

Antioxidant Effects of Aromatic Plant Essential Oils on Oxidative Stability of Ghee

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A-R-T-I-C-L-E-I-N-F-O

Article Notes:

Received: Sep 22, 2019

Received in revised form:

Dec 14, 2019

Accepted: Dec 14, 2019

Available Online: Jan 15, 2019

Keywords:

Essential oil

Ghee

Natural antioxidant

Oxidation stability

A-B-S-T-R-A-C-T

Background & Aims of the Study: The present study assessed the antioxidant properties associated with the essential oils (EOs) of four native plants, namely *Salvia officinalis* (SO), *Mentha longifolia* (ML), *Mentha spicata* (MS), and *Cuminum cyminum* (CC), during the storage of ghee, as a food model, in comparison with the features of butylated hydroxytoluene (BHT), as a synthetic antioxidant.

Materials and Methods: The EOs were prepared and analyzed for their total phenolic content, 2, 2-diphenyl-1-picrylhydrazyl, reducing power, and their effects on the oxidative stability of ghee during the accelerated storage at 65°C.

Results: The EO of SO had the highest phenolic compounds (70.06±0.72 mg gallic acid equivalents/g of EO) followed by EOs of ML, MS, and CC. Compared to other EOs, EO of SO had significantly the highest antioxidant activity ($P<0.05$). The sensory properties and overall acceptance of ghee containing 20 ppm of SO were more acceptable, compared to those reported for other treatments. The oxidation stability of ghee was preserved by all the EOs, compared to that of the control during the accelerated storage ($P<0.05$).

Conclusion: The EOs of plants could be introduced as suitable herbal sources of antioxidants to endure ghee shelf life.

Please cite this article as: Pajohi-Alamoti M, Kahledian S, Bazargani-Gilani B. Antioxidant Effects of Aromatic Plant Essential Oils on Oxidative Stability of Ghee. Arch Hyg Sci 2019;8(4):233-244.

Background

Ghee produced from sheep or cow milk has an appetizing and pleasant odor. It is generally regarded as the superb frying and cooking oil. During storage, ghee undergoes oxidative degradation, the extent of which relies on the storage temperature (i.e., controlling factor for liquid or solid-state ghee), ghee texture (i.e., solid or liquid state), and oxygen availability (i.e., packaging type). Through the reduction of consumption suitability and product's shelf life,

oxidative degradation deteriorates ghee's flavor, color, aroma, and nutritive value (1–4).

The human consumption of oxidized oils and fats could induce heart diseases, cancer, and early aging. Using antioxidants is the best way for the stabilization of oils, prevention of lipid oxidation, and preservation of herbal oils from being oxidized by free radicals (5). Antioxidants have important roles in impeding the lipid oxidation reactions of food products. The results of lipid oxidation include the formation of undesirable flavors and unstable chemical compounds (6).

Synthetic antioxidants, including butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and tert-butyl hydroquinone, are broadly used as food additives to prevent ghee oxidation in different countries. Recent reports showed that these compounds could be involved in various health hazards, such as cancer (7). Although synthetic antioxidants are used at minimum concentrations, the problems caused by the long-term consumption of these chemicals should not be dismissed (5). Therefore, the demand for the development of antioxidants with natural origin has been recently enhanced, resulting to perform various studies (8).

Natural antioxidants, such as the aromatic herbs' by-products, could be good alternatives in the food and drug industries (9–11). The extracted essential oils (EOs) from aromatic plants have been introduced as natural alternatives to synthetic antioxidants (12–14). *Salvia officinalis* L. (Lamiaceae), has different compounds, such as terpenoids and phenolics. Among them, the polyphenolic components indicated several biological impacts, including antioxidant, antiplatelet, antitumor, and antiviral activities (15).

Mentha longifolia L. is another member of the Lamiaceae extensively used in aromatic and flavored foods, perfume production, and medical purposes. Furthermore, the EOs and extracts from *Mentha* species possess antimicrobial and antioxidant properties (16–18). *Mentha spicata* L. belongs to the family Lamiaceae widely cultivated in many places around the world for the production of EO.

Recently, *Mentha spicata* (MS) has become a subject of scientific interest in viewing further potential benefits of its EO and extract, mainly as antimicrobial and antioxidant agents (19–21). *Cuminum cyminum* L. is a member of the Apiaceae family that is of Mediterranean origin. It is widely grown in Iran, China, India, and Turkey. Its seeds are utilized mostly as spices in food. However, the EO of cumin seed has appropriate antioxidant and antimicrobial

properties (22, 23).

The reported methods, for example, peroxide value (PV) and thiobarbituric acid (TBA) value for monitoring various oils' and fats' oxidative deterioration are according to the chemical changes occurring at various steps (i.e., elementary and secondary oxidation stages) (24, 25). The present study aimed to determine and compare the antioxidant capacity of the EOs from four aromatic plants, including MS, *S. officinalis* (SO), *M. longifolia* (ML), and *C. cyminum* (CC), for the first time in Iran. As a food model, the antioxidant activity of the EOs in ghee was compared in order to demonstrate their ability to be used as possible substitutes for synthetic antioxidants in the food industry.

Materials & Methods

Extraction of Essential Oils

A portion (100 g) of the dry plants of MS, SO, ML, and CC seeds were submitted for water distillation using a Clevenger-type apparatus for 3 h (yield 0.42-0.65% v/w). The EOs were dried with sodium sulfate and stored at 4°C.

Gas Chromatography-mass Spectrometry of Essential Oils

The composition of EOs was examined via gas chromatography-mass spectrometry (GC/MS) by Agilent model 6890 (USA) and completed with a flame ionization detector and HP-5 capillary column (30 m×0.25 mm; 0.25 µm film thickness) applying Mass System (Agilent 5973, USA). Using the carrier gas (i.e., helium) with a flow rate of 0.8 mL min⁻¹ was another operating condition. The injector and detector temperatures were arranged at 290 and 209°C; respectively, while taking the mass spectra at 70 eV. The composition of EOs was identified through comparing their mass spectra with those published in the literature and MS computer libraries (Wiley 275 Library). Finally, further confirmation was achieved by referring

to the Kovats index data generated from a series of alkanes (C9-C28) (26).

Total phenolic content

Phenolic component concentration in the EOs was colorimetrically determined according to methods described by Singleton and Rossi (27) with minor changes. Briefly, 0.5 mL of the diluted EOs was blended with 2.25 mL water and 0.25 mL Folin-Ciocalteu's phenol reagent and vortexed for 1 min. After 5 min, 2 mL saturated sodium carbonate solution was added to the mixture. The mixture was incubated at room temperature for 120 min, and absorbance was read at 725 nm. Gallic acid was applied for the calculation of the standard curve (0.01-0.4 mM). The results were reported as mean values±standard deviations and mg of gallic acid equivalents (GAE)/g of EO.

2,2-diphenyl-1-picrylhydrazyl assay

The assessment of 2,2-diphenyl-1-picrylhydrazyl (DPPH) was conducted based on the proposed method of Blois (28). The EOs (20 ppm) were combined with 2 mL methanol containing DPPH radicals (24 µg/mL). The mixture was rigorously shaken and kept for 60 min in the dark (until stable absorption values were acquired). The DPPH radical reduction was calculated by continuously tracking the decrease of absorption at 517 nm. The assays were conducted in triplicate, and the results were shown as mean values±standard deviations. The BHT (20 ppm) was applied as a standard antioxidant for the comparison. Radical scavenging activity (RSA) was measured as a percentage of DPPH discoloration with the use of the following formula:

$$\%RSA = [(1 - A_s / A_b) \times 100]$$

where A_s is the absorbance of the mixture when the EOs were added at a certain amount, and A_b is the absorbance of the DPPH mixture.

Reducing power assay

To determine the reducing power (RP), the EOs (20 ppm) were firstly blended with 2.5 mL sodium phosphate buffer (0.2 M, pH 6.6) and 2.5 mL potassium ferricyanide (1.0%). The combination was incubated at 50°C for 20 min. After adding 2.5 mL trichloroacetic acid (TCA) (10% w/v), the mixture was centrifuged at 1430 g for 10 min. Finally, 2.5 mL of the top layer was combined with 2.5 mL distilled water and 0.5 mL ferric chloride (0.1%). Then, the absorbance was evaluated at 700 nm. Higher absorbance showed greater RP. The assays were undertaken in triplicate, and the results were stated as mean values±standard deviations. The BHT (20 ppm) was applied as the standard.

Preparation of Treated Ghee with Essential Oils

The ghee samples were treated with 20 ppm of each EOs and BHT (20 ppm) as a positive control. All samples were incubated in glass containers at 65±1°C. The samples were taken for each treatment every 3 days up to 15 days for PV, TBA, acid value, and free fatty acid (FFA) assay.

Determination of peroxide value

The PV was measured by spectrophotometric method (29). Briefly, 0.2 g of ghee flesh was thoroughly mixed for 4 min with 9.8 mL of chloroform: methanol solution (with ratio 7:3). Then 50 µL ammonium thiocyanate solution was added and mixed for 4 min. The ferrous chloride solution (50 µL) was added and mixed for another 4 min. Incubation was performed at room temperature for 5 min. The absorbance of the samples was measured at 500 nm against a blank sample. Ferric ion was used for the preparation of the standard calibration curve and calculation of the PV in mEq active O₂/kg of fat ($y=0.3984x-0.0295$, $R^2=0.9995$).

$$PV = ([A_s - A_b] \times m) / (55.84 \times w)$$

where A_s and A_b are the absorbance of the sample and blank, respectively, m is the slope of the standard curve, and w is the lipid weight (g) of the sample (29).

Thiobarbituric acid reactive substance

To assay thiobarbituric acid reactive substance (TBARS) value in the ghee samples, 10 g of each sample was weighed and blended with 1 mL of BHT (1 mg/mL) and 35 mL of TCA (5%). The distilled water (100 mL) was added to the mixture, and the complex was distilled. The distilled mixture (50 mL) was filtered via a filter paper (No.1, WhatmanTM, UK). Then, the filtrate (5 mL) was blended with 5 mL of TBA solution (0.02 M) and put into a water bath (Teifazmateb Co., Iran) at 100°C for 60 min. After cooling the mixture, its absorbance was measured at 532 nm against water blank (30). The TBARS value was reported as the amount (mg) of malonaldehyde equivalents per kg of the sample. The 1, 1, 3, 3-tetraethoxypropane was used to prepare the standard curve ($y=0.2024x+0.0036$, $R^2=0.9989$).

Free fatty acid determination

The FFA of the samples was evaluated based on the procedure proposed by Zhang et al. (2010), with a few modifications. The sample (3 g) was mixed in neutral ether/ethanol (1:1, v/v) complex (50 mL) and shaken by hand. After cooling the mixture at room temperature, it was titrated against potassium hydroxide (0.01 M) using phenolphthalein (10 g/L). The FFA amount was measured based on the following formula:

$$\text{FFA (mg KOH/g fat)} = (V \times C \times 56.11) / m$$

where V is the level of potassium hydroxide exhausted by the samples (mL), C shows potassium hydroxide concentration (mol/L), and m stands for the sample weight (g) (31).

Sensory evaluation

The sensory properties of the ghee samples were evaluated at days 0 and 15 of the storage. The samples were tested by 10 panelists from the Department of Food Hygiene and Control in Bu-Ali Sina University in Hamedan, Iran (five females, five males; age range: 22-36 years). The sensory evaluation of ghee samples was performed for color, odor, and flavor. Then, the overall acceptance was measured using the 9-point hedonic scale (32). The sample score was given by sensory panel people from 1 to 9 as it follows:

1: dislike extremely; 2: dislike very much; 3: dislike moderately; 4: dislike slightly; 5: neither like nor dislike; 6: like slightly; 7: like moderately; 8: like very much; 9: like extremely.

Statistics

Data analysis was conducted using SPSS software (version 20) and analysis of variance. P-value less than 0.05 was considered statistically significant.

Results

Essential Oil compounds

The component analysis of the EOs by GC/MS is listed in Table 1. Ten components representing 88.45% of the total volatiles were identified for *ML* EO. The main components were pulegone (31.54% of the total essential oil) and 1, 8-cineole (15.89%). Twelve components, representing 84.13% of the total volatiles, were identified for *SO* EO. The main components were α -thujone (25.70%), camphor (18.72%) and 1, 8-cineole (14.80%). Eight components, representing 93.38% of the total volatiles, were identified for *CC* EO. The main components were cumin aldehyde (29.02%) and α -terpinen-7-al (20.70%). Nine components, representing

Table 1) Composition of essential oils analyzed by gas chromatography-mass spectrometry

Component	Percentage (%)			
	<i>Mentha longifolia</i> L.	<i>Cuminum cyminum</i> L.	<i>Salvia officinalis</i> L.	<i>Mentha spicata</i> L.
α -Pinene	1.86	-	2.18	1.30
β -Pinene	3.07	7.72	2.09	2.08
β -Myrcene	-	-	-	1.31
3-Octanol	-	-	-	1.04
Limonene	-	-	2.2	15.71
1,8-Cineole	15.89	-	14.8	6.2
Cis-Dihydrocarvone	-	4.45	-	1.53
Carvone	-	-	-	63.4
β -Bourbonene	-	-	-	1.08
Camphene	-	-	3.61	-
ρ -Cymene	-	8.55	1.16	-
α -Thujone	-	-	25.7	-
β -Thujone	-	-	4.08	-
Comphor	-	-	18.72	-
Borneol	-	-	1.8	-
α -Humulene	-	-	3.45	-
Viridiflorol	-	-	4.25	-
Myrcene	-	1.1	-	-
γ -Terpinene	-	12.94	-	-
Cumin aldehyde	-	29.02	-	-
α -Terpinen-7-al	-	20.70	-	-
γ -Terpinen-7-al	-	8.90	-	-
ρ -Menth-3-en-8-ol	7	-	-	-
Menthofuran	11.18	-	-	-
Cis-isopulegone	9.74	-	-	-
dihydrocarveol	1.78	-	-	-
Pulegone	31.54	-	-	-
2-Cyclohexan-1-one	3.80	-	-	-
1-Decene	1.58	-	-	-
Total	88.45	93.38	84.13	93.65

93.65% of the total volatiles, were identified for *MS* EO. The main components were carvone (63.4%) and limonene (15.71%).

Total phenolic content

The calculation of the total phenolic content (TPC) of EOs was carried out using the standard curve of Gallic acid ($y=0.0096x+0.6153$; $R^2=0.980$). It was presented as GAE per g of EOs (Table 2). The amounts of TPC were 70.06 ± 0.72 , 61.03 ± 0.91 , 53.77 ± 0.61 , and 46.97 ± 1.64 mg (GAE/g of EO) for *SO*, *ML*, *MS*, and *CC* EOs, respectively.

Reducing power

The RP of the EOs and BHT were evaluated

at 20 ppm concentration, as shown in Table 2. The highest amount of RP was related to BHT (0.305 ± 0.005) and *SO* (0.243 ± 0.003).

Radical scavenging activity

The RSA of the EOs and BHT was evaluated at 20 ppm concentration in the DPPH system (Table 2). The results of RSA showed that BHT had the highest percentage of RSA. The percentages of RSA for *SO*, *ML*, *MS*, and *CC* EOs were 70.06 ± 0.16 , 53.27 ± 0.95 , 41.46 ± 0.08 , and 48.73 ± 0.39 , respectively.

Ghee treated with Essential Oils

Peroxide values

The effects of natural antioxidants on the PV

Table 2) Total phenolic content, reducing power, and radical scavenging activity of essential oils and butylated hydroxytoluene

Treatment	Total phenolic content (mg acid gallic/g of the EO)	Reducing power (absorbance)	Radical scavenging activity (%)
<i>Salvia officinalis</i> L.	70.06±0.72 ^a *	0.244±0.003 ^b	70.06±0.16 ^b
<i>Cuminum cyminum</i> L.	46.97±1.64 ^d	0.194±0.005 ^e	48.73±0.39 ^d
<i>Mentha longifolia</i> L.	61.03±0.91 ^b	0.216±0.000 ^c	53.27±0.95 ^c
<i>Mentha spicata</i> L.	53.77±0.61 ^c	0.205±0.001 ^d	41.46±0.08 ^e
Butylated hydroxytoluene	-	0.305±0.005 ^a	78.19±0.31 ^a

* Small letters within each column indicating significant difference ($P < 0.05$)

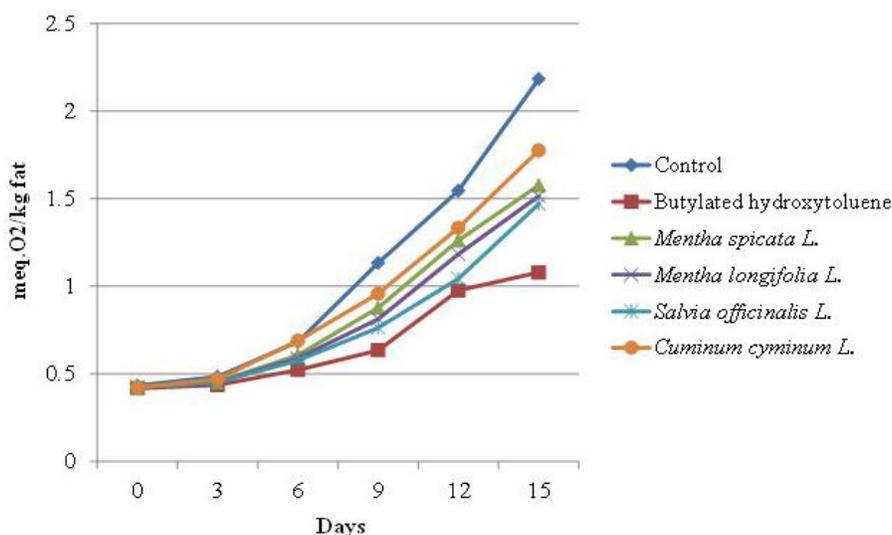


Figure 1) Peroxide values of ghee samples stored at 65±1°C as mEq O₂/kg of fat

of the ghee at 65±1°C for 15 days are shown in Figure 1. Among all treatments, BHT had the lowest PV; however, the control sample revealed the highest amount of PV during the storage time. Initial PV levels in all samples were measured to be within the range of 0.416-0.433 mEq O₂/kg of fat. The amounts of PV in the 15th day were reported for the corresponding treatment as it follows:

BHT: 1.078±0.052 mEq O₂/kg of fat; SO: 1.47±0.050 mEq O₂/kg of fat; ML: 1.515±0.032 mEq O₂/kg of fat; MS: 1.576±0.016 mEq O₂/kg of fat; CC: 1.775±0.012 mEq O₂/kg of fat.

Thiobarbituric acid value

The effects of natural antioxidants on the TBA values of the ghee at 65±1°C for 15 days are depicted in Figure 2. Initial TBA levels in

all samples were measured within the range of 0.153-0.160 mg malonaldehyde/kg fat. In the 15th day of storage, the control sample (0.402±0.010 mg malonaldehyde/kg of fat) had the highest amount of TBA. The TBA values measured on the 15th day in other samples were reported for the corresponding treatment as it follows:

BHT: 0.257±0.007 mg malonaldehyde/kg; SO: 0.286±0.002 mg malonaldehyde/kg of fat; ML: 0.308±0.006 mg malonaldehyde/kg of fat; MS: 0.323±0.001 mg malonaldehyde/kg of fat; CC: 0.333±0.002 mg malonaldehyde/kg of fat

Free fatty acid value

Figure 3 illustrates the FFA values of the ghee stored at 65±1°C for 15 days. Initial FFA values in all samples were measured at 0.149 mg

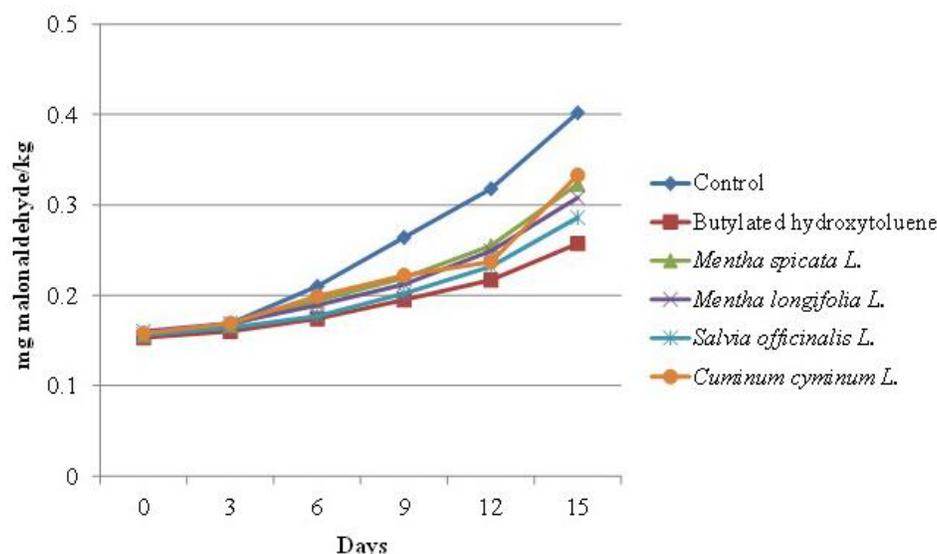


Figure 2) Thiobarbituric acid value of ghee samples stored at $65\pm 1^\circ\text{C}$ as mg malonaldehyde/kg

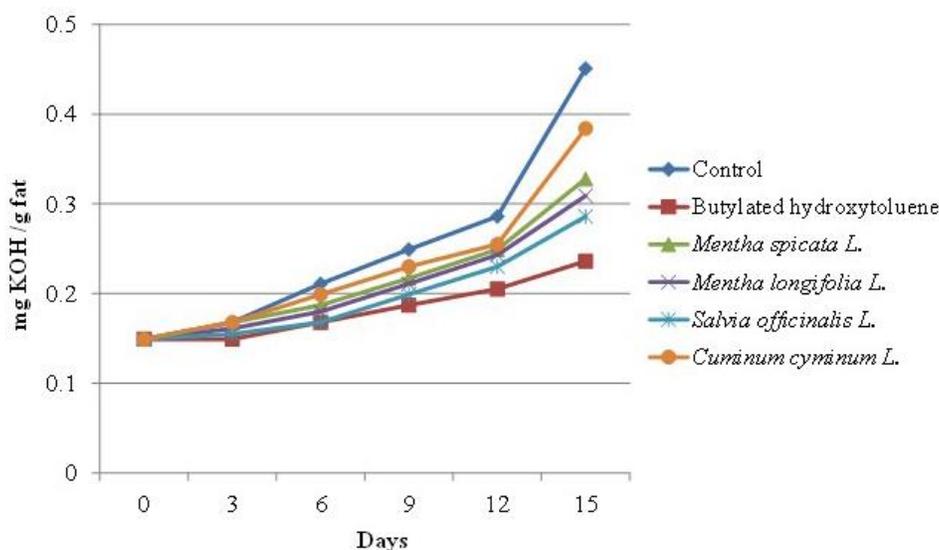


Figure 3) Free fatty acid value of ghee samples stored at $65\pm 1^\circ\text{C}$ as mg KOH/g fat

KOH/g of fat. The control samples (0.451 ± 0.05 mg KOH/g of fat) indicated the highest amount of FFA value in the storage time. The FFA values measured on the 15th day in other samples were reported for the corresponding treatment as it follows:

BHT: 0.236 ± 0.109 mg KOH/g of fat;
 SO: 0.286 ± 0.109 mg KOH/g of fat; ML: 0.309 ± 0.008 mg KOH/g of fat; MS: 0.328 ± 0.012 mg KOH/g of fat; CC: 0.348 ± 0.010 mg KOH/g

of fat.

Sensory evaluation

The mean values of color, odor, and flavor, as well as the overall acceptance scores of the samples containing EOs, BHT, and control, are shown in Table 3. Color, odor, flavor, and overall acceptance of the ghee were evaluated at days 0 and 15 of the incubation. The color score of all fresh ghee samples was the same on

Table 3) Sensory properties of ghee samples containing essential oils during storage

Storage time (days)	Treatment	Color	Odor	Flavor	Overall
0	Control	9±00 ^{aA*}	7.4±0.51 ^{aA}	7.2±0.42 ^{aA}	7.2±0.42 ^{aA}
	Butylated hydroxytoluene	9±00 ^{aA}	7.3±0.48 ^{aA}	7.2±0.42 ^{aA}	7.1±0.31 ^{aA}
	<i>Mentha spicata</i> L.	9±00 ^{aA}	8.7±0.48 ^{aA}	8.8±0.42 ^{aA}	8.7±0.48 ^{aA}
	<i>Mentha longifolia</i> L.	9±00 ^{aA}	8.6±0.51 ^{aA}	8.8±0.42 ^{aA}	8.6±0.51 ^{aA}
	<i>Salvia officinalis</i> L.	9±00 ^{aA}	7.7±0.48 ^{aA}	7.2±0.42 ^{aA}	8±00 ^{aA}
	<i>Cuminum cyminum</i> L.	9±00 ^{aA}	7.2±0.42 ^{aA}	7.2±0.42 ^{aA}	7.2±0.42 ^{aA}
15	Control	2.8±0.42 ^{eB}	3±00 ^{eB}	3±00 ^{eB}	2.8±0.42 ^{dB}
	Butylated hydroxytoluene	7.7±0.48 ^{bB}	6±00 ^{cB}	6±00 ^{cB}	7±0.66 ^{bB}
	<i>Mentha spicata</i> L.	6.3±0.48 ^{cB}	6.9±0.31 ^{bB}	6.8±0.42 ^{bB}	6.8±0.42 ^{bB}
	<i>Mentha longifolia</i> L.	6.8±0.42 ^{cB}	6.9±0.31 ^{bB}	7±00 ^{bB}	6.7±0.48 ^{bB}
	<i>Salvia officinalis</i> L.	7.7±0.48 ^{bB}	6±00 ^{cB}	6.3±0.48 ^{cB}	7.1±0.56 ^{bB}
	<i>Cuminum cyminum</i> L.	4.8±0.42 ^{dB}	4.9±0.31 ^{dB}	4.8±0.42 ^{dB}	4.8±0.42 ^{cB}

* Different capital letters in the same column within the same storage time indicating significant differences ($P<0.05$); different letters in the same column within the same treatment indicating significant differences ($P<0.05$)

day 0. However, after the storage at $65\pm 1^\circ\text{C}$ for 15 days, the order of the color score of the ghee was reported as $\text{BHT} = \text{SO} > \text{ML} > \text{MS} > \text{CC} > \text{control}$.

The odor scores of fresh ghee samples on day 0 were stated as $\text{MS} > \text{ML} > \text{SO} > \text{control} > \text{BHT} > \text{CC}$. However, after the storage at $65\pm 1^\circ\text{C}$ for 15 days, the order of the odor scores of the ghee changed to $\text{MS} > \text{ML} > \text{BHT} = \text{SO} > \text{CC} > \text{control}$. The flavor scores of fresh ghee samples on day 0 were reported as $\text{MS} = \text{ML} > \text{BHT} = \text{control} = \text{SO} = \text{CC}$. However, after the storage at $65\pm 1^\circ\text{C}$ for 15 days, the order of the flavor scores of the ghee was rearranged to $\text{ML} > \text{MS} > \text{SO} > \text{BHT} > \text{CC} > \text{control}$. The overall scores of fresh ghee samples on day 0 were reported as $\text{MS} > \text{ML} > \text{SO} > \text{CC} = \text{control} > \text{BHT}$. Nevertheless, after the storage at $65\pm 1^\circ\text{C}$ for 15 days, the order of the overall scores of ghee changed to $\text{SO} > \text{BHT} > \text{MS} > \text{ML} > \text{CC} > \text{control}$.

comprised most of the components in the EOs of *ML*, *MS*, *SO*, and *CC*. The results of the present study confirmed the findings on the common compounds of the EOs in some other studies (12, 33–35). The obtained results are also contradictory to those in some other studies (36–38). The authors believed the changes in the EO compounds observed in those studies might be due to different geological, climatic, seasonal, and geographical conditions, discussed in detail.

Total phenolic content

Among EOs, the *SO* contained a higher ($P<0.05$) amount of TPC (70.06 ± 0.72 mg GAE/g of EO), compared to *ML*, *MS*, and *CC*. Some studies reported different values for antioxidant activity and TPC (38–42). The difference in the TPC of the EOs can be ascribed to the climate of the area in which the plants are grown, their maturity at the harvesting stage, and variety (43–45).

Reducing power

The RP exhibited by *SO* ($\text{OD}=0.244\pm 0.003$) was significantly ($P<0.05$) higher than those reported for other EOs. Of course, BHT showed an RP activity higher than EOs. In conclusion,

Discussion

The results of the current study showed that pulegone (30.12 %), carvone (63.4%), α -thujone (25.7%), and cumin aldehyde (28.7%)

the antioxidant activities of the EOs and synthetic antioxidants, measured through the RP, were in the following order: BHT > SO > ML > MS > CC

Radical scavenging activity

The antioxidant activity of some plants' phenolic compounds is well known. All of these polyphenolic compounds have the ability to act as antioxidants by a free radical scavenging mechanism, as well as their known ability to chelate metals (25, 46). The RSA exhibited by *SO* (70.06%) was significantly ($P<0.05$) higher than those of other EOs. However, RSA exhibited by EOs was lower than that reported for BHT (78.19%). In the present study, the high antioxidant activity of EOs can be attributed to the polyphenolic compounds. Significant differences between the RSA data of the EOs, reported in the study, might have been caused by genotypic and environmental conditions, TPC of the plant, climatic and environmental variations, plant parts, and sampling procedures.

Peroxide values of treated ghee with essential oils

Primary oxidation products (i.e., hydroperoxides) were determined by PV measurement. The peroxide value increased during the entire storage period in all ghee samples. Kumar and Bector (1985) reported that the initial PV (International Dairy Federation method) of fresh ghee samples ranged from 0.10 to 0.47 mEq O₂/kg fat by an average of 0.21 mEq O₂/kg of fat. In the current study, the initial PV was close to the maximum level of 0.416-0.433 mEq O₂/kg of fat range (47). Moreover, Mehta et al. (2015) demonstrated the PV to be lower than that in the present study (48). Maybe, their ghee was fresh, or the primary oxidation products turned into secondary oxidation products.

Among the ghee samples, the EO of *SO* (1.47±0.050 mEq O₂/kg of fat) with 20 ppm concentration remained the most effective and

yielded the lowest PV. However, the ghee incorporated with BHT (1.078±0.052 mEq O₂/kg of fat) showed a lower PV, compared to that incorporated with EOs. The PV for the ghee samples without antioxidant (the control) reached 2.183±0.034 mEq O₂/kg of fat after 15 days of storage at $P < 0.05$. The PV of the ghee samples were followed up to the permissible limit of 10 mEq O₂/kg (49).

In the current study, at the end of the storage time, the PV was still within the permissible range. El-Shourbagy et al. (2014) demonstrated that the PV of the samples enriched with BHA and natural antioxidants had lower PV values than the control samples during the storage under accelerated incubation at 63°C for 21 days. In this regard, the findings of the aforementioned study are in line with the results of the present study (50). In other studies, by adding natural antioxidants (i.e., EO and extract), the amount of PV decreased, compared to that reported for the control sample (3, 32, 46, 51, 52).

Thiobarbituric acid value

Secondary fat oxidation can be followed by testing for TBARS, which is broadly used to identify the oxidative rancidity degree of a product during storage. A good method for the determination of different antioxidants' abilities to inhibit lipid peroxidation measures the reactive substances of thiobarbituric acid (24). The results showed that during 15 days of storage, the TBA value of the control sample reached a maximum of 0.402±0.010 mg malonaldehyde/kg fat, which was higher than that of the samples treated with the EOs ($P<0.05$).

In addition, the TBA value of BHT (0.257±0.007 mg malonaldehyde/kg) was significantly ($P<0.05$) effective in limiting the rise in TBA values, compared to that of the control ghee sample. In the ghee samples treated with EOs, the TBA value of *SO* (0.286±0.002 mg malonaldehyde/kg fat) was effective in limiting the rise of TBA values in

comparison to those of other EOs. Siwach et al (2016) reported that the control ghee samples showed higher TBA values (0.929 ± 0.003) during 12 months of storage at 30°C ; however, the use of lycopene treatment reduced the value of TBA (53).

According to the evidence, the control ghee samples indicated higher TBA values throughout the accelerated incubation period, and the use of antioxidant treatments reduced the value of TBA (50). Therefore, the increase of TBA values of ghee samples obtained in the present study was higher than those reported in the literature. This might be due to the high storage temperature in the present study. The rate of the formation of secondary products is faster during accelerated storage than normal storage (48). The results of other studies about the treatment of ghee with the EOs are similar to the findings of the present study. (4, 51, 54).

Free fatty acid value

The FFAs are formed due to hydrolysis reaction and oxidation of double bonds (32). In the 15th days of storage, a significant increase in FFA was observed in most ghee samples; however, the lowest increase of FFA was observed in the samples containing BHT (0.236 ± 0.109 mg KOH/g fat) and SO (0.286 ± 0.109 mg KOH/g fat) during the storage time. The FFA formation was significantly higher in the control sample (mg 0.451 ± 0.051 KOH /g fat) at $65\pm 1^{\circ}\text{C}$ during the storage ($P<0.05$). Yadav and Srinivasan (1992) reported that the FFA content of ghee normally ranged between 0.23% and 1.0% oleic acid (55). Therefore, the FFA content of ghee samples in the present study was under the normal range during the storage and after 15 days of storage at $65\pm 1^{\circ}\text{C}$.

The low level of FFA in the current study could be due to the freshness and lack of extra water in the ghee samples. In another study on the ghee, it was shown that ghee containing lycopene had significantly lower FFA content,

compared to the control samples (53). The BHA was significantly more effective in comparison to lycopene. The results of the current study are in agreement with those of other studies (51, 56).

Sensory evaluation

The ghee samples with different EOs showed significant differences ($P<0.05$), compared to the control samples in terms of their color, odor, and flavor scores during the storage. Generally, after the storage at $65\pm 1^{\circ}\text{C}$ for 15 days, the order of the overall scores of ghee was $SO > BHT > MS > ML > CC > \text{control}$. Therefore, the treatment of ghee with EOs could improve the sensory properties of ghee after the extended storage. Considering the sensory scores, the samples containing 20 ppm of EOs were more acceptable.

Conclusion

Ghee containing EOs was reported with a higher level of tolerance in increasing the peroxides, TBA, and FFA than the sample without EO. The results of the present study showed that among the evaluated EOs, SO can be considered a good natural antioxidant for the reduction of the oxidation rate in fat-rich foods.

Footnotes

Funding

This study was extracted from an MSc project by Saeed Kahledian, an MSc student of Food Hygiene and Quality Control in Bu-Ali Sina University and supported by Bu-Ali Sina University.

Conflict of Interest

The authors declare that there is no conflict of interest.

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