



Review article

Essential oils: in vitro antioxidant activities and their utilizations in storage life increment of foods

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A B S T R A C T

In recent years, the attention on essential oils (EOs) as natural additives of food products have been rapidly growing due to the extended health risk of synthetic preservatives. Artificial additives can minimize food spoilage, though the current generation is very health-conscious and believes in natural preservatives rather than the synthetic ones owing to their potential toxicity and other challenges. The utilization of EOs for shelf-life extension in foods is mostly due to their antioxidant attributes which can be verified by the massive number of researches published in this area. The intention of this paper is to analyze the methods which have been utilized for antioxidant activity measurement of EOs, highlighting their potential and usefulness as well as discussing the impact of EOs on oxidative stability of food products such as vegetable oils, meat and dairy products, particularly with regard to their potency, synergistic effects and principal components.

Keywords: Antioxidant activity, Essential oil, Food, Shelf-life

Received 20 August 2020; Revised 20 September 2020; Accepted 5 October 2020

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1. Introduction

Oxidation is among the most vital processes in all living beings as it generates energy crucial for their appropriate activity (Farahmandfar et al., 2018; Olszowy & Dawidowicz, 2016). However, uncontrolled oxidation derives extreme quantities of free radicals, which induce the tissue damages and fatal alterations in the Biological cells (Olszowy & Dawidowicz, 2016; Saleh et al., 2010). In recent times, living beings are progressively more targeted to free radicals from external resources, which as a result the risk of diseases such as arteriosclerosis, rheumatoid arthritis, cancer, cirrhosis as well as degenerative illnesses affiliated with aging incidences have been elevated (Olszowy & Dawidowicz, 2016; Saleh et al., 2010). Deterioration of food is another obstacle related to the destructive effects of free radicals (Farahmandfar & Ramezanizadeh, 2018). For example, lipids in foodstuffs are composed of triacylglycerol which is an appropriate supply for oxidation and its automatic chain reactions with atmospheric oxygen would lead to the extension of hazardous and off-flavor substances, which eventually would generate improper products for human usage (Hashemi et al., 2017). Furthermore, there are several factors like presence of energy input (e.g. heat or light), fatty acids (FA) profile, types of oxygen and negligible components e.g.

minerals, free fatty acids (FFA), pigments and enzymes which play significant roles in initiation of this process (Choe & Min, 2006; Hashemi et al., 2017). The detrimental activity of free radicals would be inhibited through antioxidant materials, which would scavenge the free radicals and detoxify the organism (Farahmandfar et al., 2019b). These antioxidative agents are divided into two categories: synthetic and natural (Olszowy & Dawidowicz, 2016). Over the last few decades, man-made antioxidants such as tert-butylhydroquinone (TBHQ), butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and propyl gallate (PG), have been utilized by food manufacturers due to their cost-efficiency and convenience (Farahmandfar et al., 2019a). However, it has been reported in prior literature that extent usage of these antioxidants would have teratogenic, carcinogenic and mutagenic influences, on food consumers (Ritota & Manzi, 2020). Hence, due to the expansion of consumer demands for fresh natural products without additives and the deepening concern over the safety of the artificial preservatives, the focus of food industry has been shifted towards non-chemical natural substances, in recent years (Asadi & Farahmandfar, 2020), which not only are risk-free but they possess health-promoting bioactive compounds, as well (Hashemi et al., 2017). Plants secondary metabolites are among the more promising alternatives since they produce a wide variety of

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<https://doi.org/10.22059/jfabe.2020.308599.1063>

components that often contain antioxidant characteristics (Farahmandfar et al., 2019c). Plants EOs are organic, volatile and complex components with a potent aroma which can be discovered in all parts of aromatic plants including leaves (e.g. bay leaf, eucalyptus, thyme, mint, pine needles, savory, and sage), wood or bark (cinnamon, sandalwood, rosewood), flowers (orange, pink, lavender, clove), roots (vetiver), seeds (carvi, coriander), fruits (fennel, anise, Citrus epicarps), and retained in secreting cells, canals, cavities and epidermic cells (Bakkali et al., 2008; Dhifi et al., 2016; Teixeira et al., 2013). The traditional collection method of EOs is steam or hydro-distillation which initially established in medieval times by Arabs and even today is among the most prevalent techniques of obtaining these compounds (Bakkali et al., 2008). EOs provide therapeutic aims in human medicine owing to their antioxidant, anticancer, antibacterial, antiphlogistic, antiviral and antinociceptive attributes (Bakkali et al., 2008; Farahmandfar et al., 2019c; Teixeira et al., 2013). These colourless fluids are insoluble in water and soluble in fixed oils, alcohol and ether (Dhifi et al., 2016). EOs have the potential to be applied as beneficial substitutes or supplements to artificial antioxidants, without inducing the same secondary effects, however in some cases, they might have their own limitations such as unfavorable taste and aroma as well as lower antioxidant activity than synthetic ones (Dhifi et al., 2016; Sayyari & Farahmandfar, 2017).

2. Antioxidant capacity of essential oils

The effectiveness and strength of antioxidants are generally influenced by multiple factors, such as temperature, structural characteristics, concentration, types of oxidizing substrates, physical state of the system and existence of prooxidants or synergists. Moreover, the chemical configuration of an antioxidant determines its internal reactivity towards free radicals as well as other oxygen reactant species and correspondingly its antioxidant activity (Asnaashari et al., 2015, 2016; Farahmandfar et al., 2017). There are certain approaches applied for measuring the antioxidant activities of EOs which the most popular ones are DPPH, ABTS, FRAP and β -carotene bleaching experiments (Farahmandfar et al., 2017; Olszowy & Dawidowicz, 2016). These methods are varied in oxidation substrates, oxidizing agents, reaction conditions, detection technologies and results demonstration (Shahidi & Zhong, 2015). DPPH scavenging activity is a spectrophotometric test and is among the most routinely utilized methods of antioxidant activity measurement which employs 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical as a reagent (Farahmandfar et al., 2020). DPPH is a commercially accessible radical with a strong violet color which is not required to be produced leading up to the experiment (Farahmandfar et al., 2019b). The procedure of scavenging DPPH-free radicals is relatively simple and can be employed to estimate the antioxidant capacities of EOs in a rather short time (Baser & Buchbauer, 2015). As a result of reacting with EOs, stable DPPH free radicals would change to 1, 1-diphenyl-2-picrylhydrazyl yellow-colored compounds (Alam et al., 2013). Consequently, the hydrogen donation capabilities of EOs would be measured through the discoloration degree of the reagent (Baser & Buchbauer, 2015).

ABTS essay is another colorimetric procedure similar to DPPH test which would be utilized for radical scavenging assessment of EOs. In this technique, a diode-array spectrophotometer is applied to assess the scavenging potential of the stable ABTS^{•+} radical (2, 2'-azinobis (3-ethylbenzothiazoline-6-sulphonic acid)) with a blue-green chromophore and maximum absorption at 734 nm (Alam et

al., 2013; Shahidi & Zhong, 2015). The radical-scavenging efficiency of EOs would be assessed by reduction of ABTS^{•+} to ABTS and decolorizing of the reagent. The magnitude of the blue-green reagent discoloration, quantified as a means of absorbance reduction is depended on the extent of the reaction, the innate antioxidant activity and the concentration of the EO (Shahidi & Zhong, 2015). Generally, for better comprehension, results of DPPH and ABTS essays would be expressed as IC₅₀, specified as the minimum concentration of the investigated EOs to reduce the initial amounts of DPPH or ABTS radicals up to 50% (Olszowy & Dawidowicz, 2016).

Antioxidants are well-known for their reducing activities, as well. Their electron donation capability not only enable them to scavenge free radicals, but it also provide the ability to reduce the elements with higher valence to their lower valent position (Farahmandfar et al., 2015; Shahidi & Zhong, 2015). The reducing power of antioxidants is a substantial indication of their activity and is assessed by redox reaction with varied metal ions including copper, iron, chromium, and cerium, etc. (Shahidi & Zhong, 2015). The FRAP experiment is a common technique which determines the reduction of ferric ion (Fe³⁺)-ligand complex to the extremely blue colored ferrous (Fe²⁺) complex through EOs (Alam et al., 2013). FRAP test would be conducted under acidic pH state, which would expand the redox potential by retaining the iron solubility as well as navigating the electron transport (Shahidi & Zhong, 2015).

The β -carotene bleaching experiment is another approach of assessing the antioxidant capacity of EOs (Baser & Buchbauer, 2015; Farahmandfar et al., 2015). In this technique, a spectrophotometer is used to estimate the capability of an antioxidant to reduce the combined oxidation of β -carotene and linoleic acid where the reaction with free radicals would reveal an alteration in their orange color (Baser & Buchbauer, 2015). The outcomes of β -carotene bleaching assay are superior to the DPPH test due to be more specific in lipophilic materials. The experiment is critical in food science owing to the emulsion nature of the test medium, and it is a common knowledge that many food products are presented as an oil-in-water emulsions e.g. sauces, mayonnaise, dressings, beverages, milk, ice cream and dips. Most often, these products are more sentient to oxidation than bulk oils due to their greater surface areas which elevate the interaction of the oil phase with pro-oxidants of the aqueous phase. Hence, it is momentous to also incorporate information on efficiency of an antioxidant in oil/water emulsion systems for a more inclusive evaluation of antioxidant capacity (Baser & Buchbauer, 2015; Farahmandfar et al., 2015).

Over the past few years, there were numerous studies regarding the in vitro examination of EO's antioxidant activities. For instance, in one survey, the antioxidant activity of *Lippia citriodora* EO were examined via DPPH and β -Carotene bleaching methods (Farahmandfar et al., 2018). It was found that as a result of elevation in EO's concentration (from 0 to 3200 ppm), the DPPH radical scavenging and β -Carotene/linoleic acid bleaching values were enhanced (from 10 to 55% and 6 to 50%, respectively). Likewise, via using GC-MS, 15 components in *Lippia citriodora* EO were determined which limonene (18.41%), nerol (16.1%), geranial (13.02%), β -cital (6.94%), and β -caryophyllene (4.78%) were the predominant volatile compounds and they are well-known for their substantial antioxidant capacities.

EO extracted from an Iranian wild plant (*Teucrium polium*) was analyzed with the purpose of determining chemical composition and antioxidant properties (Sayyad & Farahmandfar, 2017). GC-MS showed 6 monoterpenes, 7 sesquiterpenes and 6 oxygenated

sesquiterpenes with different percentage for each type. The major constituents of the EO were 11 acetoxyeudesman-4-a-ol (26.3%), α -bisabolol (24.6%), β -Caryophyllene (9.8%), Caryophylleneoxide (5.3%), β -Pinene (4.2%), Dehydro-sesquiceneol (3.7%), α -Pinene (3.1%) and α -Humulene (2.4%), which all possess remarkable antioxidant capacities. It was noticed that with increase in concentration (from 200 to 1200 ppm) a boost in DPPH scavenging activity of EO (from 28.74 to 89.13%) occurred. Similar trend was observed in β -carotene–linoleate system where by increasing the concentration, Inhibition raised from 32.94 to 86.91%. However, the results of positive control (Synthetic antioxidant, BHA) were 94.8 and 90.18% in DPPH and β -Carotene bleaching tests, respectively, which were all higher than those of EO.

The chemical composition and antioxidant activity (ABTS method) of EOs attained from *Ferula assa-foetida* oleo-gum-resins (OGRs) collected in three varied periods of 2011 including June 15th (OGR1), June 30th (OGR2) and July 15th (OGR3) were investigated (Kavoosi & Rowshan, 2013). It was noted that in each sample, different volatile compounds were dominant. For instance in OGR1 sample (E)-1-propenyl sec-butyl disulfide (23.9%) and 10-epi-c-eudesmol (15.1%), in OGR2 sample (Z)-1-propenyl secbutyl disulfide (27.7%) and (E)-1-propenyl sec-butyl disulfide (20.3%) and finally in OGR3 sample β -pinene (47.1%) and α -pinene (21.3%) were the major compounds of EO. Furthermore, the radical scavenging inhibitory concentration (IC50) of samples was reported and they were as follows: OGR1 (0.012-0.035 mg mL⁻¹) < OGR2 (0.025-0.047 mg mL⁻¹) < OGR3 (0.035-0.066 mg mL⁻¹). Hence, these discrepancies in antioxidant capacities could be affiliated with the differences in chemical composition of EOs.

In extracted EO of *Salvia sclareoides*, 60 components were identified (Sepahvand et al., 2014) and the primary recognized components were linalool (27.6 %), trans-caryophyllene (16.6 %), β -trans-ocimene (11.8 %) and germacrene-d (10.0 %). Moreover, the antioxidant capacity (DPPH) of *Salvia sclareoides* EO was almost 1.5 times superior to the synthetic antioxidant (BHT).

EO of *Eugenia uniflora* L. leaves was measured for its antioxidant activities via FRAP, DPPH and ABTS assays (Victoria et al., 2012). In all 3 methods with increase in EO concentration, the amount of inhibition enhanced 7.5, 3, and 6 times, respectively. Additionally, there was a remarkable correlation among the varied antioxidant activity methods.

Leaves of two types of Argentinean Rosemary plant: a narrow phenotype (NP) and a wide phenotype (WP) were employed for EO extraction and DPPH Scavenging analysis (Ojeda-Sana et al., 2013). Both EOs were capable to alter the strong violet DPPH radical into yellowish color, with IC50 quantities of 11 μ L mL⁻¹ and 25 μ L mL⁻¹ for NP and WP samples, respectively. Three major compounds of these EOs were α -pinene, myrcene and 1, 8-cineole. The results revealed a notable antioxidant activity in myrcene with an IC50 of 4.5 μ L mL⁻¹, succeeded by α -pinene with an IC50 of 18 μ L mL⁻¹. In this study thymol was utilized as a control sample since it possesses well-known antioxidant activity, and the amount of IC50 in both EOs were considerably higher than thymol (0.4 μ L mL⁻¹), which verified that these EOs were not stronger antioxidants than thymol.

In the study of Bagheri et al. (2014) antioxidant activity (DPPH) of *Piper nigrum* L. EO extracted by supercritical CO₂ was optimized and the effects of three independent variables: pressure (15–30 MPa), temperature (40–50 °C) and time (40–80 min) were evaluated. The optimum antioxidant activity (EC50) accomplished at 30 MPa, 40 min and 40 °C (103.28 μ g mL⁻¹) and it was much lower compared to the outcome of hydro-distillation extraction

(316.27 μ g mL⁻¹) which proved the superior antioxidant activity of supercritical CO₂ extraction.

An investigation was conducted to reveal the discrepancies of EOs composition, yield and antioxidant activities during three maturation stages of *Citrus medica* L. (Wu et al., 2013). In three stages of immature, intermediate and mature, EO yields were 2.39 %, 3.34 % and 3.57 % (w/w), respectively. Likewise, the chemical composition of EOs was different at various stages. The amount of limonene as the main component was at its highest in immature state (36.37%) but throughout maturation it decreased significantly (32.07%). Among the three phases, the EOs at immature stage exhibited the superior DPPH scavenging capability (78.4 %), followed by intermediate (64.7 %) and mature phases (63.8 %). Thus, with maturity the antioxidant activity of EOs reduced, as well.

The free radical scavenging capability of the EOs in both leaves and fruits of *Psidium guajava* L. were studied and compared with a control (Quercetin) sample (El-Ahmady et al., 2013). It was noticed that the IC50 of leaf sample (3.59 mg mL⁻¹) was lower than the fruit sample (8.11 mg mL⁻¹), however, both of them were considerably greater than the control sample (3.78 μ g mL⁻¹).

Impacts of microwave-assisted hydrodistillation (MAHD) and conventional hydrodistillation (HD), on the antioxidant activities of *Tetractylis articulata* EOs were analyzed (Djouahri et al., 2013). It was observed that the DPPH (IC50) and FRAP (EC50) inhibition of MAHD sample were 170 and 49.5% respectively, lower than the EO of HD, which verified the superior antioxidant activity of EOs in MAHD sample.

Antioxidant capacity of *Juniperus scopulorum* at various distillation times (1.25 to 480 min) was analyzed (Zheljzakov et al., 2013). It was observed with longer distillation time (450 min) maximum antioxidant capacity 54.7 (μ mol Trolox g⁻¹) could be achieved.

In a study of Hossain and Shah (2015), the antioxidant activities of the five extracts and EO of *Merremia borneensis* at 50 and 100 μ g mL⁻¹ concentrations were assessed via DPPH method. It was reported that at 100 μ g mL⁻¹ concentration the antioxidant activity in all the treatments was as following order: aqueous ethanol extract > chloroform extract > EO > ethyl acetate extract > butanol extract > hexane extract. Therefore, EO of *M. borneensis* had greater antioxidant capacity than most of the extracts.

DPPH radical scavenging and ferric-reducing potential of 14 thymus EOs belonging to ten species in various parts of Iran were measured at three concentrations of 5, 100 and 300 ppm (Tohidi et al., 2017). The results proved that *Thymus fedtschenkoi* (IC50 = 339.22 ppm), *Thymus daenensis* (IC50 = 273.36 ppm) and *T. vulgaris* (IC50 = 289.3 ppm) had superior antioxidant activities than other varieties. Similar to DPPH experiment, the higher reducing power was reported by *Thymus fedtschenkoi*, *Thymus daenensis* and *Thymus vulgaris* samples at 300 ppm concentration.

Antioxidant capacity and chemical composition of the rosemary (*Rosmarinus officinalis*) EOs extracted via supercritical CO₂ extraction (SCE), hydrodistillation (HYDRO), and steam distillation (SD) were analyzed and compared. The major volatile compound in different extracted EOs was varied where camphor (18.74%) in SCE, γ -cadinene (29.93%) in HYDRO and eucalyptol (12.59%) in SD were the major components. The antioxidant activity was analyzed through the ABTS test and it was 14 times higher in SCE compared to other samples (Conde-Hernández et al., 2017).

In the study of Ye, Dai, and Hu (2013) antioxidant activity of *Allium cepa* L. EO was evaluated by ABTS (0.67 mg mL⁻¹ as IC50

value) and DPPH (IC50 value = 0.63 mg mL⁻¹) methods. However, the highest concentration (1000 ppm), it was significantly inferior to the reducing potential of the EO was dose-dependent and even at the synthetic control sample (BHT).

Table 1. Application of plant essential oils in edible oils.

Essential oils	Concentration	Type of oil	Storage period	Investigated parameters	Reference
pussy willow	200-1200 ppm	Sunflower oil	60 days	Acid value, Peroxide value, Carbonyl value, Iodine value, Total phenolics. Total polar compounds, Oxidative stability index	Sayyari and Farahmandfar (2017)
<i>Lippia citriodora</i>	0-1600 ppm	Sunflower oil	60 days	Total polar compounds, iodine value, free fatty acid, Peroxide value, Carbonyl value, conjugated dienes. Oxidative stability index	Farahmandfar et al. (2018)
<i>Teucrium polium</i>	200-1200 ppm	Canola oil	60 days	Acid value, Peroxide value, Carbonyl value, Iodine value, Total polar compounds, Oxidative stability index	Savvad and Farahmandfar (2017)
<i>Thymus vulgaris</i> L.	1000 ppm	Virgin olive oil	42 days	Peroxide value, p-anisidine value, K232, K268	Keramat and Golmakani (2016)
<i>Bunium persicum</i> <i>Coriandrum sativum</i>	300-1200 ppm	Sunflower oil	24 days	Acid value, Peroxide value, Iodine value, p-anisidine. Thiobarbituric acid reactive substances, Total polar compounds	Wang et al. (2018)
<i>Ferulago aemulata</i>	125-500 ppm	Soybean oil	24 days	free fatty acid, Peroxide value, p-anisidine value	Sadeshi et al. (2016)
Black pepper Ginger	0.1-1% wt	Coconut oil	7 weeks	free fatty acid, Peroxide value, p-anisidine value, conjugated diene and triene	Chandran et al. (2017)
Cumin Savory Cardamom	0.2-0.6% v/v	Soybean oil	2-6 months	Acid value, Peroxide value, Induction period	Dolati et al. (2016)
<i>Carum copticum</i>	0.025-0.075% wt	Sunflower oil	14 days	Peroxide value, p-anisidine. Thiobarbituric acid reactive substances	Hashemi et al. (2014)

Table 2. Oxidative stability index (OSI) and iodine value (IV), total polar content (TPC) and conjugated diene of the sunflower oil as affected by the different concentrations of lemon verbena essential oil (0, 400, 800, and 1,600 ppm) during storage time (Source: Farahmandfar et al., 2018).

Treatments	Storage time				
	0	15	30	45	60
OSI					
Control	4.11 ± 0.14 ^{Ad}	4.14 ± 0.11 ^{Ad}	3.60 ± 0.35 ^{Bd}	2.94 ± 0.23 ^{Cd}	1.71 ± 0.17 ^{Dc}
400 ppm	6.25 ± 0.14 ^{Ab}	6.30 ± 0.14 ^{Ab}	5.99 ± 0.51 ^{Bc}	5.54 ± 0.15 ^{Cc}	5.37 ± 0.08 ^{Db}
800 ppm	6.75 ± 0.17 ^{Aa}	6.80 ± 0.15 ^{Aa}	6.47 ± 0.55 ^{Bb}	5.98 ± 0.16 ^{Bb}	5.80 ± 0.08 ^{Ca}
1600 ppm	6.74 ± 0.03 ^{Aa}	6.81 ± 0.08 ^{Aa}	6.78 ± 0.28 ^{Ba}	6.25 ± 0.18 ^{Ca}	5.94 ± 0.09 ^{Da}
BHT	5.12 ± 0.45 ^{Ac}	5.58 ± 0.58 ^{Ac}	5.37 ± 1.08 ^{Ac}	5.35 ± 0.34 ^{Ac}	5.67 ± 0.78 ^{Aa}
Iodine value					
Control	105.53 ± 2.43 ^{Ad}	105.77 ± 1.48 ^{Ad}	107.43 ± 5.49 ^{Ac}	104.30 ± 2.13 ^{Ad}	104.03 ± 1.13 ^{Ac}
400 ppm	109.20 ± 0.31 ^{Ac}	110.33 ± 1.51 ^{Ac}	112.60 ± 1.81 ^{Ac}	108.88 ± 1.16 ^{Ac}	108.00 ± 1.15 ^{Ac}
800 ppm	109.71 ± 0.32 ^{Ac}	110.84 ± 1.52 ^{Ac}	111.11 ± 1.82 ^{Ad}	109.38 ± 1.14 ^{Ac}	108.50 ± 1.11 ^{Ac}
1600 ppm	122.70 ± 0.35 ^{Aa}	123.97 ± 1.70 ^{Aa}	124.51 ± 2.05 ^{Aa}	122.33 ± 1.32 ^{Aa}	121.35 ± 1.29 ^{Aa}
BHT	114.67 ± 0.32 ^{Ab}	115.86 ± 1.64 ^{Ab}	114.23 ± 1.91 ^{Ab}	114.33 ± 1.22 ^{Ab}	113.42 ± 1.21 ^{Ab}
TPC					
Control	6.70 ± 0.73 ^{Ca}	8.22 ± 0.75 ^{Ba}	10.81 ± 1.16 ^{Aa}	11.18 ± 0.38 ^{Aa}	11.48 ± 0.44 ^{Aa}
400 ppm	5.19 ± 0.56 ^{Bb}	5.23 ± 0.57 ^{Bc}	8.37 ± 0.90 ^{Ac}	8.66 ± 0.29 ^{Ab}	8.90 ± 0.34 ^{Ab}
800 ppm	4.70 ± 0.61 ^{Bb}	4.74 ± 0.62 ^{Bd}	7.56 ± 0.68 ^{Ad}	7.83 ± 0.13 ^{Ac}	8.05 ± 0.47 ^{Ac}
1600 ppm	4.62 ± 0.50 ^{Bb}	4.66 ± 0.45 ^{Bd}	7.45 ± 0.80 ^{Ad}	7.71 ± 0.23 ^{Ac}	7.92 ± 0.54 ^{Ac}
BHT	6.26 ± 0.65 ^{Ca}	6.75 ± 0.73 ^{Cb}	8.22 ± 0.67 ^{Bb}	10.65 ± 1.33 ^{Aa}	11.04 ± 0.66 ^{Aa}
Conjugated diene					
Control	2.92 ± 0.66 ^{Ca}	4.00 ± 0.61 ^{Bb}	10.69 ± 1.42 ^{Aa}	11.67 ± 2.14 ^{Aa}	11.56 ± 1.51 ^{Aa}
400 ppm	2.69 ± 0.51 ^{Ea}	4.75 ± 0.52 ^{Da}	6.71 ± 0.59 ^{Cb}	7.76 ± 0.56 ^{Bc}	8.32 ± 0.18 ^{Ac}
800 ppm	1.87 ± 0.46 ^{Eb}	3.93 ± 0.46 ^{Dc}	5.90 ± 0.53 ^{Cb}	6.94 ± 0.50 ^{Bd}	7.44 ± 0.16 ^{Ad}
1600 ppm	2.84 ± 0.47 ^{Ea}	3.89 ± 0.21 ^{Dc}	5.86 ± 0.48 ^{Cb}	6.90 ± 0.49 ^{Bd}	7.41 ± 0.12 ^{Ad}
BHT	2.27 ± 0.62 ^{Da}	4.01 ± 0.49 ^{Cb}	9.36 ± 1.16 ^{Ba}	10.66 ± 1.08 ^{Ab}	10.57 ± 1.00 ^{Ab}

Means ± SD (standard deviation) within a column with the same lowercase letters are not significantly different at p < 0.05.

Means ± SD within a row with the same uppercase letters are not significantly different at p < 0.05.

The chemical composition of *Schinus molle* leaf and fruit EOs and their antioxidant attributes were specified employing DPPH free radical and β -carotene/linoleic acid experiments (do Rosário Martins et al., 2014). The main components of leaf and fruit EOs were monoterpene hydrocarbons, namely β -myrcene (11.1 and 51.3 %, respectively), α -phellandrene (14 and 25.9 %, respectively), limonene (11.7 and 14.1 %, respectively), β -phellandrene (9.2 and 10.5 %, respectively) and α -pinene (4.2 and 4.9 %, respectively). Furthermore, leaf and fruit EOs (at 16 mg mL⁻¹ concentration) possessed a free radical scavenging of 4.8 and 5.5% respectively, however, this was significantly less than the ascorbic acid standard as the control sample (14%). The results of β -carotene/linoleic acid procedure (1 mg mL⁻¹) were 57 and 19% for leaf and fruit EOs, respectively, whilst ascorbic acid displayed an impact of merely 1%. The outcomes of this investigation proved that EOs of *Schinus molle* plant were more effective in inhibiting lipid peroxidation than scavenging free radicals.

All things considered, in vitro researches on EO's antioxidant activities are not enough, they contain certain limitations such as lack of consideration for EO's physical condition and environmental situations. Hence, to achieve reliable outcomes it is better to evaluate the antioxidant activity of EOs in desired food systems.

3. Edible oils

Lipid oxidation is the leading cause of shelf-life alteration in edible fats and oils (Farahmandfar et al., 2019a). The most important outcomes of lipid oxidation are degradation of food flavor, taste, odor, texture and nutritional values especially fat soluble vitamins (vitamin A and E). Other problems regarding oxidation could be the breakdown of biological membranes, proteins and enzymes which can be a potential threat to human well-being (Farahmandfar et al., 2015; Sayyad & Farahmandfar, 2017). For the past few years, in several studies fortification of edible oils with EOs have been investigated (Table 1).

Pussy willow extract (PWE) and pussy willow EO (PWEEO) were applied to stabilized sunflower oil during atmospheric storage (25°C for 60 days) and the results were compared with synthetic antioxidant (TBHQ) (Sayyari & Farahmandfar, 2017). Initially, it was perceived that the amount of total phenolic and flavonoid of PWE (966.72 mg GAE g⁻¹, 619.45 mg CE 100 g⁻¹) were greater than PWEEO (355.84 mg GAE g⁻¹, 195.45 mg CE 100 g⁻¹), respectively. Hence, in accordance with all stabilization variables including total polar compound (TPC), peroxide value (PV), carbonyl value (CV), acid value (AV) and oxidative stability index (OSI), not only PWE had greater antioxidant activity than PWEEO, but it was superior to TBHQ, as well. Though, PWEEO was noticeably weaker than TBHQ in all parameters.

Lippia citriodora EO was applied (0 to 1600 ppm) for the purpose of elevating the stability of sunflower oil, and against this background the stability factors (TPC, iodine value (IV), free fatty acid (FFA), PV, CV, conjugated dienes, and OSI) of EO enriched samples were compared with synthetic antioxidant (BHT) at a storage period of 60 days in 60 °C (Farahmandfar et al., 2018). According to Table 1, OSI and IV of the sunflower oil samples with enriched *Lippia citriodora* EO or synthetic antioxidant (BHT) was noticeably greater ($p < 0.05$) than the blank sunflower oil and with increase in EO concentration the amount of these parameters were much higher than BHT and control sample. The level of degradation and the amount of primary oxidation compounds were

analyzed by TPC and conjugated dienes, respectively which the higher contents of these parameters would verify the higher degradation of oils. As stated in Table 2, during storage time, a notable increase in TPC and conjugated dienes at all treatments were observed. However, the most stable formulation in terms of these parameters obtained by addition of *Lippia citriodora* EO at 1600 ppm. Moreover, according to Fig. 1 and 2, the samples with 800 and 1600 ppm of *Lippia citriodora* EO revealed a remarkable decrease ($p < 0.05$) in PV, AV and CV values than other samples incorporated with *Lippia citriodora* EO (400 ppm) and synthetic antioxidants (BHT). Results of this study demonstrated that *Lippia citriodora* EO (at 1600 ppm) could provide better oxidative stability to sunflower oil than BHT and can be utilized as the prominent substitute of synthetic antioxidants.

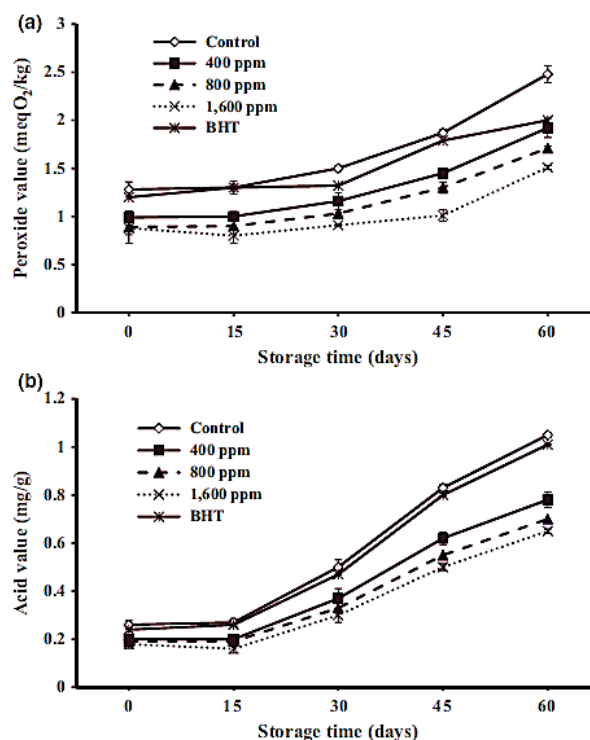


Fig. 1. (a) Peroxide value (PV) and (b) Acid value (AV) of the sunflower oil as affected by the different concentrations of lemon verbena essential oil (0, 400, 800, and 1,600 ppm) during storage time. \diamond -, Control; \blacksquare -, 400 ppm of lemon verbena essential oil; \blacktriangle -, 800 ppm of lemon verbena essential oil; \times -, 1,600 ppm of lemon verbena essential oil; \ast -, BHT (200 ppm) (Source: Farahmandfar et al., 2018).

The influence of *Teucrium polium* EO as a natural antioxidant at different doses (200 to 1200 ppm) on quality characteristics of canola oil including AV, PV, CV, IV, TPC along with OSI was studied on 60 days storage at room temperature (Sayyad & Farahmandfar, 2017). Samples enriched with EO had lower AV, PV, CV, IV and TPC than control (sample without EO), certifying the antioxidant ability of EO. However, compared to the control sample, OSI values of canola oil treated with different doses of EO had no substantial differences ($p > 0.05$) throughout storage. It was stated that OSI is not a suitable way for assessing the antioxidant activity of EOs since these substances are volatile and possibly were eliminated from canola oil by air flow during analysis.

Thymus vulgaris L. and *Bunium persicum* EOs were incorporated to the virgin olive oil at a concentration of 1000 ppm (Keramat & Golmakani, 2016). The BHT and α -tocopherol were used at 100 ppm as control samples. PV, p-anisidine (AnV), K232, and K268 values were determined for 42 days and it was noticed that both EOs notably reduced the amount of these parameters over the established period. The effect of these EOs on postponing olive oil oxidation was comparable to BHT and much better than α -tocopherol. Thus, they displayed the potential to be applied as antioxidants in virgin olive oil.

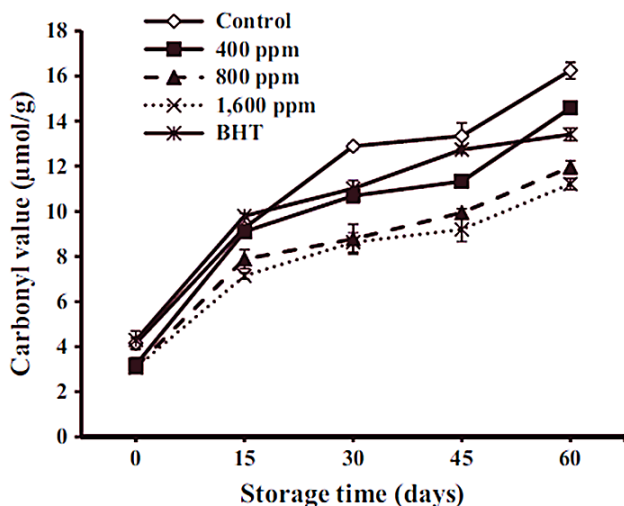


Fig. 2. Carbonyl value (CV) of the sunflower oil as affected by the different concentrations of lemon verbena essential oil (0, 400, 800, and 1,600 ppm) during storage time. —○—, Control; —■—, 400 ppm of lemon verbena essential oil; —▲—, 800 ppm of lemon verbena essential oil; ···×···, 1,600 ppm of lemon verbena essential oil; —*—, BHT (200 ppm) (Source: Farahmandfar et al., 2018).

The oxidative stability of sunflower oil following the addition of *Coriandrum sativum* EOs at concentrations of 300 to 1200 ppm throughout the 24 days storage was evaluated and the amount of AV, PV, IV, AnV, thiobarbituric acid reactive substances (TBARS) and TPC of oil samples were analyzed (Wang et al., 2018). TBHQ (200 ppm) and its mixture with EO (100 ppm EO + 100 ppm TBHQ) were applied as control samples. It was discovered that throughout storage, the contents of TBARS, AV, PV, AnV and TPC values in all treatments were significantly elevated ($p < 0.01$). However, by adding EO at 1200 ppm the oxidative stability of the sunflower oil was at better state compared to control samples.

The effect of *Ferulago angulata* EO in various concentrations (125, 250 and 500 ppm) on stabilization of soybean oil during 24 days of storage was investigated (Sadeghi et al., 2016). TBHQ (120 ppm) and a mixture with EO (60 ppm TBHQ + 60 ppm EO) were applied as control samples and FFA, PV and AnV tests were conducted for analyzing the impacts of EO soybean oil's stabilization. It appeared that the sample containing the mixture of EO and TBHQ had the highest shelf-life stability. This revealed that *F. angulata* EO is a potent antioxidant and can be utilized as an alternative of artificial antioxidant or it can be applied with TBHQ at lower concentration.

EOs of two flavoring spices namely black pepper and ginger were incorporated to the coconut oil at concentrations of 0.1-1.0% and the oxidative stability of the edible oil was determined by FFA,

PV, AnV, conjugated diene and triene experiments. The results were compared with blank coconut oil and sample containing TBHQ (Chandran et al., 2017). It was found that FFA, PV, AnV, conjugated diene and triene of both EOs at 1.0% concentration were comparable with TBHQ sample and significantly better than the blank oil. Therefore, these EOs could be utilized as the alternative natural antioxidants in coconut oil production.

Three EOs (cumin, savory and cardamom) at different doses of 0.2, 0.4 and 0.6% v/v were incorporated to the soybean oil and their impacts on AV, PV and induction period were assessed during varied storage times and temperatures (Dolati et al., 2016). Results displayed that savory and cardamom EOs at 0.4 and 0.6% v/v concentrations had remarkable effects on enhancing the induction period as well as reducing the FFA and PV.

Carum copticum EO were added to sunflower oil at varied doses (0.025%, 0.05% and 0.075%) and their oxidative stability were compared to BHA and BHT (control samples) during storage at 37 and 47 °C (Hashemi et al., 2014). The outcomes of PV, AnV and TBARS experiments exhibited that at all concentrations EO had antioxidant capacity comparable to BHA and BHT. Sample possessing EO at 0.075% concentration was the most stable oil during storage at both temperatures ($p < 0.05$) and was noticeably superior to other treatments.

4. Meat, fish and poultry

Meat, fish and poultry can be regarded as the most popular food products around the world. Therefore, the preservation against quick launch of oxidative rancidity, which leads to off-flavors and off-odors is the toughest challenge regarding these products. Hence, over the last few years, natural antioxidants extracted from plant origin like EOs have been considered to improve the durability and elevate the sensorial characteristics of meat products (Mohamed & Mansour, 2012). Although, direct addition of EOs has been the most popular technique of fortification, this method has various drawbacks which limited their applications in such products. Undoubtedly, it has been noticed that the higher doses of EOs is required to attain the similar effect of artificial antioxidants in foodstuffs (Hassoun & Çoban, 2017). However, even at low concentrations several EOs could induce a negative influence on sensory attributes (Lv et al., 2011). One of the common solutions which has been applied over the past few years is edible coating films enriched with EOs in order to diminish the required doses of these compounds (Hassoun & Çoban, 2017) and some examples of them were discussed in this section (Table 3).

EOs of *Origanum marjorana* L. and *Rosmarinus officinalis* L. at 200 mg kg⁻¹ were added to beef patties formulated with mechanically deboned poultry meat and lipid oxidation by TBARS was analyzed during frozen storage at -18 °C for 3 months (Mohamed & Mansour, 2012). BHT was used as synthetic antioxidant. It was noted that TBARS of all treatments increased over time. Though, the TBARS of formulas developed with EOs remained notably ($p < 0.05$) less than BHT samples during frozen storage which indicate the superiority of these EOs to synthetic antioxidants.

Effectiveness of packaging systems with or without active films possessing (i) 2% of an oregano EO and (ii) 1% of a green tea extract on oxidative stability (TBARS) of foal steaks during 14 days storage was examined. Samples were loaded with 80% O₂ / 20% CO₂ atmosphere and exposed through illumination at 2 °C for 14 days. Results revealed that TBARS values increased over time and they were significantly ($p < 0.01$) affected by storage period.

However, throughout the storage, the TBARS contents were less in 2% oregano active film than the other sample, verifying the superior antioxidant activity of EO (Lorenzo et al., 2014).

The oxidative stability (TBARS) of fresh pork sausages after addition of sage EO obtained from *Salvia officinalis* L. herbal dust was assessed (Šojić et al., 2018). Oxygenated monoterpenes, oxygenated sesquiterpenes and diterpene polyphenols were the predominate constituents of the EO. In all treatments, TBARS values notably raised ($p < 0.05$) within 8 days of storage, signifying the occurrence of lipid oxidation even with addition of antioxidants. However, following 6th day, sausages with enriched EO had remarkably ($p < 0.05$) lower TBARS values than control sample (without additive antioxidant), resulting in notable impediment of lipid oxidation.

Evaluation of *Ocimum basilicum* L. EO in different concentrations (0.00 to 0.25 % v/w) on lipid oxidation (TBARS) of beef burger was conducted at 4 °C for 12 days (Sharafati Chaleshtori et al., 2015). It was found that after the addition of EO at varied concentrations no noticeable differences was observed on TBARS of raw beef burger ($p > 0.05$). Therefore, this proves that not all EOs can be considered as antioxidants in meat products.

Ginger EO (at 3 and 6% wt) was included in nanoemulsion-based edible coating and then was added to chicken breast fillet for the sake of expanding its oxidative stability (Noori et al., 2018). Results displayed that the effect of EO on TBARS levels of fillets was not substantial ($p > 0.05$). However, antibacterial capacity of active coatings was greater than their antioxidant activity and it was

remarkably ($p < 0.05$) enhanced with addition of EO at 6% wt concentration.

The impact of orange dietary fiber (1 %) (ODF) and oregano EO (0.02 %) on the oxidative stability of bologna sausage was investigated and compared with the control sample (without antioxidants) (Viuda-Martos et al., 2010). At the end of the experiment (24th day), the ODF + EO sample revealed the lowest TBARS value ($p < 0.05$) which was due to high amount of polyphenols existed within both orange fibre and EO.

Rosemary EO (200 mg kg⁻¹) and varied doses of citrus fiber washing water (CFWW) (50–100 g kg⁻¹), were added to bologna sausage as a means to enhance its oxidation durability. Moreover, lipid oxidation was assessed by TBARS and DPPH methods and results were compared with the control sample (without added antioxidants) (Viuda-Martos et al., 2010). The addition of CFWW and EO caused a notable decline ($p < 0.05$) in TBARS of all samples. Whilst in favor of comparing the effect of CFWW and EO on reduction of TBARS values, treatments of CFWW 100 / EO 0 and CFWW 0 / EO 200 were applied and there was no notable variation among their TBARS values ($p > 0.05$). However, in regards to the DPPH values, the effect of CFWW and EO on inhibition of free radicals was more noticeable ($p < 0.05$) where the combination of both antioxidants at their highest concentration CFWW 100 / EO 200 led to best amount of DPPH scavenging activity. Therefore, the mixture of CFWW and rosemary EO seems to be a reliable antioxidant choice in formulation of fine paste meat products.

Table 3. Application of plant essential oils in meat products.

Essential oils	Concentration	Type of meat product	Storage period	Investigated parameters	Reference
<i>Origanum mariorana</i> L.	200 mg kg ⁻¹	Beef patties	3 months	Thiobarbituric acid reactive substances (TBARS)	Mohamed and Mansour (2012)
<i>Rosmarinus officinalis</i> L.					
Oregano	2% wt	Foal steak	14 days	TBARS	Lorenzo et al. (2014)
	0.02% wt	Bologna sausage	24 days	TBARS	Viuda-Martos et al. (2010a)
Sage	0.05-0.1 µL g ⁻¹	Fresh pork sausage	8 days	TBARS	Sojić et al. (2018)
Basil	0-0.25% v/w	Beef burger	12 days	TBARS	Sharafati Chaleshtori et al. (2015)
Ginger	3-6% wt	chicken breast fillet	12 days	TBARS	Noori et al. (2018)
Rosemary	200 mg kg ⁻¹	Bologna sausage	24 h	TBARS, DPPH	Viuda-Martos et al. (2010b)
Thyme	0.5% wt	Fresh and frozen	150 min	TBARS	Rimini et al. (2014)
Orange		chicken breast and wing			
Savory	7.80-31.25 ml g ⁻¹	Mortadella sausage	30 days	TBARS	de Oliveira et al. (2012)
Cinnamon	1.5% v/v	Rainbow trout	16 days	TBARS	Ojagh et al. (2010)

Table 4. Utilization of plant essential oils in dairy products.

Essential oils	Concentration	Type of dairy product	Storage period	Investigated parameters	Reference
Oregano	0.2% wt	Flavored cheese	35 days	Peroxide value, p-anisidine value	Olmedo et al. (2013)
Rosemary					
<i>Nigella sativa</i> L.	0.05-0.2% wt	Butter	90 days	Thiobarbituric acid reactive substances, Peroxide value	Cakmakçı et al. (2014)
Oregano	0.05% wt	Cottage cheese	30 days	Conjugated dienes	Asensio et al. (2015)
Thymol					
Oregano	0.001-0.1% wt	Dairy beverage	10 days	Conjugated dienes	Boroski et al. (2012)

In order to increase the oxidative durability of fresh and frozen chicken meat (breast and wing), a mixture of thyme and orange EOs (1:1) was utilized. Both fresh and frozen breasts and wings enriched with EOs were less sensitive to the lipid oxidation (TBARS) throughout all induced oxidation period (0-150 min at 37 °C) compared to control sample (without EOs). At the end of storage, it was noticed that the TBARS value of control frozen meat increased from 0.60 to 2.01, however this elevation for meat possessing EOs was from 0.30 to 0.79 clarifying the powerful effect of EOs on shelf-life elevation of broiler breast and wing.

Mortadella-type sausages were formulated with varied concentrations of winter savory (*Satureja montana* L.) EO (7.80, 15.60 and 31.25 ml g⁻¹) and sodium nitrite (NaNO₂) (0 to 200 mg kg⁻¹) (de Oliveira et al., 2012). Lipid oxidation was analyzed by TBARS at 25 °C for 30 days and results were compared with control sample (without NaNO₂ or EO). It was discovered that samples containing EO and NaNO₂ had lower TBARS than control sample (p<0.05). However, the antioxidant impact was merely synergistic when minimum amount of NaNO₂ (100 mg kg⁻¹) and 15.60 ml g⁻¹ EO was incorporated to the sample, verifying the potential merits of applying savory EO with minimal amounts of NaNO₂ in cured meat products.

The effect of chitosan (2 % w/v) coating enriched with cinnamon EO (1.5 % v/v) and without EO on the oxidative durability (PV and TBARS) of rainbow trout (*Oncorhynchus mykiss*) throughout 16 days storage (4 ± 1 °C) was assessed (Ojagh et al., 2010). The PV of the control and coated samples enhance remarkably (p < 0.05) with storage time and at the end of the storage, the amount of PV in control sample (4.23 meq O₂ kg⁻¹) was greater than coated samples containing EO (3.43 meq O₂ kg⁻¹) and without EO (4.60 meq O₂ kg⁻¹). Moreover, EO loaded sample had lower TBARS (0.21 mg malonaldehyde kg⁻¹) compared to control or EO-free coated samples (p < 0.05). Hence, this proved the fact that EO has a vital part on extending the shelf-life of the rainbow trout.

5. Dairy products

Dairy products are rich in polyunsaturated FAs of the n-3 family (n-3 PUFA), thus like any other lipid-containing products they would suffered from oxidation, as well. Particularly, hydrolysis of the short chain FAs available in raw milk which could be accountable for an unpleasant rancid flavor (Gad & Sayd, 2015). Therefore, in order to prolong the lipid oxidation and ameliorate the storage-life of dairy products, the incorporation of organic antioxidants like EOs has been investigated in several studies (Table 4).

The impact of oregano and rosemary EOs on the oxidative stability of flavored cheese was analyzed (Olmedo et al., 2013). Samples were evaluated for PV and AnV during storage of 35 days at 5 °C. By the end, samples enriched with EOs exhibited lower PV and AnV than the control sample (plain cheese) (p < 0.05). It was suggested that Oregano and rosemary EOs could be used as natural antioxidants in flavored cheese due to their protective influence against lipid oxidation.

Nigella sativa L. EO at various concentrations (0.05, 0.1 and 0.2 wt %) was added to the butter in order to improve its oxidative stability (Çakmakçı et al., 2014). Samples were kept at 4 °C for 90 days and antioxidant capacity of the EO was compared to BHT (100 ppm). TBARS and PV of all samples containing EO were increased over time but at the final day of storage, butter with 0.2% of EO displayed substantial oxidative stability (PV = 1.29 meq O₂

kg⁻¹ and TBARS = 0.22 mg malonaldehyde kg⁻¹), which was almost equal to that of BHT (PV = 1.23 meq O₂ kg⁻¹ and TBARS = 0.22 mg malonaldehyde kg⁻¹).

Four various Argentinean oregano (Compacto, Cordobes, Criollo, and Mendocino) and thymol EOs were added to organic cottage cheese and chemical indicators of lipid oxidation were assessed throughout 30 days of thermal storage (Asensio et al., 2015). It was observed that until 20th day, cottage cheese samples particularly the control sample (without antioxidant) did not differ noticeably (p > 0.05). However, in the final analysis (30th day), cottage cheese with Cordobes and thymol EO had lowest conjugated dienes (15.94 and 15.53, respectively; p < 0.001), whereas control sample revealed the highest content (17.54). Hence, the addition of oregano EO in cottage cheese could prolong its shelf-life.

Oregano extract (OE) and oregano EO as antioxidant agents were added at varied doses (0.001-0.1 g 100 g⁻¹) in a dairy beverage enriched with 2 g 100 g⁻¹ linseed oil and oxidation durability of the beverage was evaluated during 10 days of storage (Boroski et al., 2012). It was observed that OE was more effective than EO in inhibition of conjugated dienes. Though, after increment of EO dosage, conjugated diene were nearly steady till the end of storage, which would verify the effectiveness of EO. All things considered, researchers suggested that OE was better antioxidant than EO in regard to dairy beverage oxidation stability.

6. Conclusion

In summary, the addition of EOs on food products would improve their stability and prevent lipid oxidation as well as the development of rancid flavors. Hence, these EOs can be applied as natural antioxidants in various types of foods. However, in some cases these compounds showed lower antioxidant efficacy compared to the synthetic antioxidant or in some cases they were not suitable additive for oxidative stability. Therefore, optimum concentration of EOs or refined fractions obtained from them could increase stability of foodstuff against oxidation which must be considered in future investigations.

Acknowledgment

The authors would like to thank Sari Agricultural Sciences and Natural Resources University.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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