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Antioxidant activity of clove *(Eugenia caryophyllata* Thunb), oregano (*Oringanum vulgare* L) and sage (*Salvia officinalis* L) essential oils in various model systems

*Ghadermazi, R., Keramat, J. and Goli, S.A.H.

Department of Food Science and Technology, College of Agriculture, Isfahan University of Technology, Isfahan 84156-83111, Iran

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Abstract

The present study evaluated the antioxidant activity of clove, oregano and sage essential oils (EOs) using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging, β carotene/linoleic acid bleaching (BCB) and ferric reducing power (FRP) assays. EOs at concentrations of 600 and 1000 µg/mL and Butylated hydroxytoluene (BHT) at 100, 200 µg/mL, were added to the soybean oil at accelerated oxidation condition (60°C) for 30 days (oven test). The peroxide value (PV) and thiobarbituric acid (TBA) values of oil samples were calculated every 5 days. Amongst the investigated EOs, the clove EO was the most effective on DPPH, BCB, FRP, PV and TBA assays, which was followed by oregano and sage EOs, respectively ($P<0.05$). The results showed that the EOs as a natural antioxidant significantly reduced the oxidation of soybean oil and replaced synthetic antioxidants to increase the safety of food systems.

Introduction

Oxidation is one of the major factors in food deterioration and reduces its nutritional and qualitative values. Moreover, the free radicals produced from oxidation process are disease-promoting agents which menace the consumer health (Man and Jaswir, 2000). The addition of antioxidants is a useful way to prevent the formation of these harmful compounds. In recent years, synthetic antioxidants such as Butylated hydroxyanisole (BHA), Butylated hydroxytoluene (BHT), tertiary butyl hydroquinone (TBHQ) and propyl galates have been widely used in food industry. However, due to the risk of these compounds for health, there is a great interest in the utilization of natural antioxidants instead of synthetic ones (Abdalla and Roozen, 1999). Natural antioxidants do not have harmful effect and also can improve human health compared to synthetic antioxidants (Pokorny *et al*., 2001). Many studies have reported the antioxidant activity of natural compounds such as essential oils (EOs), herbs and spices (Farag, Badei, El Baroty 1989; Man and Tan, 1999; Politeo *et al*., 2006; Viuda-Martos *et al*., 2010). Among the major members of Lamiaceae family, *Origanum vulgare* L., and *Salvia officinalis* L., commonly known as oregano and sage respectively, are widely used in foods as flavoring agents and chutney. Oregano and sage extracts as well as their EOs have all been reported to inhibit lipid oxidation when added into various food and model systems due to the high content of phenolic compounds (Lagouri and Boskou, 1996; Abdalla and Roozen, 1999; Kulisic *et al*., 2004; Fasseas *et al*., 2008). The major compounds present in oregano EO were phenolic monoterpenes thymol and carvacrol (35.0% and 32.0%), monoterpenic hydrocarbons γ-terpinene (10.5%), p-cymene (9.1%) and α-terpinene (3.6%) (Kulisic *et al*., 2004). The most abundant components (>4%) of the sage EO were b-thujone (17.76%), 1,8-cineole (eucalyptol) (16.29%), camphor (14.19%), α-thujone (7.41%), transcaryophyllene (5.45%), viridiflorol (4.63%), β-pinene (4.41%), α-humulene (4.37%) and camphene (4.07%) (Bouaziz *et al*., 2009). Clove (*Eugenia caryophyllus*) buds also have been used in medicine (to relieve dental pain, headache and joint pain) and in food systems as flavoring and spice (Soto, 1995). Moreover, the Clove EO was reported to have fungicidal, antiviral, antitumor, insecticidal and anaesthetic properties (Chaieb *et al*., 2007).The major compounds present in Clove EO were eugenol (91.2%), β-Caryophyllene (4.1%) and eugenyl acetate (2.9%) (Dorman *et al*., 2000).The EO of Clove was found to have strong antioxidant activity using (DPPH) free radical scavenging (Chaieb *et al*., 2007), β-carotene agar diffusion (Dorman *et al*., 2000)

*Corresponding author.
Email: *r.ghadermazy@ag.iut.ac.ir*

and ferric reducing antioxidant power assays (FRAP) (Viuda-Martos *et al*., 2010). Clove EO prevents oxidation of hazelnut and poppy oils (Özcan and Arslan, 2011) and inhibits malonaldehyde formation in cottonseed oil (Farag, Badei, Hewedi *et al*., 1989). The effect of direct addition of some extracts of spice samples on the oxidative stability of linseed oil has been studied and the highest effect has been observed for allspice following by clove and nutmeg extracts, respectively (Nguyen *et al*., 2000). To our knowledge, there is very little quantitative information about the antioxidant activity of natural antioxidants with favorite aroma including clove, oregano and sage EOs in soybean oil as most widely used edible oil in the world.Therefore, the objective of this work was to study: the antioxidant activity of clove, sage and oregano EOs by using different methods (DPPH radical scavenging, β-carotene bleaching (BCB) and FRAP); and determine the correlation between their total phenolic content and antioxidant activity. Also, the effects of EOs in the oxidation rate of soybean oil during 30 days storage at 60°C was investigated by using peroxide value (PV) and thiobarbituric acid (TBA) values.

Materials and Methods

Materials

Dried clove buds (*Eugenia caryophyllataThunb*.) were purchased from Isfahan Pakan Bazr Co (Isfahan Province, Iran). Dried aerial parts of oregano (*Oringanum vulgare* L.) and sage (*Salvia officinalis* L.) were obtained from Shahrekord area (Chaharmahal and BakhtiariProvince, Iran). Linoleic acid, β-carotene and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma Chemical Co. (Sigma– Aldrich GmbH, Sternheim, Germany). Chemicals such as methanol, acetic acid, sodium iodide, sodium thiosulphate, thiobarbituric acid, 1-butanol, chloroform, Folin-Ciocalteu reagent, BHT were analytical grade, obtained from Merck (Darmstadt, Germany). Soybean oil without any antioxidant was obtained from Naz Oil Refinery (Isfahan Province, Iran).

Essential oil extraction

One hundred gram of crushed plant material was hydro-distilled for 3 h, using Clevenger-type apparatus, according to the method recommended by Özcan and Arslan (2011). The extraction was conducted in three times. Oils were dried over anhydrous sodium sulfate and stored at 4–6°C in amber glass bottle for further analyses.

Total phenolic content

Total phenolic content of the EOs was determined as described by Viuda-Martos *et al*. (2010) with minor modification. The 0.3 ml of a methanolic solution (50 mg/ml) of EOs was mixed with 2.5 ml Folin-Ciocalteu reagent (diluted 10 times with water) and 2 ml Na_2CO_3 (7.5% w/v). After incubation at 50°C for 5 min, the absorbance of the mixture was measured at 760 nm (M350 Double Beam UV-Visible Comspec Spectrophotometer). Total phenolic were expressed as mg tannic acid equivalents (TAE) per gram EO.

Radical scavenging activity using DPPH

Radical scavenging activity of the EOs was evaluated using the DPPH free radical according to the method of Bozin *et al*. (2007), with some modifications. Briefly, 3 ml of methanolic solutions of EOs at different concentrations (100, 200, 400, 600, 800 and 1000 µg/ml) and BHT (100, 200 µg/ml) as positive control were added to 1 ml of a freshly prepared 90 µM DPPH solution. The mixtures were incubated in dark for 60 minutes at room temperature and then their absorbance was recorded at 517 nm. The percentage of DPPH radical inhibition was calculated according to the following equation (Eq. 1):

$$\%\ \text{Radical scavenging capacity} = \frac{A_b - A_s}{A_b} \times 100 \quad (1)$$

Where A_b represents the absorbance of the blank and A_s is the absorbance of the samples containing antioxidant. Blank sample contained all reagents except the antioxidant (Bamdad *et al*., 2006).

β-carotene/linoleic acid bleaching assay

Antioxidant activity of EOs was determined according to β-carotene bleaching method of Kulisic *et al*. (2004) with some modifications. One mg of β-carotene was dissolved in 10 ml of chloroform. Four milliliter of this solution was added into a boiling flask containing 400 mg of Tween 80 and 40 mg of linoleic acid. Chloroform was completely evaporated at 37°C by a rotary evaporator. Then, 100 ml of oxygenated distilled water was added to the residue along with vigorous shaking to form a clear yellowish emulsion. Five ml of this emulsion were added into test tubes containing 0.2 ml of the methanolic solutions of EOs at different concentrations (100, 200, 400, 600, 800 and 1000 µg/ml) and BHT (100, 200 µg/ml) as positive control was mixed thoroughly. The test tubes were incubated in a water bath at 37°C for 180 min together with a negative control (blank) and the absorbance was measured at 470 nm every 30 min. Antioxidant activity was calculated according to the following equation (Eq. 2):

$$\% \text{ Antioxidant activity} = 1 - \frac{A_{s_0} - A_{s_t}}{A_{c_0} - A_{c_t}} \times 100 \quad (2)$$

Where A_{s0} and A_{st} were the absorbance values of test samples for 0 and 180 min, respectively. A_{c0} and A_{ct} were the absorbance values of blank for 0 and 180 min.

Reducing power

The ferric reducing power of the EOs was determined according to the method of Oyaizu (1986). One ml of methanolic solutions of EOs at different concentrations (100, 200, 400, 600, 800 and 1000 µg/ml) and BHT (100, 200 µg/ml)as positive control were applied to 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml potassium ferricyanide (1%). The mixtures were incubated at 50°C for 20 min. Then 2.5 ml trichloro acetic acid (10%) was added to the mixture and it was centrifuged at 1500 × g for 10 min. Then 2.5 ml of the upper layer was mixed with 2.5 ml of distilled water and 0.5 ml of ferric chloride (1%). The absorbance was measured at 700 nm after allowing the solution to stand for 30 min at room temperature.

Preparation of soybean oil samples

Soybean oil samples containing EOs were prepared by the method of Özcan and Arslan (2011), with some modifications. The clove, oregano and sage EOs at concentrations of 600 and 1000 µg/mL and BHT at 100, 200 µg/mL, were added to the soybean oil and completely mixed by homogenizer (IKA-T25 digital ultra-Turrax) for 15 min at the speed of 5000 rpm. The samples were stored in accelerated conditions at 60°C and in a dark place for 30 days (oven test). The soybean oil without any antioxidant was used as control sample.

Antioxidant activity of EOs in soybean oil

In order to evaluate antioxidant activity of the essential oils, peroxide value (PV) and thiobarbituric acid (TBA) of the oil samples were determined at interval of five days during the storage. PV and TBA were measured by the methods of cd 8-53 and cd 19-90 (AOCS), respectively.

Statistical analysis

All experiments were carried out in triplicate and the data was reported as mean± standard deviation. The data were statistically analyzed by ANOVA program (using by the statistical analysis system (SAS, 2002) software package) and the means evaluation was done using least significant difference (LSD) at confidence level of 95%.

Table 1. Yield and phenolic contents of clove, oregano and sage essential oils

Plant Type	Essential Oil Yield* (% v/w)	Phenolic Content (mg TAE /g EO)
Clove	14.79	217.51 ± 1.74[a]
Oregano	0.89	53.51 ± 0.54[b]
Sage	1.39	22.55 ± 0.96[c]

Values expressed are the mean ± SD of three replications
Data with different letters in column indicated significant differences ($p<0.05$)
* ml/100 g dry weight

Results and Discussion

Extraction yield and total phenolic content

The average yield of EOs from clove buds, oregano and sage leaves and their total phenolic contents are shown in Table 1. The content of the EOs expressed was as follows: clove, oregano and sage. Phenolic compounds are a group of natural antioxidants having radical scavenging ability due to their hydroxyl groups (Viuda-Martos *et al*., 2010).Clove EO showed the highest amount of total phenolic whereas the lowest amount of total phenolic belonged to oregano and sage EOs, respectively. Viuda-Martos *et al*. (2010) found that clove EO had high phenolic content (898.89) than both oregano EO (763.97) and sage EO (122.98 mg GAE/l). The content of phenolic compounds as an acceptor of free radicals by interrupting chain oxidation reactions or by chelating metals could be an indicator of the antioxidant capacity of EOs (Fernandes de Oliveira *et al*., 2012).

Free radical scavenging activity

Table 2 shows DPPH radical scavenging activity of clove, oregano and sage EOs and BHT at different concentrations. All of EOs were efficient in DPPH radical scavenging ($P<0.05$). The results indicated that free radical-scavenging activity was enhanced by increasing the concentration of three EOs. Clove EO and BHT, showed the highest DPPH radical inhibition. Clove EO showed higher DPPH radical scavenging than BHT at the same dose. At 200 µg/mL concentration, both of clove EO and BHT showed 100% antioxidant activity; therefore, higher doses were not tested. The most activity was obtained for oregano EO (57.89%) and was followed by sage EO (50.62%) at 1000 µg/ml concentration. Generally, as shown in Table 2, the antioxidant activity decreased in the order of clove EO > BHT > oregano EO > sage

Table 2. Antioxidant activity (mean ± SD) of the clove, oregano, sage essential oils and BHT at different concentrations Using DPPH, β-carotene bleaching and reducing power assays

Sample	concentration (µg/ml)	DPPH radical scavenging (%)	β-carotene bleaching (inhibition %)	reducing power (absorbance at 700 nm)
Clove	100	94.18 ± 2.12^{b}	56.79 ± 7.12^{gh}	0.95 ± 0.03^{f}
	200	100.00 ± 0.08^{a}	73.80 ± 6.79^{e}	1.10 ± 0.08^{e}
	400	-	82.53 ± 6.20^{cd}	2.04 ± 0.11^{d}
	600	-	86.29 ± 1.52^{cd}	2.68 ± 0.02^{c}
	800	-	95.64 ± 1.16^{ab}	2.80 ± 0.04^{b}
	1000	-	96.32 ± 1.86^{ab}	2.92 ± 0.03^{a}
Oregano	100	11.20 ± 1.08^{i}	7.02 ± 1.49^{i}	0.10 ± 0.03^{ab}
	200	20.25 ± 2.09^{hi}	36.98 ± 2.90^{i}	0.17 ± 0.06^{ab}
	400	37.18 ± 2.73^{fg}	51.40 ± 1.95^{hi}	0.29 ± 0.07^{ab}
	600	41.42 ± 2.67^{ef}	57.29 ± 2.35^{gh}	0.33 ± 0.68^{ab}
	800	51.24 ± 1.98^{de}	66.16 ± 0.83^{fg}	0.45 ± 0.06^{a}
	1000	57.89 ± 2.05^{c}	76.53 ± 1.61^{ef}	0.57 ± 0.07^{a}
Sage	100	9.40 ± 0.34^{i}	6.74 ± 2.92^{i}	0.08 ± 0.08^{b}
	200	15.38 ± 1.03^{i}	30.99 ± 0.85^{ij}	0.14 ± 0.06^{ab}
	400	31.73 ± 1.29^{gh}	45.61 ± 3.13^{i}	0.20 ± 0.07^{ab}
	600	34.68 ± 2.12^{fg}	51.69 ± 2.84^{hi}	0.21 ± 0.07^{ab}
	800	44.49 ± 2.08^{ef}	55.80 ± 2.14^{gh}	0.26 ± 0.08^{ab}
	1000	50.62 ± 0.62^{e}	59.34 ± 4.79^{gh}	0.34 ± 0.08^{a}
BHT	100	92.15 ± 3.23^{b}	91.85 ± 2.60b^{b}	0.74 ± 0.05^{b}
	200	100.00 ± 0.03^{a}	98.84 ± 1.42^{a}	0.90 0.07^{a}

Data with different letters in column indicated significant differences ($p<0.05$)

EO.

This remarkable antioxidant capacity of clove EO could be attributed to higher content of phenolic components such as eugenol and eugenyl acetate and to their hydrogen donating ability by which they are considered to be potent free radical scavengers (Dorman *et al*., 2000; Chaieb, Zmantar, Ksouri *et al*., 2007). Politeo *et al*. (2006) studied the antioxidant activity of twelve herbal plant EOs and observed that 5 g/l EO of clove had highest (93%) power DPPH radical scavenging followed by basil (85%), laurel (68%), coriander (30%), nutmeg (24%), black pepper (14%), everlast (11%), marjoram (9%), mint (8%), cinnamon (6%), sage (5%) and fennel (2%). Some researchers have reported the low free radical scavenging capacity of sage EO. Miguel *et al*. (2011) determined a maximum of 60–70% inhibition in DPPH assay at 22 g/l of sage EO. It is reported that antioxidant activity of sage EO is mainly due to its phenolic compounds (Pokorny *et al*., 2001) such as β-thujone, 1,8-cineole and camphor (Bouaziz *et al*., 2009) whereas in oregano EO, it is related to the high (more than 50%) amount of phenolic monoterpenes like thymol and carvacrol (Lagouri and Boskou, 1996; Kulisic *et al*., 2004).Viuda-Martos *et al*. (2010) reported that antioxidant activity of clove EO, ascorbic acid, BHT, thyme, oregano, sage and rosemary EOs at 5 g/l concentration was 97.85%, 96.61%, 95.93%, 62.87%, 51.79%, 51.17% and 47.54%, respectively. Quiroga et al. (2013) showed that Oregano EO had higher phenolic content (12.47 mg gallic acid/mL) and DPPH scavenging activity (IC50= 0.357 µg/mL) than Lippia EO (7.94 mg gallic acid/mL and IC50= 0.400 µg/mL, respectively). Zengin and Baysal (2014) demonstrated that in DPPH assay, Clove EO (IC50= 0.14 µL/mL) showed the higher antioxidant activity than thyme EO (IC50= 9.88 µL/mL). Mushrooms treated with clove, cinnamaldehyde or thyme had markedly lower radical signals for DPPH and ABTS than those of the control, indicating that the treated mushroom had higher scavenging activities for these radicals (Jiang *et al*., 2015).

β- Carotene bleaching method

The presence of different antioxidants can hinder β–carotene bleaching by quenching free radicals in the system. The inhibition percentage of different concentrations of clove, oregano, sage EOs and BHT on β-carotene bleaching are shown in Table 2 and the dose-dependent activity was observed for all samples. All samples containing EO showed antioxidant activity and oxidation rate reduction of linoleic acid. BHT at 200 µg/ml concentration showed the highest antioxidant activity which was followed by 1000 and 800 µg/ml of clove EO with inhibition percentages of 96.3%, 95.6%, respectively

(P>0.05). Oregano and sage EOs showed the lowest antioxidant activity compared to clove EO and BHT at the same concentration of 1000 µg/ml antioxidant activity was estimated to be 76.53% and 59.34%, respectively. Generally, as shown in Table 2, the antioxidant power decreased in the order: BHT > clove EO > oregano EO > sage EO.

Dorman *et al*. (2000) showed that among phenolic compounds of clove EO, only eugenol and terpinolene demonstrate the ability to inhibit the oxidation of β-carotene agar. Antioxidant activity depends on the assay methods, the concentration and the physicochemical attribute of the studied antioxidants. Therefore, it is necessary to evaluate the antioxidant activity of clove EO using different assays. Kulisic *et al*. (2004) demonstrated that the antioxidant power of some standard compounds and oregano EO in β-carotene assay decreased in the order of BHT > α-tocopherol > oregano EO > ascorbic acid. Also they illustrated that carvacrol is a fraction with stronger effect than thymol in oregano EO. Şahin *et al*. (2004) showed that both methanol extract and EO of oregano were not able to effectively inhibit linoleic acid oxidation and only 24% and 36% inhibitions were achieved at 2 mg/ml concentrations, respectively, which were far below than that of BHT (89%) at the same concentration. Hussain *et al*. (2011) showed that the antioxidant activity in the β-carotene assay for sage EO (65.2%) was lower than value of BHT (more than 90%) at the 4 mg/ml.

Determination of reducing power

Table 2 shows the reducing power of different concentrations of clove, oregano, sage EOs and BHT to reduce Fe^{3+} to Fe^{2+}. Higher absorbance indicates that the antioxidant was active in reductive ability. Clove EO showed the highest ferric reducing capacity at all concentrations followed by BHT, but oregano and sage EOs have less effectiveness compared to clove EO at the same dose (P<0.05). However, at lower concentrations, oregano and sage EOs had no significant difference (P>0.05).

In a study by Viuda-Martos *et al*. (2010) on clove EO, it showed the highest ferric reducing capacity in terms of Trolox concentrations, followed by oregano, sage and rosemary EOs. The strong activity of clove EO can be due to the presence of eugenol (90%), which is known to have antioxidant activity (Dorman *et al*., 2000; Politeo *et al*., 2006). Gülçin *et al*. (2012) showed reducing power of clove EO and standard antioxidants (at 15-45 µg/ml) in the following order: Clove oil > BHA ≈ BHT > α-tocopherol > trolox. Politeo et al. (2006) demonstrated that clove EO had highest (88 mmol/l) reducing power which followed by basil EO (7 mmol/l), laurel EO (2 mmol/l), black pepper EO (1 mmol/l), and EOs of sage, coriander, nutmeg, everlast, marjoram, mint, cinnamon and fennel showed the weakest capacity (<1 mmol/l). Zengin and Baysal (2014) demonstrated that in FRAP assay, clove EO (4,357.45 mmol Trolox/mL) showed the higher antioxidant activity than thyme EO (2,150.72 mmol Trolox/mL).

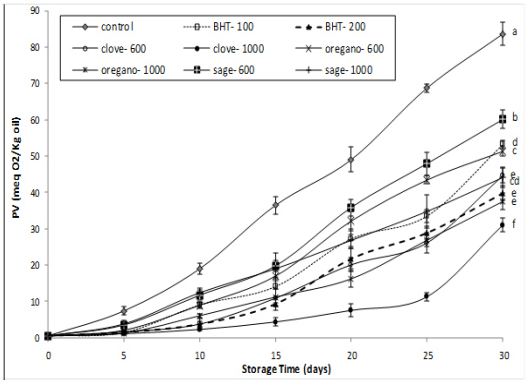

Figure 1. Effect of clove, oregano and sage essential oils and BHT on the PV of soybean oil stored at 60°C. Different letters (a–f) within the lines indicate significant differences between formulations (p<0.05).

Correlation coefficient between antioxidant activity and phenolic content of EOs

Correlation coefficient (R^2) was calculated between the total phenolic content and DPPH radical scavenging activity; There was a positive linear correlation between antioxidant activity and total phenolic of EO (R^2 = 0.995, Y= 0.051X + 43.88). Where Y and X were the antioxidant activity and phenolic content, respectively. Results shows that there is normal correlation between antioxidant activity in β-carotene bleaching method and phenolic content (R^2 = 0.906, Y = 0.033X + 61.63). Also, high correlation was observed between total phenolic content with FRAP of EOs at 1000 µg/ml concentration (R^2 = 0.991, Y = 0.002X – 0.067).

Effect of EOs on soybean oil oxidation

Peroxide value (PV) is a measure of the primary compounds produced through lipid oxidation. Figure 1 represents the antioxidant effect of clove, oregano and sage EOs in comparison to BHT and control (the sample without antioxidant) on oxidation of soybean oil as determined by the PV. In accelerated condition at 60°C, the PV of all samples increased during storage. The PV of oil in onset of storage were 0.56 which at 30th day of storage increased to 83.69, 53.24 and 39.83 meq O_2/Kg oil for control and samples containing 100 and 200 µg/mL BHT, respectively.

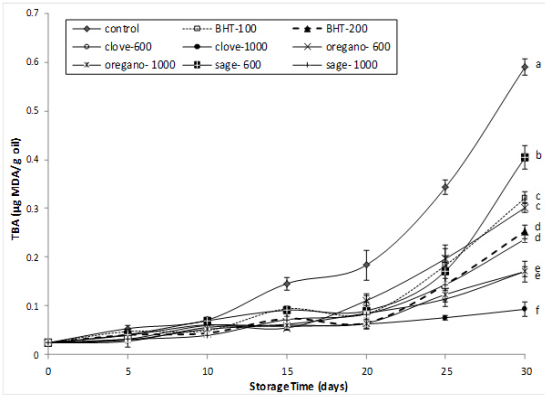

Figure 2. Effect of clove, oregano and sage essential oils and BHT on the TBA of soybean oil stored at 60°C. Different letters (a–f) within the lines indicate significant differences between formulations ($p<0.05$).

The samples with natural antioxidants also had lower PV than control. Until 5th day, growth rate of PV was not remarkable ($P>0.05$) but at the end of storage, the samples with high concentrations of EOs showed the highest antioxidant effect in soybean oil. During storage, EOs (except sage) had a significantly higher efficiency to reduce oxidation than BHT at 100 µg/mL. The PV for samples 600 and 1000 µg/mL clove EO at 30th days storage was calculated as 44.63 and 31.08 (meq O_2/Kg oil), respectively. Samples containing 600 µg/mL of clove EO were not significantly different with 200 µg/mL BHT ($P<0.05$). Clove EO at 1000 µg/mL was very stronger than BHT at 200 µg/mL and other EOs. The stability of the sample with 1000 µg/mL clove EO was considerably higher than that of all samples. Initially, increase in PV was in slow and retarding manner, but it was observed that it's increasing speeds up after 5th days of storage and increased further with the increase in storage period. In the 600 µg/mL of clove EO, an increase was observed after 10th days and for 1000 µg/mL clove EOs, increase was recorded after 15th day's storage. Antioxidant activity of oregano EOs was comparable to BHT in storage period. Oregano (37.55) and sage (44.17) EOs at 1000 µg/mL concentration were not significantly different from BHT (39.83 meq O_2/Kg oil) at 200 µg/mL ($P>0.05$) in final day of storage.

Thiobarbituric acid (TBA) index is defined as the quantity of malondialdehyde (MDA) in microgram present in one gram of oil (µg MDA/g oil). TBA demonstrates the quantity of secondary oxidation products (aldehydes and carbonyls) which may contribute to off-flavor and degeneration of oxidized oil. The TBA value of control oil in initial stage of storage was 0.024 which was calculated 0.59, 0.32 and 0.25 µg MDA/g oil oil at the end of storage for the control and samples containing 100 and 200 µg/mL BHT, respectively. Figure 2 illustrates the effect of clove, oregano and sage EOs at 600 and 1000 µg/mL and BHT at 100 and 200 µg/mL concentrations on TBA values of soybean oil during 30-day storage at 60°C. The TBA value as well as PV showed an increase during storage. Moreover, as concentration of EOs was increased, TBA value was decreased. Clove EO strongly inhibited the formation of TBA. TBA value for the oils containing 600 and 1000 µg/mL clove EO was calculated 0.17 and 0.09 (µg MDA/g oil) at the 30th day of storage. Samples with both 600 and 1000 µg/mL clove EO were significantly different ($P<0.05$) from 200 µg/mL BHT. Oregano EO had a desirable potential to prevent secondary compounds formation but it was weaker than clove EO at the same doses. At 600 µg/mL concentration, there was no significant difference between oregano EO and 100 µg/mL of BHT and oregano EO at 1000 µg/mL a having higher ability than that of BHT at 200 µg/mL.

Clove EO is very stronger antioxidant than oregano and sage EOs and these results are mainly due to the high content of eugenol in clove EO (Özcan and Arslan, 2011). Fasseas *et al*. (2008) studied the antioxidative properties of oregano and sage EOs (3% w/w) in meat treated, and observed that oregano EO was more effective than sage EO at the same dose. Farag, Badei, Hewedi *et al*. (1989) showed that clove EO had better performance than thyme EO to exhibit antioxidant activity in cottonseed oil at room temperature.

Baratta *et al*. (1998) showed the antioxidant activity of EO of some herbs by thiobarbituric acid reactive species (TBARS) assay, using egg yolk and rat liver as oxidable substrates. The antioxidant activity of the EOs at 1000 µg/mL in egg yolk was followed in this order: oregano > BHT > coriander > laurel > rosemary > a-tocopherol > sage, whereas in rat liver the order of BHT > coriander > laurel > rosemary > a-tocopherol > oregano > sage was obtained. Özcan and Arslan (2011) evaluated the effect of clove, rosemary and cinnamon EOs on the stability of hazelnut and poppy oils at 50°C, cinnamon oil at 0.5% was the most effective EO on retarding lipid oxidation of hazelnut oil, followed by clove and rosemary oils. Farag, Badei, Hewedi *et al*. (1989) showed that antioxidant activity of some spice EO in linoleic acid which emulsified in aqueous phase following this order: caraway > sage > cumin > rosemary > thyme > clove. Ibrahium et al. (2013) showed that the clove EO was able to retard the oxidation rate and reduction of formed preliminary and secondary oxidation products in

cakes compared with synthetic antioxidant, except the sample containing 400 ppm clove EO had lower values than control and almost equal with the sample containing BHT. Zengin and Baysal (2014) showed that thyme and clove EOs retarded lipid oxidation during 9 days of storage at 4°C. Although clove EO showed the higher in vitro antioxidant activity than thyme EO in minced meat application, both of the EO treatments showed significant reduction ($P<0.05$) in TBA value comparing with control.Quiroga *et al*. (2013) showed that Both oregano and lippia EO had similar antioxidant indexes (about 1.2) determined by rancimat.

Conclusion

The results obtained from different methods to evaluate the antioxidant activity showed that clove, oregano and sage EOs might be considered as good sources of natural compounds with significant antioxidant activity. The antioxidant activity depends on the assay used, the concentration and the physicochemical attributes of the studied antioxidant compounds. It is generally concluded that clove EO was a more powerful antioxidant than oregano and sage EOs compared to BHT. In the terms of oil stability, it is concluded that EOs from clove (both 600 and 1000 µg/mL concentrations), oregano (both 600 and 1000 µg/mL concentrations) and sage (1000 µg/mL concentration) which can stabilize soybean oil up to a greater extent than commonly employed synthetic antioxidants (100 µg/mL BHT). Therefore, clove, oregano and sage EOs can be taken as a relative potent source of natural antioxidants for the stabilization of oils in food systems. But clove EO showed strong protective ability against oil oxidation and highest antioxidant activity in different model system.

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