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Chemical Composition and Antioxidant Activity of Clove Essential Oil and its Effect on Stability of Sesame Oil under Accelerated Condition

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ABSTRACT

Background: Recently due to adverse effects of synthetic antioxidants, there has been a growing interest in the application of natural essential oil in vegetable oils. The present study investigated the chemical composition and antioxidant activity of clove essential oil (CEO) and its addition to sesame oil. **Methods**: composition and antioxidant activity of clove essential oil The CEO was prepared and analyzed by GC-MS. Then, total phenolic content (TPC), antioxidant activity and ferric reducing antioxidant power (FRAP) were determined. The CEO at different concentrations (0.02, 0.04, 0.06, and 0.08%) and TBHQ (0.02%) were added to sesame oil and samples were stored at 60 °C for 5 weeks. Peroxide value (PV), p-Anisidine value (p-AV), total oxidation (TOTOX) value and Thiobarbituric acid reactive substances (TBARs) were determined in sesame oil samples every week for 35 days. A total of 5 components including eugenol (96.25%), eugenol acetate (1.88%), trans-Caryophyllene (1.66%), α-humulene (0.16%), and caryophyllene oxide (0.06%) were determined as the main components of CEO. **Results:** The TPC of CEO was 345.95±7.85 mg GAE/g. Moreover, the antioxidant activity of CEO for DPPH (IC_{50}) and FRAP methods was estimated 0.83 ± 0.11 mg/ml and 112.37±8.81 mM Fe_2SO_4. It was shown that peroxide, p-AV, TOTOX, and TBARS values of all sesame oil samples increased during 5 weeks of storage at accelerated conditions. TBHQ showed better function in preventing oil oxidation, but CEO had acceptable function especially in 0.08% concentration. **Conclusion**: The CEO in vegetable oil due to high phenolic content could retard lipid peroxidation. It could be mentioned that CEO could be considered as an alternative of synthetics ones in vegetable oils**.**

Keywords: *Sesame oil; Clove essential oil; Antioxidant activity; Lipid peroxidation*

Introduction

Sesame seed (*Sesamum indicum L.*) is the oldest oilseed crop known to humans, which has been widely cultivated in Asia, Africa, and South America (Abou-Gharbia *et al.*, 2000). Sesame seed

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is a nutritive crop containing a lot of edible oil and proteins which have therapeutic properties (Majdalawieh *et al.*, 2017). Sesame oil also benefits from various biologically active phytochemicals such as phytosterols, tocopherols, sesamin, sesamoline, and sesaminol, which have high potential antioxidant activity and are known to play an important role in maintaining stability against oil oxidation (Bopitiya and Madhujith, 2013). Although the presence of sesamin and sesamoline in sesame seeds makes the extracted oil stable to oxidation at high temperatures, several factors will increase the procedure of oxidation in this oil (Carrasco-Pancorbo *et al.*, 2005, Shahidi, 1997). The oxidation of unsaturated fatty acids is considered as one of the most important factors affecting oil deterioration. Lipid oxidation causes undesirable flavor, off-odors,, discoloration, and loss of nutritional value, leading to oil rancidity (Darughe *et al.*, 2012, Hraš *et al.*, 2000). During the oxidation process, the formation of break down products such as peroxides, aldehydes, and ketones decreases the oil shelf life (Özcan and Arslan, 2011). These changes make the degraded oil unacceptable for consumption (Özcan and Arslan, 2011). In order to retard oil oxidation, synthetics antioxidants such as butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT), propyl gallate (PG) and tertbutyl hydroquinone (TBHQ) are commonly used in oil industry (Hraš *et al.*, 2000, Özcan and Arslan, 2011). In recent decades, the safety of synthetic antioxidants has raised the public concerns about their possible toxicity (Özcan and Arslan, 2011). Given the destructive effects of these antioxidants including toxicity, liver damage, and carcinogenicity, the replacement of natural alternatives have been proposed as a beneficial approach with fewer adverse outcomes in order to overcome the negative health effects of the synthetic antioxidants (Özcan and Arslan, 2011).

Clove (*Syzigium aromaticum L., family Myrtaceae*) as a common traditional herb consists of a variety of bioactive compounds that has been reported to be effective in preventing oxidation process (El-Maati *et al.*, 2016, Özcan and Arslan, 2011). The safety and antimutagenic activity of cloves have been approved in previous studies (Vijayasteltar *et al.*, 2016). Also, the Food and Drug Administration (FDA) classified eugenol as generally recognized as safe (GRAS) (El-Maati *et al.*, 2016). Hence, it can be hypothesized that application of clove essential oil (CEO) as an antioxidant agent may positively affect the oxidative stability of vegetable oils without potential negative health effects on consumers. The present investigation aims to determine the antioxidant effects of different concentrations of CEO on sesame oil stored at 60 °C.

Methods and Materials

Preparation of CEO: CEO was prepared by hydrodistillation reported by Özcan *et al.* (Özcan and Arslan, 2011). Approximately 100 g of clove was powdered and 1.5 L of distilled water was added. The mixture was put in Clevenger system for 4 h to extract the essential oil. The extracted CEO was dehydrated by anhydrous sodium sulphate. The CEO was kept at -18 °C for further analysis.

GC-MS analysis: Qualification of volatile oil was analyzed by a gas chromatography-mass spectrometer (manufactured by Thermo Quest Finningan, TRACE MS model). The DB-5 column with length of 30 meters, inner diameter of 0.25 mm, and a thickness of 0.25 µm was utilized. The carrier gas Helium with a flow rate of 1.1 ml / min was used. The injection temperature and detection of 250 °C and a temperature program of 40 to 460 °C and an increasing trend of 5 °C per minute was applied. The injection volume of 0.2 microliters and electronic ionization detector with ionization energy 70 Electron volts were performed.

Preparation of methanol extract of CEO: The methanol extract of CEO was prepared by adding 5 ml of methanol to 0.5 ml CEO sample. The solution was shaken for 2 h and centrifuged at 2500 ×g for 10 min. The supernatant solution was used for antioxidant activity and total phenolic analysis.

Determination of total phenolic content (TPC):

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The TPC of CEO was measured using the Folin-Ciocalteu method explained by El-Maati *et al.* (El-Maati *et al.*, 2016) with some modifications. A total of 500 µl diluted sample was added to 2.5 ml of 10-time diluted Folin-Ciocalteu reagent. After 8 min, 2 ml of 7.5% Na_2CO_3 was added and kept in room temperature for 1 h. The absorbance of samples was recorded at 765 nm in contrast to distilled water as a blank. The TPC was determined as gallic acid equivalent (mg GAE/g) based on calibration curve of y=0.007x-0.065 and R^2=0.99 (y is absorbance, x is the concentration of TPC and R^2 is the correlation coefficient).

DPPH free radical scavenging activity: The radical scavenging activity of CEO was measured by 1,1-diphenyl-2-picryl-hydrazil (DPPH) according to Gülçin *et al.* with slight modification (Gülçin *et al.*, 2004). Five hundred microliter of CEO diluted extract was added to 2.5 ml of 0.1mM DPPH dissolved in methanol (A_{sample}). For control sample, 500 µl methanol and 2.5 ml of 0.1mM DPPH were used ($A_{control}$). The absorbance of samples after 30 min at room temperature was measured at 517 nm against methanol as a blank.

Antioxidant activity (inhibition %) = $[(A_{control}-A_{samples})/A_{control}] \times 100$

The IC_{50} which shows the amount of CEO needed to reduce 50% of DPPH radicals was measured.

Ferric reducing antioxidant power (FRAP) assay: The FRAP method was used according to the study of Li Y *et al.*. The FRAP reagent was prepared by mixing 2, 4, 6-triyridyl-s-triazine (TPTZ, 10 mM in 40mM HCl) solution, $FeCl_3$.6H2O (20 mM), and acetate buffer (300 mM, pH=3.6) in a ratio of 1:1:10, respectively. A total of 150 µl of diluted CEO was mixed with 3 ml FRAP reagent and the absorbance was measured after 5 min at 593 nm against methanol as a blank (Li *et al.*, 2006). The standard curve was prepared by different concentrations of $FeSO_4.7H_2O$ (y=0.704x-0.066, R2=0.997).

Addition of CEO to the sesame oil: CEO in concentrations of 0.02, 0.04, 0.06, and 0.08% was added to sesame oil. The samples with TBHQ (0.01%) and virgin sesame oil samples were used for comparison. The samples were stored in a dark oven at 60 °C for 6 weeks. Peroxide value (PV), p-Anisidine value (p-AV), total oxidation value (TOTOX) and Thiobarbituric acid reactive substances (TBARs)) were performed in 7 days intervals for 5 weeks.

PV: The PV was measured according to the method by Khalili (Khalili *et al.*, 2020). The oil samples were (5 g) dissolved in acetic acid-chloroform solution in ratio of 3:2 (30 ml). After adding 500 µl of saturated solution of potassium iodide, the samples were kept in dark for 2 min. Then, 30 ml distilled water was added and the mixture was titrated by sodium thiosulphate in the presence of starch indicator. The PV was evaluated according the following equation:

$PV= (V-V_b) \times N \times 1000/W$

In this equation, V and V_b are the volume of used sodium thiosulfate by sample and blank (ml), N is concentration of sodium thiosulfate, and W is the oil weight (g) (Khalili *et al.*, 2020).

p-AV: The p-AV was calculated based on the AOCS method Cd 18-90 (Chong *et al.*, 2015). Approximately, 0.5 g of oil samples was dissolved in 25 ml isooctane. Then, 5 ml of the prepared solution was mixed with 1 ml p-anisidine (25 g p-anisidine in 1000 ml acetic acid, As). The absorbance of samples was assessed after 10 min at 350 nm against isooctane as a blank (Ab). The p-AV was calculated according to the following equation:

p-AV= 25 × [(1.2As - Ab)]/weight (g)

TOTOX value: The TOTOX value of samples were measured according to the method described by kheirati *et al.* (Kheirati Rounizi *et al.*, 2021) using the following equation:

TOTOX= p-AV + (PV × 2)

TBARs **:** The TBARs was evaluated according to Chong *et al.* (Chong *et al.*, 2015) with slight modification. Five hundred microliter of oil sample was mixed with 2 ml TBA-TCA solution (15% TCA and 20 mM TBA) and 500 µl distilled water. The mixture was shaken and incubated at 95 °C for

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30 min. The samples were cooled and centrifuged for 15 min at 4500 rpm. The absorbance of samples was measured at 532 nm against distilled water as a blank. The standard curve was prepared by 1,1,3,3-tetraethoxypropane (TEP) and expressed as mg of MDA equivalents per kg.

Data analysis: All data were tested using analysis of variance (ANOVA) followed by repeated measure tests using SPSS 9.0.

Results

The GC-MS analysis: The chemical composition of the CEO was determined by GC-MS (**Table 1**). A total of 5 constituents were identified in CEO as eugenol (96.25%), eugenol acetate (1.88%), trans-Caryophyllene (1.66%), α-humulene (0.16%), and caryophyllene oxide (0.06%).

TPC and antioxidant activity: The results of TPC and antioxidant activity (FRAP and DPPH) are given in **Table 2**. The TPC of the CEO was measured 345.95±7.80 mg GAE/g. The antioxidant activity of the CEO by DPPH (IC_{50}) and FRAP methods was evaluated 0.83±0.11 mg/ml and 112.37±8.81 mM, respectively.

CEO in sesame oil at accelerated condition: The sesame oil oxidation contained different concentrations of CEO (0.02, 0.04, 0.06 and 0.08%) and TBHQ (0.02%) and sesame oil without antioxidants was investigated at accelerated condition for 5 weeks. In general, the peroxide (**Figure 1**), p-AV (**Figure 2**), TOTOX (**Figure 3**), and TBARS value (**Figure 4**) of all sesame oil samples increased during 5 weeks of storage at accelerated condition. Sesame oil samples contained 0.04, 0.06, and 0.08% CEO and TBHQ had PV lower than 20 mEq/kg during storage time.

The p-AV of sesame oil in the first day ranged from 3.196 to 3.256. Then, p-AV was rapidly increased from the 7th day of storage. At the end of the 35th day of storage, the highest p-AV was in a sample without antioxidants (17.89) and 0.02% CEO (17.47) ($P < 0.05$). The p-AV of sesame oil samples containing TBHQ and 0.04%, 0.06%, and 0.08% CEO was significantly ($P < 0.05$) lower than others.

The result of TOTOX value was in line with PV and p-AV. TOTOX value of samples containing TBHQ and 0.06% and 0.08% CEO was significantly ($P < 0.05$) higher than other samples.

The TBARs value increased during storage time, but the increase was accelerated rapidly after the 3rd week. Sesame oil samples contained 0.08% CEO and TBHQ had significantly ($P < 0.05$) lower TBARs value in comparison with other samples.

Table 1. Chemical composition of CEO.

Peak	Compound	Peak area (%)	Retention time (min)
1	Eugenol	96.25	14.63
2	Trans-Caryophyllene	1.66	16.30
3	α-humulene	0.16	17.21
4	Eugenol acetate	1.88	18.79
5	Caryophyllene oxide	0.06	20.37

CEO: composition and antioxidant activity of essential oil.

Table 2. Phenolic content and antioxidant activity of CEO.

Phenolic content (mg GAE/g)	FRAP (mM Fe_2SO_4)	DPPH (IC_{50}, mg/ml)
345.95 ± 7.85	112.37 ± 8.81	0.83 ± 0.11

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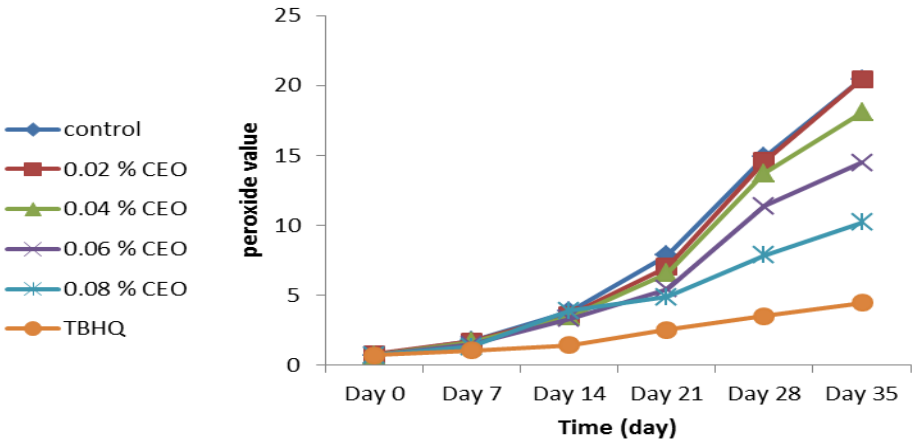

Figure 1. Effect of CEO addition on PV of the sesame oil under accelerated storage at 60 °C for 35 days.

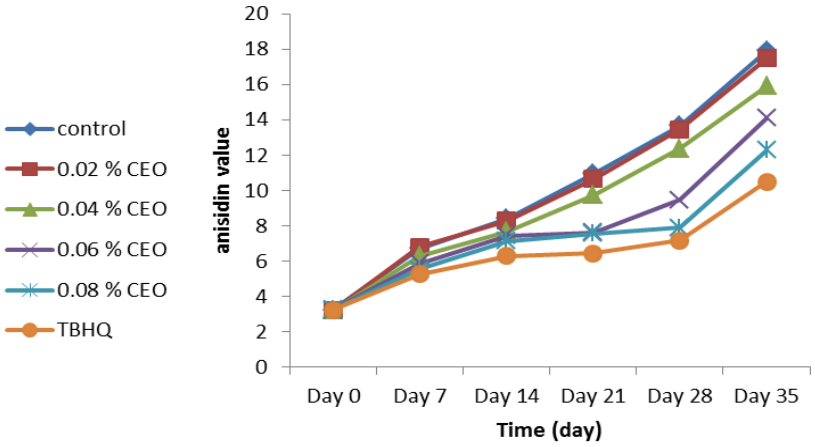

Figure 2. Effect of CEO addition on p-AV of the sesame oil under accelerated storage at 60 °C for 35 days.

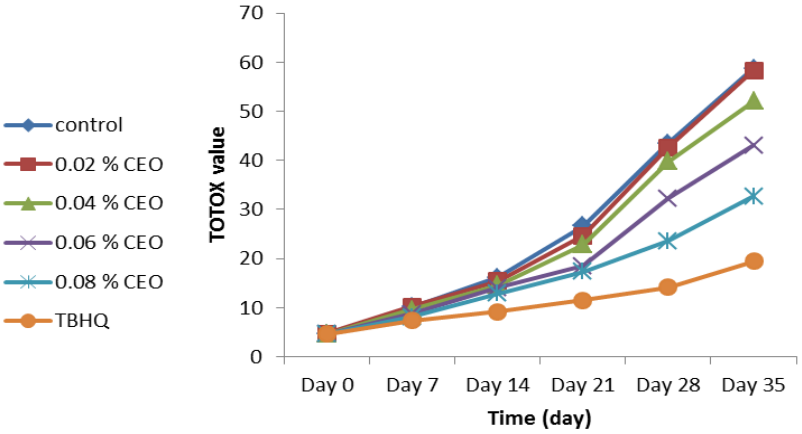

Figure 3. Effect of CEO addition on TOTOX value of the sesame oil under accelerated storage at 60 °C for 35 days.

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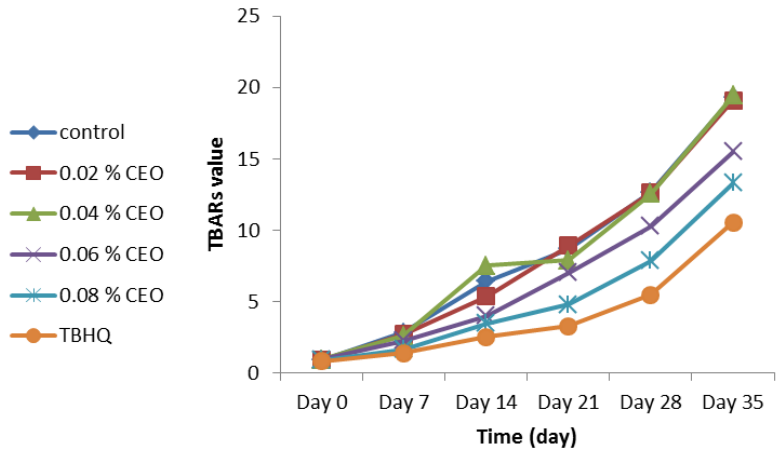

Figure 4. Effect of CEO addition on TBARs value of the sesame oil under accelerated storage at 60 °C for 35 days.

Discussion

The GC-MS analysis: In the current study, about five constituents were identified in CEO as eugenol (96.25%), eugenol acetate (1.88%), trans-Caryophyllene (1.66%), α-humulene (0.16%), and caryophyllene oxide (0.06%). Özcan (Özcan, 2003) and Golmakani (Golmakani *et al.*, 2021) reported eugenol and eugenyl acetate as the major constituents of clove buds, which is consistence with current results. Radünz *et al.* reported eugenol (56.06%), caryophyllene (39.63%), and α-caryophyllene (4.31%) as the terpenic compounds of CEO (Radünz *et al.*, 2019). Zangin *et al.* reported the composition of CEO as follows: eugenol (75.2%), benzyl salicylate (14.74%), propylene glycol (6.02%) and caryophyllene (21.3%) (Zengin and Baysal, 2015). The volatile compounds of clove bud oil in the study by El-Mesallamy *et al.* included eugenol (78.5%), eugenol acetate (18.9%), β-caryophyllene (4.2%), λ-cadinene (0.3%), caryophyllene oxide (0.3%), αhumulene (0.1%), methyl salicylate (0.1%), chavicol (less than 0.05), 2-Methoxy-3- (2-propenyl) phenol (less than 0.05), and α copaene (less than 0.05) (El-Mesallamy *et al.*, 2012). Lee *et al.* identified 21 compounds in clove bud extract which the major compounds were eugenol (24.37 mg/g) and eugenyl acetate (2.35 mg/g) (Lee and Shibamoto, 2001). Tomaino *et al.* reported phenylpropanoids eugenol (82.6%) as the most abundant constitute in clove oil (Tomaino *et al.*, 2005). It could be seen that in most studies, eugenol has been introduced as the main constituent of the clove. The variation in the chemical composition of clove is attributed to ecological, geographical condition, climate, and soil conditions (Zengin and Baysal, 2015) as well as method and extraction time of essential oils (Radünz *et al.*, 2019). Due to low molecular weight and volatile characteristics of chemical compounds, evaporation of some components during harvesting and production could be occurred (Al Nomaani *et al.*, 2013).

TPC and antioxidant activity: TPC of CEO was 345.95 mg GAE/g. The phenolic contents of clove in Bamdad *et al.* (Bamdad *et al.*, 2006) and Radünz *et al.* (Radünz *et al.*, 2019) were reported 243.9 and 9.07 mg GAE/g, respectively, which were lower than results of the current study. According to the results of Ivavovic *et al.*, phenolic content of clove extract ranged from 161.98 to 530.56 mg GAE/g (Ivanovic *et al.*, 2013). According to Zangin *et al.*, TPC in CEO was estimated to be 635.32 mg GAE/g (Zengin and Baysal, 2015), which is higher than the current results. The variation in the phenolic content of essential oils depends on the biological and geographical distribution, as well as climate change and soil conditions. It is suggested that the method of oil extraction and characteristics of samples are

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important in phenolic content (Radünz *et al.*, 2019).

In order to determine antioxidant activity, several tests according to different mechanisms should be used (El-Maati *et al.*, 2016). The antioxidant activity of essential oils is attributed to their ability to scavenge free radicals, donate hydrogen atoms, or chelate metal ions (Chen *et al.*, 2014). In the present study, two methods of FRAP and DPPH were applied to study the CEO antioxidant activity. The CEO antioxidants can react with $DPPH^{\bullet}$ and reduce the amount of $DPPH^{\bullet}$ radicals. The DPPH radical scavenging ability of CEO was reported as IC_{50}, which indicated the concentration of CEO that can inhibit 50% of the total DPPH radicals. The IC_{50} of CEO was evaluated 0.83 mg/ml (**Table 1**). Radünz *et al.* studied antioxidant activity of un-encapsulated and encapsulated CEO. They reported 94.86% of DPPH scavenging at a concentration of 484.7 µg/ml, which is lower than the current study (Radünz *et al.*, 2019). High antioxidant activity of CEO was due to synergist effect of phenolic compound (Radünz *et al.*, 2019). Jirovetz *et al.* reported the concentration of 0.08 µg/ml for inhibition of 50% DPPH radical (Jirovetz *et al.*, 2006). The substitution of hydroxyl groups of aromatic rings and the ability to donate the hydrogen are contributed to radical scavenging ability (Chen *et al.*, 2014). The ability of CEO to reduce Fe^{3+} is considered as electron donation potential and high antioxidant activity. The FRAP was reported 112.37 mM Fe_2SO_4. It was shown that by increasing CEO concentration, the antioxidant activity increased. According to Zangin *et al.*, the antioxidant activity of CEO was calculated 4357.45 mmol trolex/ml and 0.14 µl/ml according to FRAP and DPPH, respectively (Zengin and Baysal, 2015). The antioxidant activity of herbal essential oils varied due to temperature, hydrophobic or amphipathic properties, and the concentration and presence of synergists (Özcan, 2003). In the current study, a positive correlation was observed between FRAP and phenolic content of CEO. Therefore, the antioxidant activity of CEO are related to concentration of phenolic in the CEO which is consistence with results of the study by El-Maati *et al.* (El-Maati *et al.*, 2016). Generally, the chemical structure and reducing functions of phenolic compounds could promote their antioxidant activity (Golmakani *et al.*, 2021) which is confirmed in the present study.

CEO in sesame oil at accelerated condition: The PV of all samples increased, indicating the formation of primary oxidation products or hydro-peroxides during storage. The highest PV value after 3 weeks was measured in samples with 0, 0.02 and 0.04 % CEO. Different studies reported PV of 10 to 20 mEq/kg as acceptable limit for consumption (Alrefaie and Bostan, 2017). During 35 days storage, the samples contained 0.04, 0.06, 0.08% CEO and TBHQ had PV lower than 20 mEq/kg. Oil samples with 0.08% CEO and TBHQ showed better function in retarding the oxidation. The results are in accordance with the study of Alrefaie and Bostan which used clove and lemongrass essential oil in cake and indicated that samples with high concentrations of essential oils had lower PV (Alrefaie and Bostan, 2017). Abozid and Asker showed the efficiency of thyme and rosemary essential oil in inhibiting peroxide formation of sesame oil under accelerated condition compared to the control group (Abozid and Asker, 2013). Ibrahium et al. reported that except for 400 ppm concentration, other concentrations (600 and 800 ppm) decreased the PV of cake samples (Ibrahium *et al.*, 2013). This can be attributed to the presence of eugenol in CEO which could control the lipid peroxidation by inhibiting the iron formation and OH radicals (Golmakani *et al.*, 2021). It was observed that phenolic hydroxyl groups of antioxidants could be resulted in production of stable free radicals which can inhibit the initiation or propagation of lipid oxidation (Nassar *et al.*, 2007). During storage time, peroxides could be decomposed to secondary oxidative products which could be determined by p-AV (Quiroga *et al.*, 2011). In the current study, p-AV of sesame oil samples supplemented with TBHQ, 0.04%, 0.06%, and 0.08% CEO was significantly ($P < 0.05$) lower than others. The

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TOTOX indicated the total oxidative degradation of oils, since it is obtained from two compounds of aldehyde and peroxide. The result of TOTOX value was in accordance to PV and p-AV. TOTOX value of TBHQ, 0.06 and 0.08% CEO samples were significantly higher than other samples. The reaction of hydro-peroxides and oxygen form malondialdehyde (MDA) which could be determined by TBARs assay (Chen *et al.*, 2014). It was shown that TBARs rapidly increased after 21 days of storage. The results of TBARs were similar to the results of p-AV and PV.

The antioxidant effects of essential oils varied due to concentration, hydrophobic and amphipathic features, temperature, as well as the chemical nature of used food (Özcan, 2003). During 35 days of storage of sesame oil at accelerated condition, all variables increased in all samples. These results are in line with Ibrahium *et al*. which reported the increase in TBA and PV of cake samples contained different concentrations of CEO during 28 days of storage (Ibrahium *et al.*, 2013). In general, according to the results of oxidation test, the synthetic antioxidant had better function in preventing oxidation, which is consistent with the study of Zhang *et al*. (Zhang *et al.*, 2010) and Chen *et al*. (Chen *et al.*, 2014). High preventive activity of TBHQ could be related to the presence of two para-hydroxyl groups which can easily make phenols donate hydrogen atom to free radicals in oxidation reaction (Zhang *et al.*, 2010). However, in the present study, sesame oils containing higher CEO concentration played a role in preventing peroxidation. It could be suggested that higher concentration of CEO is better to be examined for stabilizing sesame oil.

Conclusion

During the past decade, application of natural plants with high antioxidant activity in foodstuff has been increased. It was shown that CEO was a good source of natural phenolic compounds such as eugenol. The current study showed high antioxidant activity of CEO. Although TBHQ showed higher retardation in sesame oil oxidation, CEO especially in 0.08% concentration showed good antioxidant potency. Therefore, application of CEO in sesame oil could retard lipid peroxidation. Therefore, CEO could be considered as an antioxidant preservative and alternative of synthetics ones in vegetable oils.

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Author's contributions

Khalili Sadrabad E and Akrami Mohajeri F conceived of the presented idea. Sarrami S, Khalili Sadrabad E, Akrami Mohajeri F and Sadeghizadeh J developed the theory and performed the experiments. Sarrami S and Jambarsang S developed the theoretical formalism, performed the analytic calculations and performed the numerical simulations. All authors provided critical feedback and helped shape the research, analysis and manuscript. All authors read and approved the final manuscript.

Conflict of interest

There is no conflict of interest.

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