http://www.cjmb.org
doi 10.34172/cjmb.2024.3003
Open Access Original Article
Crescent Journal of Medical and Biological Sciences
Vol. x, No. x, xx 2024, x–x
eISSN 2148-9696

Effect of Some Herbal Essential Oils on the Content of Compounds With Pharmacological Properties and Oxidative Stability of Black Cumin Seed Oil

Rogaieh Soltani[1], Sodeif Azadmard-Damirchi[2*], Mahdi Gharekhani[1], Mohammadali Torbati[3]

Abstract

Objectives: Thymoquinone is present in Nigella sativa L. seeds which is responsible for the majority of the pharmacological properties. Although synthetic antioxidants are effective in retarding oil oxidation, their use is being limited due to their potential negative health effects.

Materials and Methods: In this study, the effect of essential oils of rosemary, pennyroyal, thyme, mint, peppermint, mastic gum, and frankincense as natural antioxidants in concentrations of 250, 500, 750, and 1000 ppm in comparison with tert-butyl-hydroquinone (TBHQ) (100 ppm) on the quality characteristics of black seed oil and its thymoquinone content during 90 days of storage was studied.

Results: The results showed that the incorporation of essential oils was more effective than TBHQ in increasing oxidative stability and reducing peroxide and acid values. Among essential oils, rosemary was more effective in increasing the oxidation stability index. Rosemary (750 ppm) and mastic gum (500 ppm) were more effective in controlling the peroxide value. The addition of essential oils to black seed oil did not result in a significant alteration ($P > 0.05$) in the thymoquinone content. However, it did inhibit the decomposition and loss of this compound.

Conclusions: It was concluded that essential oils could be a good alternative to TBHQ for increasing the black seed oil stability, shelf life and preservation. Given the numerous pharmaceutical applications of black seed oil, the findings of this study can be utilized to improve the stability of black seed oil and formulations containing it, as well as to enhance the stability of thymoquinone, which holds therapeutic potential, during storage.

Keywords: Natural antioxidants, Essential oils, Black cumin seed oil, Oxidative stability, Therapeutic

Introduction

Due to its distinctive compounds, black cumin (*Nigella sativa* L.) seed oil possesses numerous pharmaceutical, medical, and food applications. It has health-promoting effects, such as anti-hypertensive, anti-microbial, anti-cancer, and anti-inflammatory properties. It is used traditionally for the treatment of disorders such as rheumatoid arthritis, malnutrition, and diseases such as asthma and bronchitis (1). The problem with black cumin seed oil is its low oxidative stability. It has a high peroxide value compared to other vegetable oils, even at the time of extraction (2). A method proposed to enhance oxidative stability is the application of synthetic antioxidants such as *tert*-butyl-hydroquinone (TBHQ), Butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA). However, increasing consumer awareness of the potential health risks of using synthetic antioxidants has led manufacturers to replace synthetic antioxidants with natural ones (3). Plant-derived extracts and essential oils contain high amounts of terpenoids and polyphenols and have become very popular for use as natural antioxidants.

Plants belonging to the *Lamiaceae* family are aromatic and medicinal, with their essential oils being utilized in both modern and traditional medicine, as well as in the food industry (4). *Rosmarinus officinalis* is a shrub of the *Lamiaceae* family and the main compounds of its essential oil are 1,8-cineolel and α-pinene, comprising about 26.5% and 20%, respectively (5). Rosmarinic acid, carnosic acid, and carnosol are the main diterpenes of the rosemary essential oil with a great antioxidative property (6).

Mentha is a genus of the *Lamiaceae* family with 25-30 species. *M. spicata*, *M. piperita*, and *M. pulegium* are important species of the genus *Mentha*. The major compounds of the *M. spicata* (mint) essential oil are limonene, 1,8-cineol, and dihydrocarvone. Carvone, which is found in high amounts in *M. spicata* causes a special odor and makes *M. spicata* a widespread spice. *M. piperita* (peppermint) is a hybrid spp. (*aquatica* ×*spicata*). Menthol, menthone, and their derivatives are the major compounds of *M. piperita* essential oil that give a cooling feeling. *M. piperita* has application in flavoring confectionery, chewing gums, and pharmaceuticals like toothpaste, cold candy, and mouthwash (7). *M. pulegium*, commonly known as pennyroyal, is native to Europe,

Received 17 June 2022, **Accepted** 26 March 2023, **Available online** 8 December 2023

[1]Department of Food Science and Technology, Tabriz Branch, Islamic Azad University, Tabriz, Iran
[2]Department of Food Science and Technology, Faculty of Agriculture, University of Tabriz, Tabriz, Iran
[3]Department of Food Science and Technology, Faculty of Nutrition, Tabriz University of Medical Sciences, Tabriz, Iran
*Corresponding Author: Sodeif Azadmard-Damirchi, Tel: +98-41 33392032, Email: sodeifazadmard@yahoo.com

Key Messages

- *Nigella sativa* L. seeds has high content of thymoquinone which is responsible for the majority of the pharmacological properties of these seeds and its oil.
- *Nigella sativa* L. seeds has high content of oil with several positive health effects, but with low stability. Incorporating essential oils to this oil can enhance its stability and formulations containing this oil and also increasing stability of thymoquinone with a therapeutic potential during storage.

North Africa, and the Middle East. According to the region of cultivation, three main groups of components are identified in *M. pulegium* essential oil, including pulegone, piperitenone (and/or piperitone), and isomenthone/neoisomenthol (8). In addition to being used traditionally for the treatment of cold, *M. pulegium* methanolic extract is applicable in increasing the oxidative stability of vegetable oils (9).

Zataria multiflora Boiss. (Shirazi thyme) belongs to the genus *Thymus* and the main compounds of its essential oil are thymol, carvacrol, *ρ*-cymene, and *γ*-terpinene (6). Mastic gum of the *Pistacia atlantica* and frankincense oleogum resin of the *Boswellia serrata* are aromatic resins with antioxidative properties that have been considered in recent years (10). This study aimed to compare the effect of natural antioxidants including the essential oils of rosemary, pennyroyal, thyme, mint, peppermint, mastic gum, and frankincense, with a synthetic antioxidant (TBHQ) on the oxidative stability of black cumin seed oil.

Materials and Methods

Nigella sativa L. seeds, *R. officinalis, M. spicata, M. piperita, M. pulegium, Z. multiflora,* mastic gum, and frankincense were purchased from the local market (Tabriz, Iran). Thymoquinone, KOH, KI, starch, chloroform, acetic acid, methanol, TBHQ were obtained from Sigma–Aldrich Chemie (Steinheim, Germany).

Extraction of Essential Oils From Herbs

Dried herbs were powdered using a hand mill and passed through a 40-mesh sieve. To extract the essential oils, the powdered herbs were heated with the distilled water in a Clevenger apparatus and were dehumidified using sodium sulfate anhydrous. Obtained essential oils were kept at -18 ºC (11).

Extraction of Black Cumin Seed oil and Preparation of the Samples

Black cumin seeds were cleaned from debris and were cold-pressed using a screw press (model 85 mm) at 10 MPa (12,13). Immediately after the oil extraction, the synthetic antioxidant TBHQ at a concentration of 100 ppm and essential oils obtained from *R. officinalis, M. spicata, M. piperita, M. pulegium, Z. multiflora,* mastic gum, and frankincense at the concentrations of 250, 500, 750, and 1000 ppm were added to the oil samples. The oil sample with no added antioxidant (synthetic or natural) was used as a control sample. For further analysis, all the oil samples were kept in dark glass bottles in a dark place at room temperature for 90 days. They were analyzed at day of preparation and in intervals of 30 days.

Rancimat Test

The oxidative stability index (OSI) of the samples was measured using a Metrohm Rancimat (model 734, USA). 2.5 g of oil samples were subjected to 110 ºC and the induction time was measured. The protection factor (PF) was calculated as the ratio of induction time of the antioxidant-containing sample to the induction time of the control sample (the black cumin seed oil with no added antioxidant) (14).

Determination of the Acid Value

The AOCS method was used to evaluate the acid value every 30 days (14). In berif, oil sample (20 g) was dissolved in 100 ml of methanol: chloroform (50:50 v/v), then few drops of phenolphthalein were added. The mixture then titrated by NaOH (0.1 N) to appear the pink color.

Acid value = (V*N*40)/m

Where V: volume of the KOH used for titration, N: Normality of NaOH, m: Oil sample weight

Determination of the Peroxide Value

The peroxide value (PV) of the samples was evaluated according to the AOCS standard method (Cd 8-53) at intervals of 30 days (14). In brief, oil sample (5 g) were dissolved in chloroform and acetic acid. Next, KI (0.5 mL) was added to the mixture and kept in dark for 1 minute. Following this, 30 mL of water and 1 mL of starch solution were added to the mixture and mixed very well. Then the mixture was titrated with *sodium thiosulphate* (0.1 N).

PV = (a-b)*N*1000/m

Where a: volume of the *sodium thiosulphate* used for oil sample, b: volume of the *sodium thiosulphate* used for oil sample, N: Normality *sodium thiosulphate*, m: Oil sample weight

Determination of the Thymoquinone Content

The High-performance liquid chromatography (HPLC) equipment (Agilent 1200, Palo Alto, USA) was used for the quantification of thymoquinone content of samples (15).

Statistical Analysis

Experiments were performed in triplicate and the data were expressed as the mean values. The SPSS software (SPSS Inc., Chicago, IL) and a completely randomized design were used for the analysis of data, and the differences among the mean values were evaluated using a

Duncan test at the probability level of 5%.

Results

Results indicated that the control sample without added antioxidants had the lowest oxidative stability (13 hours). The addition of the synthetic antioxidant and/or essential oils increased the oxidative stability of the black seed oil significantly ($P < 0.05$) from 17 hours to 20 hours (Table 1).

Acid value at day of oil extraction was 1.8 (mg KOH/g oil) (Table 2). The free fatty acids formation increased in all samples as a result of hydrolysis of triacylglycerols in the black cumin seed oil during the storage to the value of 7.7 (mg KOH/g oil) (Table 2).

In this study, the peroxide value of the black seed oil was 4.2 (meq O_2/kg oil) on the first day. The peroxide value of all samples increased during the storage. The peroxide value of the control sample increased 14.5-fold during 3 months of storage and reached to the 61(meq O_2/kg oil), while it was increased 1.58-3.7-fold in samples incorporated with essential oils. Results indicated that essential oils successfully prevented a sharp increase in the peroxide value during storage (Table 3).

Results of thymoquinone by HPLC showed that its amount in the oil samples at extraction day was 1660-1684 (mg/kg oil). It was decreased during the storage (Table 4). The reduction in the control sample was higher and the amount of thymoquinone reached to 1230 (mg/ kg oil). Higher amount of thymoquinone after 90 days of storage was for oil samples containing pennyroyal, frankincense and mastic gum essential oil (Table 4).

Discussion

The Rancimat test is an accelerated method to determine the oxidative stability and quality of oils, in which the concurrent effect of heat and oxygen exposure is studied. Determination of OSI or induction time can also be used to compare the efficiency of different antioxidants in oils (11). Higher values of OSI or PF > 1 indicate better protection against oxidation (16).

Based on our results, control sample had the lowest oxidative stability (13 hours), which indicates that this sample is not suitable for high temperatures. All of the

Table 1. Effect of the Addition of Essential Oils and TBHQ Into the Black Cumin Seed Oil on the OSI Value (h) and PF

Sample	Concentration (ppm)	OSI	PF
Control	Without antioxidant	13.1^{f}	1^{f}
TBHQ	100	17.6^{e}	1.34^{e}
Rosemary	250	20.9^{a}	1.59^{a}
	500	20.9^{a}	1.59^{a}
	750	21^{a}	1.6^{a}
	1000	20.4^{ab}	1.55^{ab}
Pennyroyal	250	19.2^{c}	1.46^{c}
	500	20.1^{b}	1.53^{b}
	750	20.3^{b}	1.54^{b}
	1000	20.1^{b}	1.53^{b}
Thyme	250	20.5^{ab}	1.56^{ab}
	500	20^{bc}	1.52^{b}
	750	20^{bc}	1.52^{b}
	1000	18.16^{d}	1.38^{d}
Mint	250	18.48^{c}	1.43^{c}
	500	19.36^{bc}	1.47^{c}
	750	19.04^{b}	1.45^{c}
	1000	19.04^{b}	1.45^{c}
Peppermint	250	19.28^{b}	1.47^{c}
	500	17.92^{d}	1.36^{d}
	750	19.04^{b}	1.45^{c}
	1000	17.28^{de}	1.31^{e}
Mastic gum	250	18.45^{c}	1.4^{d}
	500	19.05^{b}	1.45^{c}
	750	16.88^{ef}	1.28^{g}
	1000	17.92^{d}	1.36^{d}
Frankincense	250	20.1^{a}	1.53^{b}
	500	19.28^{b}	1.47^{c}
	750	19.1^{b}	1.45^{c}
	1000	19.44^{ab}	1.48^{c}

Means within a column with different letters are significantly different ($P < 0.05$) according to the Duncan test.
OSI: Oxidative stability index; PF: Protection factor.

Table 2. Effect of the Incorporation of Essential Oils and TBHQ on the Acid Value (mg KOH/g oil) of Black Cumin Seed Oil During Storage

Sample	Concentration (ppm)	Storage Time (days)			
		1	30	60	90
Control	Without antioxidant	1.8^{aD}	2.5^{cdC}	4.4^{dB}	7.7^{aA}
TBHQ	100	1.8^{aD}	2.6^{cC}	4.3^{dB}	7.8^{aA}
Rosemary	250	1.8^{aD}	2.7^{cC}	4.2^{deB}	7.5^{bA}
	500	1.8^{aD}	2.7^{cC}	4.1^{eB}	7.5^{bA}
	750	1.9^{aD}	2.6^{cC}	4.4^{dB}	7.5^{bA}
	1000	1.9^{aD}	2.8^{bcC}	4.2^{deB}	7.5^{bA}
Pennyroyal	250	1.8^{aD}	2.4^{dC}	4^{eB}	6.8^{cA}
	500	1.7^{aD}	2.3^{dC}	3.8^{fB}	6.8^{cA}
	750	1.8^{aD}	2.5^{cdC}	3.9^{efB}	6.9^{cA}
	1000	1.8^{aD}	2.5^{cdC}	3.9^{efB}	6.8^{cA}
Thyme	250	1.7^{aD}	2.6^{cC}	4.3^{dB}	7.4^{bA}
	500	1.7^{aD}	2.5^{cdC}	4.3^{dB}	7.8^{aA}
	750	1.8^{aD}	2.5^{cdC}	4.2^{deB}	7.5^{bA}
	1000	1.8^{aD}	2.7^{cC}	4^{eB}	7.4^{bA}
Mint	250	1.9^{aD}	2.7^{cC}	3.9^{efB}	7^{cA}
	500	1.9^{aD}	2.7^{cC}	4.2^{deB}	7.37^{bA}
	750	1.8^{aD}	2.8^{bcC}	4.5^{cdB}	6.69^{cA}
	1000	1.9^{aD}	2.6^{cC}	3.8^{fB}	7.29^{bA}
Peppermint	250	1.9^{aD}	3^{abC}	4.3^{dB}	7.78^{aA}
	500	1.8^{aD}	2.8^{bcC}	4.1^{eB}	7.07^{cA}
	750	1.8^{aD}	2.8^{bcC}	4.2^{deB}	7.51^{bA}
	1000	1.9^{aD}	2.7^{cC}	3.5^{gB}	6.83^{cA}
Mastic gum	250	1.8^{aD}	2.8^{bcC}	4.3^{dB}	7.15^{cA}
	500	1.8^{aD}	3^{abC}	4.8^{cB}	7.79^{aA}
	750	1.8^{aD}	2.9^{bC}	4.1^{eB}	7.16^{cA}
	1000	1.9^{aD}	2.4^{dC}	4^{eB}	7.07^{cA}
Frankincense	250	1.9^{aD}	2.9^{bC}	4.2^{deB}	7.78^{aA}
	500	1.8^{aD}	2.9^{bC}	5.8^{bB}	7.2^{bcA}
	750	1.8^{aD}	3^{abC}	6^{abB}	6.7^{cA}
	1000	1.9^{aD}	3.2^{aC}	6.2^{aB}	7.4^{bA}

Means within a column with different lowercase letters and means within a row with different capital letters are significantly different ($P < 0.05$) according to the Duncan test.

Table 3. Effect of the Incorporation of Essential Oils and TBHQ on the Peroxide Value (mEq O_2/kg oil) of Black Cumin Seed Oil During Storage

Sample	Concentration (ppm)	Storage Time (days)			
		1	30	60	90
Control	Without antioxidant	4.2^{aD}	20.7^{aC}	48.3^{aB}	61.13^{aA}
TBHQ	100	4.1^{aD}	5.2^{gC}	6^{gB}	7.3^{hA}
Rosemary	250	4.1^{aD}	5.4^{gC}	6.3^{gB}	8.19^{gA}
	500	4.3^{aD}	5.5^{gC}	7.1^{fB}	8.41^{gA}
	750	4.2^{aD}	4.9^{gC}	6.2^{gB}	7.1^{iA}
	1000	4.2^{aD}	7.4^{eC}	10^{cB}	13.1^{cA}
Pennyroyal	250	4.1^{aD}	6.3^{fC}	7.5^{fB}	11.6^{deA}
	500	4^{aD}	7^{eC}	11.2^{bB}	14.8^{bA}
	750	4.2^{aD}	6.9^{eC}	10.8^{bB}	13.1^{cA}
	1000	4.2^{aD}	7.1^{eC}	9.6^{dB}	12.7^{cA}
Thyme	250	4.1^{aD}	5^{gC}	7.1^{fB}	8.3^{hA}
	500	4.3^{aD}	5.4^{gC}	7.5^{fB}	8.8^{fB}
	750	4.2^{aD}	5.1^{gC}	6.2^{gB}	7.8^{hA}
	1000	4^{aD}	8^{cdC}	10.3^{cB}	13.3^{cA}
Mint	250	4.1^{aD}	7.3^{eC}	9.4^{dB}	11.45^{eA}
	500	4.15^{aD}	7.1^{eC}	$9..25^{dB}$	10.75^{fA}
	750	4.11^{aD}	7.2^{eC}	9.2^{dB}	10.7^{fA}
	1000	4.11^{aD}	6.8^{fC}	7.9^{eB}	9.5^{gA}
Peppermint	250	4.25^{aD}	8.2^{cC}	9.5^{dB}	11.66^{deA}
	500	4.3^{aD}	7.9^{dC}	9.9^{cdB}	12^{dA}
	750	4.2^{aD}	8.7^{bC}	10^{cB}	12.41^{dA}
	1000	4.2^{aD}	8^{cdC}	9.3^{dB}	12.5^{cdA}
Mastic gum	250	4.1^{aD}	5.1^{gC}	6.9^{fB}	7.3^{hA}
	500	4.11^{aD}	5.2^{gC}	6^{gB}	6.5^{iA}
	750	4.2^{aD}	7.7^{dC}	8.14^{eB}	11.31^{eA}
	1000	4.1^{aD}	8.1^{cC}	9.52^{dB}	12.8^{cA}
Frankincense	250	4.3^{aD}	8.2^{cC}	9.1^{dB}	10^{fA}
	500	4.1^{aD}	6.2^{fC}	8.5^{eB}	9.46^{gA}
	750	4.2^{aD}	6.4^{fC}	8^{eB}	9.79^{gA}
	1000	4^{aD}	7.2^{eC}	9.2^{dB}	10.85^{fA}

Means within a column with different lowercase letters and means within a row with different capital letters are significantly different ($P < 0.05$) according to the Duncan test.

essential oils at all concentrations were more effective than TBHQ in increasing the OSI and had higher PF than it (Table 1).

It has been shown that the rosemary essential oil (400 ppm) was more effective in retarding the oxidation in vegetable oils such as soybean, cottonseed, and rice bran, compared to the 50:50 mixture of BHA and BHT (200 ppm) (16). It has also been reported that rosemary essential oil and/or peppermint essential oil at a concentration of 3000 ppm were more effective in increasing the oxidative stability of pistachio oil than BHT (100 ppm). Evidence shows that rosemary extract had the highest protective effect against oxidation of pistachio oil and in combination with peppermint essential oil, synergistically increased the protective effect of peppermint essential oil (11).

Reports indicate that antioxidants resistant to volatility or heat loss are more effective in retarding oxidation under extreme conditions of a Rancimat (17). Among the essential oils, rosemary (at all added concentrations) and thyme (at 250 ppm) had the greatest effect on the oxidative stability of the black cumin seed oil. With exceeding a certain concentration, the protection effect of thyme, peppermint, mastic gum, and frankincense essential oils on the black cumin seed oil was decreased. It could be related to the pro-oxidative effect of some essential oils and phenolic compounds at higher concentrations (18).

Results indicated that the addition of essential oils did not have a great effect on the acidity of black cumin seed oil (Table 2). It has been reported that despite the increase in acidity during the 7-month storage period, there was no significant difference between the acidity of the control sample of extra virgin olive oil and the oil-flavored with rosemary, hot pepper, garlic, and mint (18). Black cumin seed oil samples had an acid value less than the maximum allowed limit established by Codex Alimentarius for cold-pressed oil samples (4 > mg KOH/g oil) by day 30, but from day 60 the amount of acid value exceeded the maximum limit (19). Therefore, more studies are needed to control the increase in acidity in black seed oil. It has been reported that roasting the seeds before oil extraction, by inactivating lipase and lipoxygenase enzymes, can be used as a way to control the increase in acid value in black cumin seed oil (20).

The black cumin seed oil has many health-promoting

Table 4. Effect of the Incorporation of Essential Oils and TBHQ on the thymoquinone Content (ppm) of Black Cumin Seed Oil During Storage

Sample	Concentration (ppm)	Storage time (days)			
		1	30	60	90
Control	Without antioxidant	1670^{aA}	1450^{dB}	1400^{cC}	1230^{dD}
TBHQ	100	1660^{aA}	1581^{bB}	1504^{bC}	1458^{bcD}
Rosemary	250	1672^{aA}	1550^{cB}	1502^{bB}	1408^{cC}
	500	1684^{aA}	1557^{bB}	1500^{bC}	1401^{cD}
	750	1680^{aA}	1604^{bB}	1564^{abB}	1499^{bC}
	1000	1677^{aA}	1602^{bB}	1580^{aB}	1491^{bC}
Pennyroyal	250	1670^{aA}	1650^{aA}	1600^{aB}	1573^{aB}
	500	1667^{aA}	1650^{aA}	1602^{aB}	1586^{aB}
	750	1665^{aA}	1641^{aAB}	1610^{aBC}	1580^{aC}
	1000	1660^{aA}	1653^{aA}	1624^{aAB}	1591^{aB}
Thyme	250	1681^{aA}	1594^{bB}	1541^{bC}	1493^{bC}
	500	1674^{aA}	1590^{bB}	1583^{aB}	1492^{bC}
	750	1676^{aA}	1602^{bB}	1598^{aB}	1490^{bC}
	1000	1661^{aA}	1625^{abA}	1601^{aB}	1499^{bC}
Mint	250	1682^{aA}	1551^{cB}	1523^{bB}	1419^{cC}
	500	1687^{aA}	1568^{bcB}	1523^{bB}	1414^{cC}
	750	1678^{aA}	1604^{bB}	1564^{abB}	1499^{bC}
	1000	1679^{aA}	1602^{bB}	1580^{aB}	1491^{bC}
Peppermint	250	1674^{aA}	1650^{aA}	1610^{aB}	1580^{aB}
	500	1668^{aA}	1600^{bB}	1509^{bC}	1480^{bC}
	750	1675^{aA}	1607^{bB}	1524^{bC}	1450^{bcD}
	1000	1669^{aA}	1610^{bB}	1571^{aB}	1400^{cC}
Mastic gum	250	1684^{aA}	1691^{aA}	1541^{bB}	1493^{bC}
	500	1684^{aA}	1600^{bB}	1583^{aBC}	1550^{aC}
	750	1679^{aA}	1559^{cB}	1507^{bB}	1442^{cC}
	1000	1681^{aA}	1600^{bB}	1581^{aC}	1519^{bD}
Frankincense	250	1671^{aA}	1609^{bB}	1588^{aBC}	1564^{aC}
	500	1664^{aA}	1601^{bB}	1572^{aBC}	1550^{aC}
	750	1673^{aA}	1600^{bB}	1562^{abB}	1561^{aB}
	1000	1670^{aA}	1613^{bB}	1554^{bC}	1570^{aC}

Means within a column with different lowercase letters and means within a row with different capital letters are significantly different ($P < 0.05$) according to the Duncan test.

and nutritional properties. However, it has low oxidative stability and has a higher peroxide value than other vegetable oils from the very beginning of extraction (2). Therefore, it is recommended to control the increment of peroxide value in this oil. The high peroxide value of black seed oil may be due to the high activity of the lipoxygenase enzyme in the seed (21). It has been reported that pre-treatment of seeds by microwave radiation and roasting are useful methods in controlling peroxide value and oxidation in black seed oil (12,13).

Mastic gum at a concentration of 500 ppm and rosemary essential oil at a concentration of 750 ppm had the highest efficiency in controlling the peroxide value. The inhibition of peroxide formation by essential oils was concentration-dependent and increased with increasing concentration, but sometimes this trend was reversed after a critical concentration. It may be due to the pro-oxidant activity of the essential oils at concentrations higher than the critical concentration (18). This critical concentration was 750 ppm for rosemary and thyme essential oils and 500 ppm for mastic gum. These results were consistent with the results obtained from the Rancimat test. It has been reported that rosemary essential oil with the high antioxidative property was able to retard the oxidation of flaxseed oil compared to α-tocopherol and/or BHT (22).

According to the maximum acceptable level of peroxide value for cold-pressed oils (<15 meq O_2/kg oil) established by CODEX Alimentarius, all the samples containing natural (essential oils) and synthetic (TBHQ) antioxidants had an acceptable peroxide value during the whole 3-months storage (19). However, the control sample containing no antioxidant committed the CODEX standard only on the first day of storage.

Thymoquinone, the main component of black seed essential oil, is a volatile phenolic compound. It has a strong antioxidative property and is responsible for the biological and health-promoting effects of black seed and its oil (22, 23). As thymoquinone is a unique compound of the black seed oil, incorporation of essential oils and/or the synthetic antioxidant did not have a significant ($P > 0.05$) effect on the thymoquinone content of samples (Table 4).

Thymoquinone is a highly sensitive compound that decomposes rapidly under high temperature

and light exposure and produces free radicals (1, 22). The thymoquinone content of all samples decreased significantly ($P < 0.05$) during the 90 days of storage. The same trend has been reported for black seed oil and its blends with sunflower oil (21). The rate of thymoquinone reduction was highest in the control sample and decreased from 1670 ppm to 1230 ppm after 90 days of storage. The addition of essential oil to black seed oil significantly reduced ($P < 0.05$) the decomposition rate of thymoquinone during storage. This may be due to the phenolic compounds of essential oils with antioxidative properties that had a protective effect on thymoquinone.

Also, black cumin seed and its oil have many applications in pharmaceutical and cosmetic industries, results of this study could also be used in the food industry as well. Using different oilseeds in the formulations of foods has been increasing to enhance their functional and health promoting effects (24-27) and results of this study could give more wide opportunity to use black cumin seed oil in the different food product, pharmaceutical and cosmetic formulations.

Conclusions

The incorporation of herbal essential oils into the black cumin seed oil increased oxidative stability. In some concentrations, essential oils performed even better than TBHQ. Rosemary was more effective in increasing the OSI and controlling the hydroperoxide formation. The antioxidative effect of essential oils was concentration-dependent. However, rosemary, thyme, and mastic gum acted as pro-oxidants when used further than a certain concentration and caused an increase in peroxide value compared to the lower concentrations. It was concluded that herbal essential oils increased the oxidative stability of the black cumin seed oil during storage at dark and room temperature. In addition, herbal essential oils were proposed as good alternatives for the synthetic antioxidant, TBHQ. Results of this study could be used in the future research on the stability of black cumin seed oil in the formulations of the different medicinal and cosmetic products.

Directions for Future Research

In future research, it is recommended to assess the impact of distinct and unadulterated forms of black cumin seed bioactive compounds on various diseases. Additionally, it is suggested to investigate the influence of incorporating essential oils on the detailed composition of black cumin seed oil.

Authors' Contribution

Conceptualization: Sodeif Azadmard-Damirchi and Mehdi Gharakhani.
Formal analysis: Roghayeh Soltani.
Investigation: Sodeif Azadmard-Damirchi and Mehdi Gharakhani.
Methodology: Roghayeh Soltani, Sodeif Azadmard-Damirchi and Mehdi Gharakhani.
Project administration: Sodeif Azadmard-Damirchi and Mehdi Gharakhani.
Supervision: Sodeif Azadmard-Damirchi and Mehdi Gharakhani and Mohammadali Torbati.
Validation: Sodeif Azadmard-Damirchi and Mehdi Gharakhani.
Writing–original draft: Roghayeh Soltani.
Writing–review & editing: Mohammadali Torbati.

Conflict of Interests

Authors have no conflict of interest.

Ethical Issues

This study was a part of Ph.D. thesis approved by Islamic Azad University, Tabriz Branch with a Registration No: 162285902.

Financial Support

This research received no funding.

References

1. Salem ML. Immunomodulatory and therapeutic properties of the *Nigella sativa* L. seed. Int Immunopharmacol. 2005;5(13-14):1749-1770. doi:10.1016/j.intimp.2005.06.008
2. Kıralan M. Changes in volatile compounds of black cumin (*Nigella sativa* L.) seed oil during thermal oxidation. Int J Food Prop. 2014;17(7):1482-1489. doi:10.1080/10942912.2012.723231
3. Augustyniak A, Bartosz G, Cipak A, et al. Natural and synthetic antioxidants: an updated overview. Free Radic Res. 2010;44(10):1216-1262. doi:10.3109/10715762.2010.508495
4. Mamadalieva NZ, Akramov DK, Ovidi E, et al. Aromatic medicinal plants of the Lamiaceae family from Uzbekistan: ethnopharmacology, essential oils composition, and biological activities. Medicines (Basel). 2017;4(1):8. doi:10.3390/medicines4010008
5. Jiang Y, Wu N, Fu YJ, et al. Chemical composition and antimicrobial activity of the essential oil of rosemary. Environ Toxicol Pharmacol. 2011;32(1):63-68. doi:10.1016/j.etap.2011.03.011
6. Keramat M, Golmakani MT, Aminlari M, Shekarforoush S. Oxidative stability of virgin olive oil supplemented with *Zataria multiflora* Boiss. and *Rosmarinus officinalis* L. essential oils during accelerated storage. J Food Process Preserv. 2017;41(3):e12951. doi:10.1111/jfpp.12951
7. Singh P, Pandey AK. Prospective of essential oils of the genus *Mentha* as biopesticides: a review. Front Plant Sci. 2018;9:1295. doi:10.3389/fpls.2018.01295
8. Mahboubi M, Haghi G. Antimicrobial activity and chemical composition of *Mentha pulegium* L. essential oil. J Ethnopharmacol. 2008;119(2):325-327. doi:10.1016/j.jep.2008.07.023
9. Sarikurkcu C, Eryigit F, Cengiz M, Tepe B, Cakir A, Mete E. Screening of the antioxidant activity of the essential oil and methanol extract of *Mentha pulegium* L. from Turkey. Spectrosc Lett. 2012;45(5):352-358. doi:10.1080/00387010.2012.666701
10. Murugesan K, Ravi kumar G, Jain SH, Mohan S, Nair SG. Wood exudates: an overview on aromatic gums and resins. J Indian Acad Wood Sci. 2011;8(2):72-75. doi:10.1007/s13196-012-0046-7
11. Esmaeili M, Goli SAH, Shirvani A, Shakerardakani A. Improving storage stability of pistachio oil packaged in different containers by using rosemary (*Rosmarinus officinalis* L.) and peppermint (*Mentha piperita*) essential oils. Eur J Lipid Sci Technol. 2018;120(4):1700432. doi:10.1002/ejlt.201700432
12. Mazaheri Y, Torbati M, Azadmard-Damirchi S, Savage GP. Oil extraction from blends of sunflower and black cumin seeds by cold press and evaluation of its physicochemical properties. J Food Process Preserv. 2019;43(10):e14154. doi:10.1111/jfpp.14154
13. Mazaheri Y, Torbati M, Azadmard-Damirchi S, Savage GP. Effect of roasting and microwave pre-treatments of *Nigella sativa* L. seeds on lipase activity and the quality of the oil. Food Chem. 2019;274:480-486. doi:10.1016/j.foodchem.2018.09.001
14. American Oil Chemists' Society (AOCS). Official Methods and

Recommended Practices of the AOCS. 5th ed. Champaign: AOCS; 2009.
15. Ghosheh OA, Houdi AA, Crooks PA. High performance liquid chromatographic analysis of the pharmacologically active quinones and related compounds in the oil of the black seed (*Nigella sativa* L.). J Pharm Biomed Anal. 1999;19(5):757-762. doi:10.1016/s0731-7085(98)00300-8
16. Yang Y, Song X, Sui X, et al. Rosemary extract can be used as a synthetic antioxidant to improve vegetable oil oxidative stability. Ind Crops Prod. 2016;80:141-147. doi:10.1016/j.indcrop.2015.11.044
17. Farhoosh R, Niazmand R, Rezaei M, Sarabi M. Kinetic parameter determination of vegetable oil oxidation under Rancimat test conditions. Eur J Lipid Sci Technol. 2008;110(6):587-592. doi:10.1002/ejlt.200800004
18. Gambacorta G, Faccia M, Pati S, Lamacchia C, Baiano A, La Notte E. Changes in the chemical and sensorial profile of extra virgin olive oils flavored with herbs and spices during storage. J Food Lipids. 2007;14(2):202-215. doi:10.1111/j.1745-4522.2007.00080.x
19. Codex Alimentarius. Standard for Named Vegetable Oils-CXS 210-1999. Codex Alimentarius; 2019.
20. Suri K, Singh B, Kaur A, Yadav MP, Singh N. Impact of infrared and dry air roasting on the oxidative stability, fatty acid composition, Maillard reaction products and other chemical properties of black cumin (*Nigella sativa* L.) seed oil. Food Chem. 2019;295:537-547. doi:10.1016/j.foodchem.2019.05.140
21. Kiralan M, Ulaş M, Özaydin A, et al. Blends of cold pressed black cumin oil and sunflower oil with improved stability: a study based on changes in the levels of volatiles, tocopherols and thymoquinone during accelerated oxidation conditions. J Food Biochem. 2017;41(1):e12272. doi:10.1111/jfbc.12272
22. Lutterodt H, Luther M, Slavin M, et al. Fatty acid profile, thymoquinone content, oxidative stability, and antioxidant properties of cold-pressed black cumin seed oils. LWT Food Sci Technol. 2010;43(9):1409-1413. doi:10.1016/j.lwt.2010.04.009
23. Ashrafi A, Azadmard-Damirchi S, Hesari J. Quality of oil extracted by cold press from *Nigella sativa* seeds incorporated with rosemary extracts and pretreated by microwaves. Green Process Synth. 2023;12(1):20228149. doi:10.1515/gps-2022-8149
24. Mazandarani M. Aut ecology, total phenol and total flavonoid content, antioxidant activity and ethno-pharmacological survey of *Nigella sativa* Linn. in traditional medicine of Golestan province, north of Iran. Crescent J Med Biol Sci. 2015;2(3):95-99.
25. Hashempour-Baltork F, Torbati M, Azadmard-Damirchi S, Savage GP. Quality properties of sesame and olive oils incorporated with flaxseed oil. Adv Pharm Bull. 2017;7(1):97-101. doi:10.15171/apb.2017.012
26. Hashempour-Baltork F, Torbati M, Azadmard-Damirchi S, Savage GP. Quality properties of puffed corn snacks incorporated with sesame seed powder. Food Sci Nutr. 2018;6(1):85-93. doi:10.1002/fsn3.532
27. Ghafouri-Oskuei H, Javadi A, Saeidi Asl MR, Azadmard-Damirchi S, Armin M. Quality properties of sausage incorporated with flaxseed and tomato powders. Meat Sci. 2020;161:107957. doi:10.1016/j.meatsci.2019.107957