloon, a 2-L sanded lid volume balloon, 50 volume balloons, 25 dark glasses, and a Falcon tube.

Extract and essential oil preparation. *A. absinthium* extract was obtained according to Amin *et al*. [14] and *A. absinthium* essential oil was extracted according to Msaada *et al*. [15]. For this, 50 g of the dry plant was mixed with 500 mL of water, and essential oil was extracted for 4 h by the distillation method using a Cloninger device. Then the oil was dehydrated with anhydrous sodium sulfate and stored in dark glass containers at –18°C until the test.

The composition of the essential oil was determined by gas chromatography according to Iranian National Standard No. 5192 and International Standard No. 7359.

Measurement of total phenolic content. The total phenolic content was determined spectrophotometrically using a Folin-Ciocalteau reagent as described by Iqbal *et al*. [16]. For this, 0.5 mL of the extract was mixed with 2.5 mL of 10-fold diluted Folin-Ciocalteau's reagent and 2 mL of 7.5% Na_2CO_3 in a volumetric flask, and purified water was added to reach the final volume of 50 mL. The samples were stored overnight and spectrophotometric analysis was performed at 765 nm. The results were expressed in microgram of gallic acid per mL of extract.

Measurement of flavonoids content. To measure the number of flavonoids in *A. absinthium* essential oil, a 1:1 ratio of a sample or standard and 2% aluminum chloride was poured into a test tube (1.5 mL of sample or standard + 1.5 mL of 2% aluminum chloride), kept in the dark for 60 min, and then read at 415 nm. The results were expressed as mg of quercetin per gram of extract, i. e. the final number obtained in terms of mg/L was divided by 1,000 and multiplied by 5 mL of the solvent volume, and then divided by 0.0015 g of extract. If needed, the extract was diluted 2–3 times (100 µL of extract with 100 µL of methanol or 50 µL of extract with 150 µL of methanol) [17].

Gas chromatography-mass spectrometry test. A mass spectrum was obtained by converting the components of a sample into fast-moving gaseous ions and

separating them based on mass-to-charge ratios. The essential oil prepared by the microwave method was dried again with sodium sulfate and diluted with 1 mL of hexane. After mixing, the sample with 1 µL of the solvent was mass-injected into the gas chromatography device connected to the spectrometer. Spectra of compounds were identified based on their inhibition index and compared to the spectra of standard compounds in the reference books and in the computer library. In this way, we identified the chemical compounds present in each sample of the essential oil. They were compared by drawing graphs based on their sub-peak levels.

Polyphenolic compounds by high-performance liquid chromatography. High-performance liquid chromatography (Agilent Technologies, 1200 series, Germany) was used to separate and determine the amounts of polyphenolic compounds in *A. absinthium* extract. The system was equipped with a Zarbax eclipse (XDB) C18 column with a length of 150 mm and an internal diameter of 4.6 mm. The column was filled with 5-µm particles and its temperature was 30°C. The wavelength of the UV detector was 280 nm. The flow rate was 1 mL/min, and the volume of each sample injected was 20 µL. The mobile phase consisted of two parts: A (methanol) and B (1% formic acid). The data were analyzed using the ChemStation software (version B.03.01, Santa Clara, CA, USA).

Antioxidant activity by DPPH. The DPPH free radical inhibition evaluation method was used to determine the antioxidant activity of the oil samples. The oil samples were prepared at concentrations of 10, 1, 0.1, 0.01, and 0.001 mg/mL chloroform. The DPPH solution was prepared at a concentration of 0.5×10 M in chloroform. To determine the antioxidant activity, 2 mL of each concentration was mixed with 1 mL of the DPPH test solution and homogenized with a stirrer. The prepared samples were kept for 1 h at 20°C in the dark. The spectrophotometer was zeroed using chloroform. After 1 h, the absorption of the oil samples was read using a UV2100PC spectrophotometer at a wavelength of 517 nm, and the absorption of the DPPH solution after 1 h was considered as a control. Finally, the inhibition percentage of the DPPH free radical activity was measured according to the modified method of Ozen *et al.* [17].

The inhibition concentration index (IC_{50}) was used to better evaluate the antiradical activity of the oil samples. To check the amount of total phenols in the essential oil, a certain volume of the solvent was added to a certain amount of the powdered plant (for example, 60 mg with 20 mL of solvent). The sample was sonicated for 20–30 min, kept for 24 h, and filtered. Then, 1.2 mg (0.0012 g) of the extract was taken and dissolved in 3 mL of distilled water. After that, 200 µL of methanol was poured and made up to volume with water, and the amount of methanol was subtracted from the final volume of 3 mL. In a glass vial, 0.0012 g of gallic acid + 200 µL of methanol + 3800 µL of distilled water were added to a volume of 4 mL to prepare a concentration of 300 mg/L.

Sesame oil preparation and storage conditions. Sesame oil was prepared and stored as described by Kahraman & Özdemir [18] with some changes. *A. absinthium* extract was added to the sesame oil samples at concentrations of 0.5, 1.0, and 1.5% (w/v). Tert-butyl hydro quinone (TBHQ) was added as a synthetic antioxidant (0.1%). The sesame oil sample without antioxidants (control) was considered a reference. All the samples were poured into dark glass bottles and kept in an incubator at 40°C for 35 days. The samples were evaluated on days 0, 7, 14, 21, 28, and 35 of storage to assess their physicochemical properties and oxidative stability. Each treatment had three replications [18].

Control treatment – Sesame oil without *A. absinthium* extract;

Treatment 1 = Sesame oil + 0.5% ethanolic extract of *A. absinthium*;

Treatment 2 = Sesame oil + 1% ethanolic extract of *A. absinthium*;

Treatment 3 = Sesame oil + 1.5% ethanolic extract of *A. absinthium*; and

Treatment 4 = Sesame oil + 0.1% TBHQ antioxidant.

Peroxide value. First, 1 g of a sample was weighed and mixed with 25 cm^3 of chloroform acetic acid with a 3:2 ratio of chloroform to acetic acid. Then, 0.5 cm^3 of a saturated potassium iodide solution, which was freshly prepared and placed in the dark, and 30 cm^3 of distilled water were added to the Erlen contents. The Erlenmeyer lid was closed and the flask was kept in the dark for 1 min. Then, 0.5 cm^3 of starch reagent 1 was added to it. The lid was closed and the solution was shaken vigorously. The released iodine changed the color of the solution, which was titrated with 0.01 normal thiosulfate solution. Then, the peroxide value (PV) was calculated as milliequivalent O_2/kg ($meqO_2$/kg) by using the following Eq. (1) [19]:

$$PV = \frac{\text{Volume of thiosulfate} \times \text{normality} \times 100}{\text{Sample weight}} \quad (1)$$

Acid degree value. American Oil Chemists' Society Standard No. 63-Cd 3d was used to determine the acid number of the oil. For this, 5 g of an oil sample was weighed in a 200 mL Erlenmeyer flask and mixed with 1,025 mL of a neutralized ethanol mixture and 2 mL of phenolphthalein. The mixture was stirred well so that the oil was completely dissolved in the resulting solution with 0.1 normal standard sodium hydroxide solution. The end point was tested. Then it was determined by the appearance and stability of the purple color, then the acid value (2% of oleic acid) was calculated using the following Eq. (2).

$$\text{Acid degree value} = \frac{\text{mL consumption benefit} \times \text{The benefit of normality} \times 56.1}{\text{Sample weight}} \quad (2)$$

***p*-anisidine value.** American Oil Chemists' Society Standard No. Cd 18-90 was used to determine the *p*-anisidine value. For this, 0.5 g of a sample was weighed in a 10-mL flask and then made up to volume with isooctane. The absorbance of the solution was read at 350 nm using a spectrophotometer. Isooctane was used as a control. Then, 5 mL of that solution was poured into one test tube, and 5 mL of the solvent was poured into another test tube. 1 mL of anisidine reagent was added to each test tube and mixed. After 10 min of absorption, the solvent in the first test tube was read at 350 nm (As), while the second tube solution was used as a control (Ab). The *p*-anisidine value (*p*AV) was calculated by the following Eq. (3):

$$pAV = \frac{25 \times (1.2As - Ab)}{m} \quad (3)$$

Total oxidation value (Totox index). The total oxidation value is used as a measure of antioxidant activity, making the evaluation more accurate. The Totox index was calculated by the following Eq. (4):

$$\text{Totox index} = 2PV + pAV \quad (4)$$

where PV is the peroxide value; *p*AV is the anisidine value [20].

The thiobarbituric acid reactive substances were determined according to the method described by Drinić *et al*. [21].

The conjugated dienoic acid in the sesame oil samples was measured according to the method described by Na *et al*. [22]. The amount of conjugated dienoic acid was calculated by the following Eq. (5):

$$\text{Conjugated dienoic fatty acid} = 0.84\left\{\frac{As}{(b \times c)} - 0.03\right\} \quad (5)$$

where *b* is the length of the cuvette, cm; and *c* is the concentration of the test sample, g/L [22].

Thermal oxidative stability. The autoxidation resistance was measured using a Rancimat apparatus (Metrohm, 743 Rancimat, Switzerland) at 110°C according to the method described by Mohanan *et al*. [23].

Statistical analysis. The experiments were performed in two replications, and the results were analyzed in a completely randomized design. Analysis of variance and statistical analysis were performed using SPSS23 and Excel software. The means were compared by using Duncan's multi-range test at a 5% level.

RESULTS AND DISCUSSION

Gas chromatography of *Artemisia absinthium* L. essential oil. Table 1 summarizes the gas chromatography-mass spectrometry (GC–MS) results for the *A. absinthium* essential oil. The test revealed 26 compounds that made up 100% of the total composition. The highest percentages were those for phenol, 2, 3, 5, 6-tetramethyl and caryophyllene (24.61 and 22.64%, respectively). The other compounds included columbin (5.8%), 2-dodecen-1-yl (–) succinic anhydride (4.52%), hexacosane (4.49%), *β*-guaiene (4.38%), phytol (3.3%), dodecanoic acid (2.91%), di-n-decylsulfone (2.78%), tetradecanoic acid (2.48%), and others. The lowest percentage was shown by bicyclo [3.3.1] nonan-3-ol,7-methylene (0.28%).

A. absinthium is one of the most widely distributed and commonly used aromatic plants in the world. Its volatile constituents have been investigated in Egypt, Tunisia, Algeria, Spain, Germany, Siberia, Lithuania, Turkey, and other countries. However, the countries' varying environmental conditions affected the plant's volatile constituents and, subsequently, the quality and biological activities of the oil [24]. Taherkhani *et al*. [25] identified 19 components in *A. absinthium* oil, including 1,8-cineole (36.46%), borneol (25.99%), and camphor (10.20%). Mohammed [24] reported the presence of 34 volatile constituents (based on GC-MS and GC-FID) in the distilled essential oil of *A. absinthium* in central Saudi Arabia. *Cis*-davanone had the highest percentage (52.51%) among all the constituents [24]. Another study found thujone and *trans*-sabinyl acetate as major constituents of the essential oils of *A. absinthium* [26], unlike our study.

Table 1 Chemical composition of *Artemisia absinthium* L. essential oil (GC–MS)

Chemical components	Amount, %
Bicyclo[3.3.1] nonan-3-ol, 7-methylene	0.28
Eucalyptol	1.45
Acetophenone	0.42
Santolina triene	1.37
(1S,3S,4S,5R)-1-Isopropyl-4-methylbicyclo[3.1.0]hexan-3-ol	1.31
Bicyclo[4.1.0] heptane, 7-(1-methylethylidene)	1.56
Nonanoic acid	1.08
Phenol, 2,3,5,6-tetramethyl	24.61
1-Octadecanesulphonyl chloride	1.46
n-Decanoic acid	1.89
(–)-*β*-Bourbonene	0.73
Z,Z,Z-1,4,6,9-Nonadecatetraene	0.75
Caryophyllene	22.65
Columbin	5.80
5,8,11,14-Eicosatetraenoic acid, methyl ester, (all-Z)	1.91
Dodecanoic acid	2.91
β-Guaiene	4.38
2-Dodecen-1-yl(–)succinic anhydride	4.52
Tetradecanoic acid	2.48
1-Decanol, 2-octyl	1.298
2-Methyltetracosane	0.92
n-Hexadecanoic acid	1.86
Phytol	3.30
Tetratetracontane	0.76
Hexacosane	4.49
Di-n-decylsulfone	2.78
Total	100.00

Other studies identified *β*-caryophyllene (9%) in *Artemisia annua* L. (*Asteraceae*) [27], *β*-caryophyllene (0.1%) in *Artemisia dracunculus* L. [28], and *trans*-*β*-caryophyllene (7.02%) in Romanian *A. annua* [29]. These contents, however, were lower than those in our study. The most abundant compounds reported by Khan *et al*. [30] were 7-Hydroxy-bicyclo[3.3.1] non-2-en-9-one (17.05%) and tocopherol *β*-d-mannoside (16.21%) in *Artemisia scoparia* extract. In the same study, GC-MS revealed oleic acid (41.45%) as most abundant in *A. absinthium* extract, while 2-furancarboxaldehyde, 5-(hydroxymethyl) was only 14.24%. The authors also analyzed *Artemisia indica* essential oil and found such compounds as ketone (42.1%), germacrene D (8.6%), borneol (6.1%), chrysanthenyl acetate (4.8%), *p*-cymene (2.7%), *α*-thujone (2.7%), and *β*-pinene (2.4%) [30]. A review indicated a wide chemical diversity in the composition of *A. indica*, which explains the variety of chemical structures identified in the oils of this genus [31].

As shown above, the concentrations of active constituents in *Artemisia* essential oils are seasonally and geographically different and some genotypes have particularly high contents of these components.

Liquid chromatography of *A. absinthium* extract. Table 2 shows 4 polyphenolic compounds identified by high-performance liquid chromatography in the *A. absinthium* extract. In particular, they were caffeic acid (63.40), *p*-coumaric acid (120.00), carvacrol (359.00), and *trans*-ferulic acid (275.37). Msaada *et al*. [15] also found similar polyphenolic compounds in *A. absinthium*, namely *p*-coumaric acid [leaves (3.84 ± 0.05%) and inflorescence (27.90 ± 0.06%)] and ferulic acid [leaves (4.54 ± 0.03%) and inflorescence (4.60 ± 0.03%)]. However, these compounds showed higher contents in our study.

Table 2 Polyphenolic compounds in *Artemisia absinthium* L. extract identified by high-performance liquid chromatography

Polyphenolic compound	Content, mg/L	Retention time, min
Sinapic acid	n. d.	16.50
Gallic acid	n. d.	3.30
Catechin	n. d.	8.30
Caffeic acid	63.40	11.60
Quercetin	n. d.	21.60
p-Coumaric acid	120.00	15.60
Coumarin	n. d.	17.40
Carvacrol	359.00	28.40
Vanilin	n. d.	13.50
trans-ferulic acid	275.37	16.30
Hesperedin	n. d.	18.50
Ellagic acid	n. d.	19.02
Eugenol	n. d.	23.70
Hesperetin	n. d.	22.40
Rosmarinic acid	n. d.	19.20
Thymol	n. d.	28.90

n. d. – not detected

Total phenolic and flavonoid contents and IC_{50} in *A. absinthium* extract. According to Table 3, total phenolics, total flavonoids, and IC_{50} in *A. absinthium* extract amounted to 0.028, 38.74, and 196.24 mg/L, respectively. The IC_{50} level in the extract was higher than in the gallic acid, which indicates lower antioxidant activity of the extract. Boudjelal *et al*. [32] measured total phenolic and flavonoid contents in *A. absinthium* extract as 180.33 ± 16.25 mg GAE/g DE and 165.47 ± 13.32 mg RE/g DE, respectively. These values were higher than those obtained in our study.

These differences may be related to many factors, including the extraction method, cultivation conditions, the type of extraction solvent, maturity of the plants, geographical location, environmental conditions, genetics, variety, part of the plant used, and the harvesting season [33].

Peroxide value. Figure 1 shows the comparison between average amounts of peroxide in different treatments (sesame oil samples containing *A. absinthium* extract). The results indicate a significant difference between the treatments ($p < 0.05$). Also, the larger the amount of the extract in the sample, the lower its peroxide value, which was statistically significant ($p < 0.05$).

On day 0 of storage, the peroxide values for all the treatments were almost equal and very insignificant ($p < 0.05$). With the passage of storage time, the peroxide values increased in all the treatments. However, the control treatment saw a significant rise in peroxide – from 0.029 ± 0.20 meq O_2/kg on day 0 to 12.06 ± 0.76 meq O_2/kg on day 35. At the end of storage, the lowest peroxide value belonged to the samples with tert-butyl hydro quinone (TBHQ) (0.934 ± 0.86 meq O_2/kg) and 1.5% *A. absinthium* extract (2.754 ± 0.86 meq O_2/kg).

The peroxide number is expressed as milliequivalents of oxygen per kg of oil. This index provides a measure of the amount of hydroperoxides based on the amount of active oxygen in the fat [34]. According to our results, the peroxide value of the control sample increased significantly until 35 days of storage. The peroxide value of the experimental samples (with *A. absinthium* extract and with TBHQ) did not grow significantly after 21 days of storage, which is because the rate

Table 3 Total phenolics, total flavonoids, and IC_{50} in *Artemisia absinthium* L. extract

Sample	Total phenolics, mg/mL	Total flavonoids, mg/mL	IC_{50}, mg/L
Artemisia absinthium L. extract	0.028 ± 0.001	38.74 ± 0.54	196.24 ± 0.49
Gallic acid	–	–	25.43

of decomposition of hydroperoxides is higher than the rate of their formation. Due to the instability of primary oxidation products, hydrogen peroxides are usually converted into secondary products such as aldehydes, alcohols, ketones, acids, dimers, trimers, polymers, and cyclic compounds [35]. Hras *et al.* [36] reported that the peroxide value of the sunflower oil sample containing 0.1% α-tocopherol decreased after one day of storage at 60°C. The rate of formation of hydroperoxides in that phase was reduced by their decomposition into secondary products.

Acid degree value. Figure 2 shows the comparison between average acid degree values in different treatments of sesame oil containing *A. absinthium* extract. On the 0 day of storage, the acidity values for all the treatments were almost equal and very insignificant ($p < 0.05$). Throughout storage, however, there was a significant difference between the treatments in terms of acid degree values ($p < 0.05$). Also, as the amount of the extract increased, the acid degree values decreased ($p < 0.05$). These values increased in all the treatments with the passage of storage time. Yet, the increase was the highest in the control treatment, from 0.038 ± 0.01 mL/g on day 0 to 11.99 ± 0.09 mL/g on day 35. In treatment 1 (0.5% ethanolic extract of *A. absinthium*), the acid degree values showed a significant increasing trend with increasing storage time. In treatment 2 (1.0% ethanolic extract of *A. absinthium*), the increase was lower compared to the other 2 treatments and in treatment 3 (1.5% ethanolic extract of *A. absinthium*), it was lower than in the other 3 treatments. Therefore, the protective effects of 1.5% *A. absinthium* extract were nearly the same as those of the widely used synthetic antioxidant TBHQ. On storage day 35, the lowest acid degree value belonged to the samples with TBHQ (0.85 ± 0.01 mL/g) and with 1.5% *A. absinthium* extract (1.15 ± 0.06 mL/g), with no significant difference. The acid number, which indicates the amount of free fatty acids, is obtained from the breakdown of oil triglycerides and free fatty acids during storage, which is an important parameter for measuring pungency in foods [37]. The study by Eshghi *et al.* [38] showed that all the treatments containing different concentrations of curcumin were more effective in preventing an increase in acidity than the control sample without antioxidants. The authors also found that higher concentrations of curcumin increased the antioxidant effect and reduced the acidity of the samples. According to Mohd Nor *et al.* [39], *Curcuma longa* L. leaf extract was effective in reducing the amount of free fatty acids after 24 h of frying. Although the occurrence of hydrolysis was very low, their amount was 0.25% after 32 h of storage at 180°C and 0.39% after frying at 180°C for 40 h, which was significant.

***p*-Anisidine value**. Figure 3 compares average amounts of *p*-anisidine in different treatments of sesame oil containing *A. absinthium* extract. On the 0 day of storage, the amounts of *p*-anisidine in all the treatments were almost equal and very insignificant ($p < 0.05$). Yet, our results showed a significant difference between the treatments throughout storage ($p < 0.05$). Also, the sam-

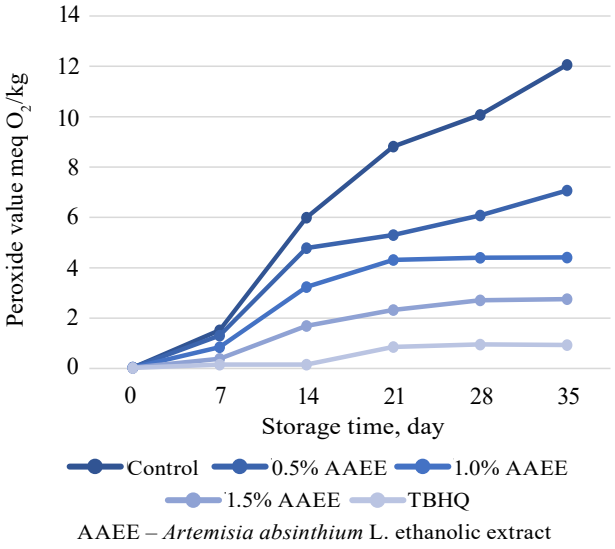


Figure 1 Peroxide values in sesame oil during storage

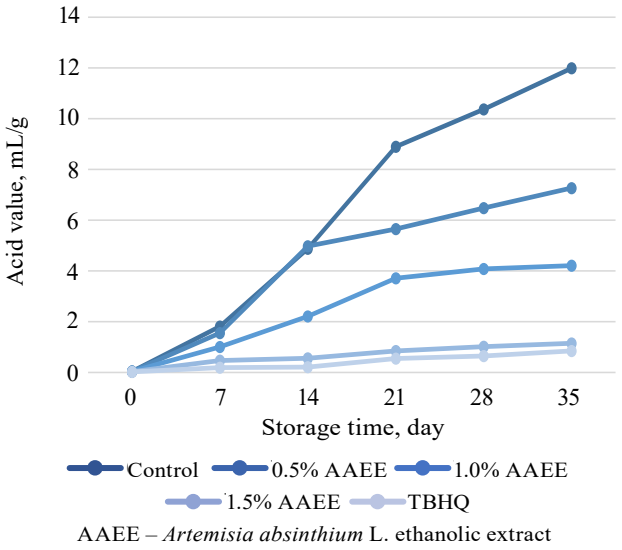


Figure 2 Acid degree values in sesame oil during storage

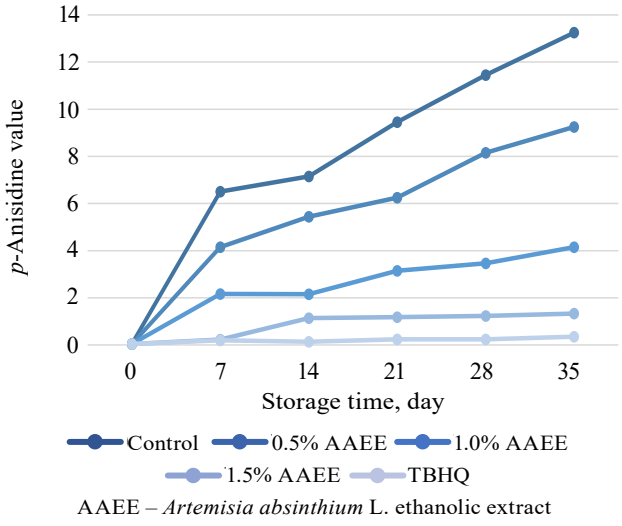


Figure 3 *p*-Anisidine values in sesame oil during storage

ples with larger amounts of the extract had lower *p*-anisidine values, with a significant difference between the samples ($p < 0.05$). With the passage of storage time, *p*-anisidine values increased in all the treatments. The control treatment showed a significant rise in this index, from 0.04 ± 0.02 on day 0 to 13.25 ± 0.59 on day 35. The lowest amounts of *p*-anisidine were found in the sesame oil samples with TBHQ (0.35 ± 0.03) and those with 1.5% *A. absinthium* extract (1.33 ± 0.23). According to the results, all the concentrations of *A. absinthium* extract (0.5%, 1%, and 1.5%) were able to reduce the *p*-anisidine values of the samples. They also decreased the rate of oxidation in terms of the formation of hydroperoxides. Higher concentrations of the extract contributed to lower *p*-anisidine values, with a significant difference between the samples ($p < 0.05$).

The *p*-anisidine value plays an important role in the oxidation of edible oils and fats. Its calculation is one of the oldest methods for measuring secondary oxidation products. This method is based on the reactivity of the bond of carbonyl aldehyde with the amino group of *p*-anisidine, leading to the formation of compounds that absorb at the wavelength of nanometers [35]. Ayoughi *et al.* [40] stated that secondary oxidation products, especially in the aldehyde structure, were not significantly formed until the storage time of 12 days. The *p*-anisidine value of the control sample and citric acid increased more rapidly on the 12 day. During this time, the formation of hydroperoxides was reduced, and they were broken down into secondary oxidation products. Kalahrodi *et al.* [41] reported a higher antioxidant effect of fennel seed extract on the stability of sunflower oil in the first week (concentration 300 ppm), compared to that of the synthetic antioxidants. These antioxidants decreased the number of anisidine (first week = 7 and fourth week = 17.5) and peroxide in the oil samples.

Total oxidation value (Totox index). Figure 4 compares average total oxidation values in different treatments of sesame oil containing *A. absinthium* extract.

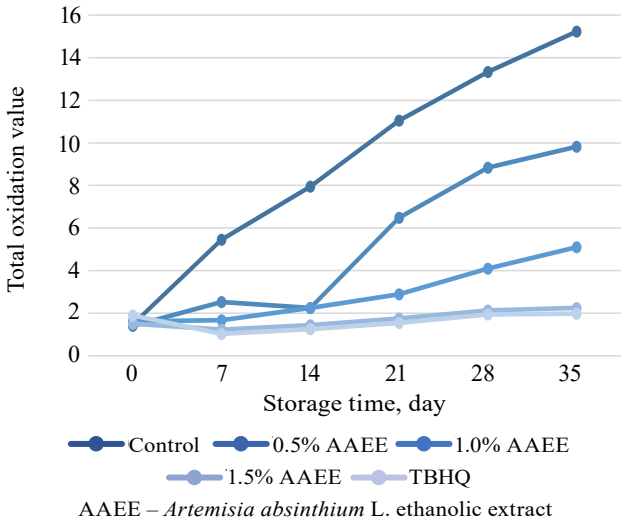


Figure 4 Total oxidation indexes in sesame oil during storage

On the 0 day of storage, the Totox index values were almost equal in all the treatments and very insignificant ($p < 0.05$). Yet, there was a significant difference between the treatments throughout storage ($p < 0.05$). Also, larger amounts of the extract decreased the Totox index, with a significant difference between the samples ($p < 0.05$). With the passage of storage time, the values of this index increased in all the treatments. However, the highest rise was observed in the control treatment, from 1.47 ± 0.01 on day 0 to 15.23 ± 0.22 on day 35 ($p < 0.05$). At the end of the storage time, the lowest Totox index values were found in the samples with TBHQ (1.98 ± 0.86) and 1.5% *A. absinthium* extract (2.25 ± 0.81), with no significant difference between the samples.

The Totox index measures the amount of hydroperoxides and their decomposition products, showing the progress of oxidation of oils and fats. This index is a combination of peroxide and *p*-anisidine value, and the antioxidant activity of essential oils depends on their constituent compounds [3, 42]. Ruberto & Baratta [43] reported that thymol and carvacrol are more effective in reducing the rate of primary and secondary oxidation of lipid than other oxygenated monoterpenes. The activity of thymol and carvacrol was similar to that of *α*-tocopherol. In fact, the antioxidant activity of many essential oils is due to the presence of thymol and carvacrol. Kalhorodi *et al.* [44] similarly stated that the use of fennel seed extract (at ppm levels of 100, 200, 300, 400, 500, 600, 700, and 800) in soybean oil reduces the Totox index. In their research, the 300 and 400 ppm levels of the extract had higher antioxidant activity than butylated hydroxyl toluene and butylated hydroxyl anisole, and the Totox index after 14 days was reported between 200–300 ppm for different samples.

Thiobarbituric acid index. Figure 5 shows the analysis of the thiobarbituric acid index in the sesame oil samples fortified with different concentrations of *A. absinthium* extract during 35 days of storage. The values of the thiobarbituric acid index significantly increased in all the sesame oil samples ($p < 0.05$). However, the highest increase was observed in the control

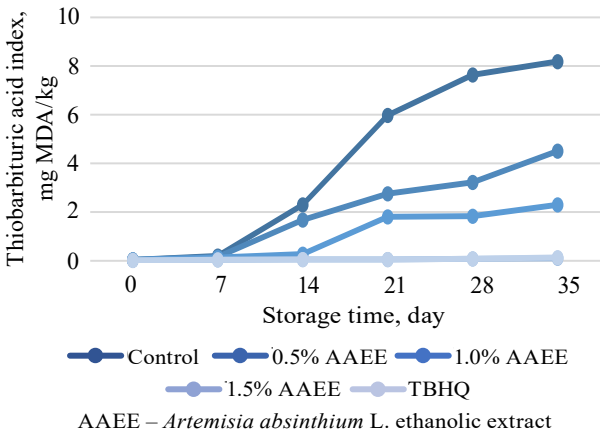


Figure 5 Thiobarbituric acid indexes in sesame oil during storage

treatment, from 0.037 ± 0.02 mg MDA/kg on day 0 to 8.188 ± 0.71 mg MDA/kg on day 35. At the end of storage, the lowest thiobarbituric acid index belonged to the sample with 1.5% *A. absinthium* extract (0.102 ± 0.03 mg MDA/kg) and with TBHQ (0.138 ± 0.03 mg MDA/kg).

Our results were consistent with those reported by Samad Louei *et al.* [45], who evaluated the antioxidant activity of phenolic compounds extracted from pomegranate seeds with acetone solvent (at 100, 200, and 350 ppm levels) in the stabilization of soybean oil in accelerated conditions (at 60°C). In their study, the highest level of antioxidant activity was related to the highest level of phenolic compounds (350 ppm), and the amount of TBA on day 12 was 0.3587 mg/kg/MA. In a similar study, Ayoughi *et al.* [46] evaluated the antioxidant activity of dill (*Anethum grareolens* Boiss.) essential oil in soybean oil by measuring peroxide and TBA values. They concluded that dill essential oil at 0.6 mg/mL could prevent the production of primary and secondary oxidation products in crude soybean oil, which is almost equal to butylated hydroxyl anisole chemical oxidation at 0.1 mg/mL. Özcan & Arslan [13] presented evidence that hazelnut oil samples containing clove, cinnamon, and rosemary essential oils at 0.25 and 0.5% could effectively delay the formation of primary oxidation products compared to the control sample. Furthermore, peppermint extract enhanced the stability of extra virgin olive oil. Hashemi *et al.* [47] also observed that the essential oil of ajwain (aniseed) reduced the oxidation rate of sunflower oil.

Conjugated dienoic acid. Figure 6 compares average amounts of conjugated dienoic acid in different treatments of sesame oil containing *A. absinthium* extract. On the 0 day of storage, the conjugated dienoic acid values were almost equal in all the treatments and very insignificant ($p < 0.05$). Yet, there was a significant difference between the treatments throughout storage ($p < 0.05$). Also, larger amounts of the extract decreased the amount of conjugated dienoic acid, with a significant difference between the samples ($p < 0.05$).

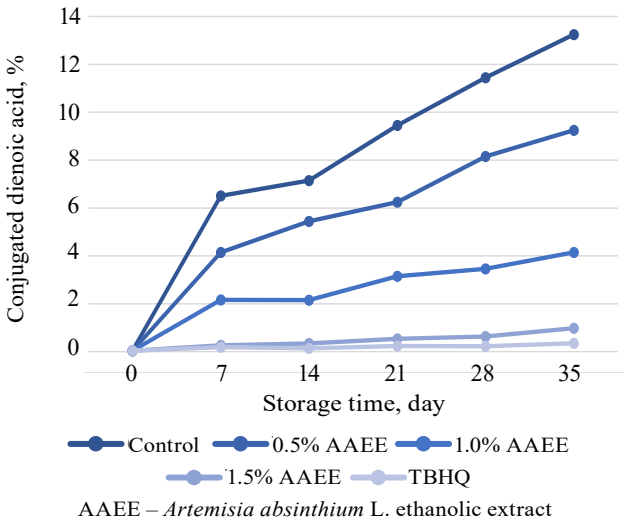


Figure 6 Conjugated dienoic acid in sesame oil during storage

At each storage level (day 7, 14, 21, 28, and 35), the values of conjugated dienoic acid significantly increased in all the treatments ($p < 0.05$). However, the highest increase was found in the control treatment, from 0.04 ± 0.03 on day 0 to 13.25 ± 0.45 on day 35. The lowest values of the acid were observed in the samples containing the antioxidant TBHQ (0.35 ± 0.02) and 1.5% *A. absinthium* extract (0.98 ± 0.05), with no significant difference between them.

Conjugated dienoic acid forms and accumulates quickly in polyunsaturated oil. The oil compounds react with oxygen to form conjugated hydroperoxides [48]. The measurement of conjugated dienoic acid is a good indicator of the oxidation stability of oil samples. When the rate of degradation of hydroperoxides or conjugated dienes exceeds the rate of their formation, peroxide or conjugated diene numbers can decrease, even if oxidation increases during storage [42]. The conjugated diene number is a measure of primary oxidation products created during the oxidation of fats, with a high correlation with the peroxide number [49]. The structure of a conjugated diene is similar to that of hydroperoxide. Therefore, the absorbance at 232 nm shows a good correlation with the peroxide number due to the formation of primary oxidation products [50]. The conjugated diene number increases or decreases in a similar way to the peroxide number [48]. As a result, the process of peroxide formation coincides with the formation of conjugated dienes [51].

Oxidative stability of sesame oil with *A. absinthium* extract measured by Rancimat method. Table 4 shows the average oxidative stability of sesame oil samples containing *A. absinthium* extract, as determined by the Rancimat method. On the 0 day of storage, the oxidative stability of the samples increased with larger amounts of the extract, which was statistically significant ($p < 0.05$). The highest level of oxidative stability was observed in the samples containing TBHQ and 1.5% *A. absinthium* extract, while the lowest stability was found in the samples without the extract ($p < 0.05$).

Oil stability determined by the Rancimat method is a direct measure of changes in oxidation resistance of

Table 4 Effects of *Artemisia absinthium* L. extract on the oxidative stability, h, of sesame oil during storage

Treatment	1	2
Control	6.40 ± 0.34^{Ae}	5.80 ± 0.75^{Be}
Treatment 1	11.50 ± 0.19^{Ad}	9.70 ± 0.46^{Bd}
Treatment 2	15.70 ± 0.25^{Ac}	13.50 ± 0.34^{Bc}
Treatment 3	19.90 ± 0.15^{Ab}	17.30 ± 0.52^{Bb}
Treatment 4	23.50 ± 0.05^{Aa}	22.50 ± 0.42^{Ba}

The numbers are expressed as mean ± standard deviation. Different lowercase letters indicate significance in the column ($p < 0.05$). Different uppercase letters indicate significance in the row ($p < 0.05$). Control (without *A. absinthium* extract), Treatment 1 – 0.5% ethanolic extract of *A. absinthium*, Treatment 2 – % ethanolic extract of *A. absinthium*, Treatment 3 – 1.5% ethanolic extract of *A. absinthium*, and Treatment 4 – containing the antioxidant TBHQ

oils. This parameter shows the degree of degradation of the oil during heating. In general, the Rancimat method is often used to evaluate or predict oxidative stability in thermal conditions, and determines the induction time or resistance time [52]. Until today, most researchers have attributed the significant oxidative stability of sesame oil to the presence of non-saponifiable lignans in it. However, Langyan *et al*. [53] reported that when sesamol alone was combined with vegetable oils, the stability of the resulting oil increased slightly, but this stability was much lower than that obtained by adding sesame oil extract containing the same amount of sesamol. This shows that sesame extract contains other antioxidant compounds besides sesamol, which participate in the stability of oils. Previously, Gharby *et al*. [54] reported a powerful stabilizing effect of compounds such as sesamulin (an antioxidant precursor), sesamol, sesaminol and its isomers, which are exclusively present in sesame seed oil, during the frying process. They showed that sesaminol and related isomers are formed from sesamulin by intermolecular transfer under anhydrous conditions and in the presence of an acid. During cooking and in the presence of moisture, sesamolin breaks down into sesamol through protonolysis and an oxonium ion is formed. When the moisture is removed by heat, these compounds are bonded at carbon number 2 with the carbon-carbon bond to form sesaminol and related isomers through intermolecular transfer. It was also proven that both sesamol and sesaminol have an important synergistic effect with tocopherol during the thermal oxidation of oils. It should be mentioned that sesaminol and sesamol act preferentially as radical scavengers and therefore reduce the degradation of tocopherol in the oil used in the food preparation process.

CONCLUSION

The growing concern regarding the adverse effects of synthetic antioxidants on human health has increased the desire to use effective and affordable natural antioxidants. In this study, *Artemisia absinthium* L. extract improved the oxidation stability of sesame oil during storage. Our results showed that, under different accelerated conditions, the oxidation level of sesame oil increased with increasing storage time. The addition of the extract significantly reduced the oxidation rate of the oil samples compared to the control.

Our study showed that *A. absinthium* extract can be used as a natural antioxidant in vegetable oils such as sesame oil because of its bioactive compounds (e. g., carvacrol). Also, larger amounts of the extract decreased the values of peroxide, acid degree, total oxidation, thiobarbituric acid, conjugated dienoic acid, and *p*-anisidine, with a significant difference between the samples ($p < 0.05$). The protective effects of 1.5% *A. absinthium* extract were nearly the same as those of the widely used synthetic antioxidant tert-butyl hydro quinone (TBHQ). This means that the extract has nearly the same potential as TBHQ to delay the formation of secondary oxidation products and preserve free fatty acids during the storage period. Thus, our study demonstrated that *A. absinthium* extract can be used as an effective natural antioxidant to increase the shelf life of sesame oil.

CONTRIBUTION

The authors were equally involved in writing the manuscript and are equally responsible for any potential plagiarism.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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