



Original research

Antioxidant activity of the rosemary (*Rosmarinus officinalis* L.) extract on the stability of sesame (*Sesamum indicum* L.) oil during accelerate storage time

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ABSTRACT

Rosemary (*Rosmarinus Officinalis* L.) extract is widely recognized as a natural antioxidant due to the potent activity of its constituents. This study evaluated the efficacy of different concentrations of rosemary extract against lipid oxidation in refined sesame oil during a 30 day of storage at 60 °C. The leaves of *R. Officinalis* and refined sesame oil were obtained from Yazd province. An ethanolic extract was prepared from the leaves. The antioxidant activity of the rosemary extract (RE) was assessed using DPPH radical scavenging capacity, Rancimat analysis, Peroxide value (PV), and Thiobarbituric acid (TBA) assay. Butylated hydroxyanisole (BHA), a synthetic antioxidant, was used as a positive control. The radical-scavenging capacity (DPPH) of RE was significantly higher than that of the control and was comparable to oils with synthetic antioxidants. The induction period (IP) of sesame oil inoculated with rosemary extract was significantly higher than that of the control group and oils with synthetic antioxidants. All tested concentrations of the ethanolic Rosemary extract significantly reduced the peroxide and TBA values, particularly during long-term storage. Furthermore, the optimum concentrations of rosemary extract in refined sesame oil under accelerated storage conditions were determined to be 50 and 100 µg/ml. Although the synthetic antioxidant demonstrated significantly higher antioxidant activity than the rosemary extract. ($P \leq 0.05$, the results suggest that ethanolic rosemary extract can serve as an effective natural alternative to synthetic antioxidants.

Keywords: Antioxidant activity; Lipid oxidation; Sesame oil; Rosemary extract; *Rosmarinus officinalis*.

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1. Introduction

Lipid oxidation of vegetable oils with high unsaturated fatty acids content is augmented through the elimination of hydrogen atoms and the subsequent addition of oxygen at alpha positions to a fatty acid double bond; this leads to the formation of primary peroxides, which are then susceptible to further decomposition into secondary lipid oxidation byproducts such as aldehydes, ketones, and malondialdehyde (Gad & Sayd, 2015; Kamkar et al., 2014; Musakhanian et al. 2022). When these molecules react with oxygenated components in foods, flavor, taste, nutritional value, and overall quality are adversely affected. This process may also pose

health hazards and act as harmful agents compromising human and animal safety (Kamkar et al., 2014; Mohdaly et al. 2010; Suja et al. 2004).

Antioxidants prevent free radical formation, scavenge radicals, and promote their decomposition. Generally, they are classified into two main groups: synthetic and natural antioxidants. Among synthetic types, the most frequently used include butylated hydroxyanisole (BHA), butylated hydroxyl toluene (BHT), propyl gallate (PG), and tert-butyl hydroquinone (TBHQ) (Pourghanbari et al. 2021; Rašković et al., 2014). Although synthetic antioxidants have demonstrated beneficial anticarcinogenic and antimutagenic properties (Musakhanian et al., 2022; Taghvaei & Jafari, 2015;

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Wang et al. 2023), their safety has increasingly been questioned due to potential adverse health effects (Ammar, 2016; Chen et al., 2014). Several studies indicate that synthetic antioxidants may exert toxic effects at high concentrations, leading to ongoing debate regarding their safe application in foods (Ammar, 2016; Taghvaei & Jafari, 2015). Recent interest has therefore shifted toward natural antioxidants derived from herbs and plant sources. Many of these contain phenolic compounds with notable antioxidant activity (Ammar, 2016; Erdmann et al. 2017; Mohammadi et al. 2016).

Rosemary (*Rosmarinus officinalis* L.) extract is a well-known natural antioxidant rich in phenolic diterpenes such as carnosic acid, carnosol, and rosmanol. Yang et al. (2016) reported that rosemary extract demonstrated stronger antioxidant activity than BHT and BHA. Moreover, rosemary extracts have shown potent inhibitory effects on lipid oxidation, offering protection to oils by increasing antioxidant capacity and delaying the degradation of tocopherols and polyunsaturated fatty acids (Yang et al. 2016; Yong et al. 2016). Palm oil enriched with rosemary ethanol extract exhibited superior oxidative stability compared to oil containing synthetic antioxidants under frying conditions (Guo Qing et al., 2016).

Sesame (*Sesamum indicum* L.) seeds consist of 45–50% lipid, along with moisture, carbohydrates, fiber, protein, and ash (Mohdaly et al., 2010). Sesame oil is widely valued both as an edible oil and a functional ingredient in foods (Mohdaly et al. 2011). Its notable stability is attributed to natural antioxidants such as sesamin, sesamol, sesaminol, sesamol, and α -tocopherol (Taghvaei & Jafari, 2015; Wei et al., 2022). Despite these inherent antioxidants, sesame oil contains approximately 85% unsaturated fatty acids, primarily oleic acid (39.09%) and linoleic acid (40.39%), making it susceptible to oxidative degradation under thermal stress (Heidari Soureshjani et al., 2017; Lourenço et al. 2019).

A considerable number of investigations have examined natural antioxidants particularly rosemary extract for their protective efficiency in foods and vegetable oils (Mezza et al., 2018; Taghvaei & Jafari, 2015). However, most previous studies have primarily evaluated rosemary extracts in oils such as sunflower, palm, soybean, or blends, and data on its performance in refined sesame oil (RSO), especially under high-temperature conditions and varying exposure durations, remain limited. Sesame oil has a unique endogenous antioxidant profile and unsaturated fatty acid composition; therefore, its interaction with external antioxidants such as rosemary extract may differ significantly from other edible oils. The limited literature addressing rosemary extract's capacity to enhance thermal oxidative stability in RSO highlights a gap that warrants further investigation. Therefore, the present research was conducted to evaluate the antioxidant activity of *R. officinalis* ethanol extract as a natural antioxidant source on the stability and oxidation properties of refined sesame oil (RSO) under high-temperature conditions during different exposure times. Furthermore, the study aimed to determine how rosemary extract interacts with the natural antioxidants of RSO and whether its incorporation can significantly improve the oil's thermal oxidative resistance. A comprehensive set of oxidation indices was measured to assess the protective efficacy of the extract under prolonged heating conditions.

2. Materials and Methods

2.1. Materials

Sesame oil (*Sesamum indicum* L., with Herbarium voucher

number 903 from Faculty of Natural Resources, Yazd University) was obtained from Barsam Ardakan Sesame Products Company and Rosemary (*R. officinalis* with Herbarium voucher number 149 from Faculty of Natural Resources, Yazd University), spectrophotometer (UV-Visible S-2,150 UNICO Model, USA), rancimat 892 (Metrohm, Herisau, Switzerland) instrument, centrifuge (HB110, Behsan Company, Iran), Soxhlet extractor Pyrex® Soxhlet extraction apparatus. BHA, acetic acid, chloroform, potassium iodide and thiocylglycerate were purchased from Sigma Chemical Co. (St. Louis, MO, USA). 1,1-Diphenyl-2-picrylhydrazyl (DPPH) was obtained from Fluka (Buchs, Switzerland). Folin-Ciocalteu reagent, Ethanol, Methanol, tri-chloroacetate (TCA), thiobarbituric acid and sodium carbonate were purchased from Merck, Germany. All the chemical reagents and solvents were of highest analytical grade.

2.2. Extraction

The whole plant of *R. officinalis* was freshly collected from a cultivation farm in the Yazd State of Iran. First, the plant was washed, dried at 40 °C, and ground into powder. One hundred g of sample was initially defatted with hexane (500 ml three times), at room temperature. The defatted residue was water-washed using distilled water (500 ml three times) and dried at 70°C 10 g of the above residue was extracted with 150 ml methanol for 16 h in a Soxhlet extractor. The Extract was filtered. The solvent was evaporated under vacuum, and the resulting residue was dried and weighed, and the residue (0.5 g) was redissolved in 100 ml of methanol to produce an antioxidant solution of known concentration, and stored in refrigerator until further experiments (Hussain et al., 2018).

2.3. Total phenolic content measurement

The total phenolic content of oils was measured using the Folin-Ciocalteu method according to the work of Wafaa et al. (2016). In order to measure the total phenolic content of the extract, a 1: 200 dilution of extract was prepared in distilled water. After that, 100 mg aliquot of oil sample was mixed with the concentrated Folin-Ciocalteu reagent (2 mL) and then 2 mL of sodium carbonate (Na_2CO_3) was added into the mixture, followed by shaking for 30s. Then, the mixture was incubated at room temperature in the dark for 20 min. The absorbance was measured at 750 nm using a spectrophotometer and the results were expressed as mg of Gallic acid equivalents (mg GAE) per 1 g of powdered extract using standard curve prepared from Gallic acid (0.1 mg/ml) solution.

2.4. Rancimat analysis

The Rancimat analysis was evaluated using a Rancimat 892 (Metrohm, Herisau, Switzerland) instrument. Rosemary extract (2.5 g) was accurately weighed into reaction vessel. The target temperature was set at 120 °C and airflow rate was 20 L/h. Synthetic antioxidant was added at a legal limit of 200 mg/kg as positive control. Oils without added antioxidants were considered as blank control. The air flow and heating caused the electrical conductivity of the solution to change by producing oxidative materials; it was evaluated and displayed in a curve. The inflection point of the curve was the highest resistance value of the oil sample. The result was expressed as an induction period (IP) (Yang et al., 2016).

2.5. Determination of DPPH radical scavenging capacity

The DPPH antioxidant assay was performed according to Zhou & Yu, (2004). The antioxidant properties of extracts based on DPPH activity were measured by spectrophotometry at 517 nm wavelengths at the end of experiment time (day 30). The working solution was prepared by dissolving 3.5 ml of DPPH stock solution (0.1 mM) with 100 μ l (1×10^{-2}) diluted sample and stored for 30 min at room temperature. The control sample was made in accordance with the above method but water was used instead of sample. The absorbance of the solution was measured using a spectrophotometer in a 517 nm wavelength.

2.6. Peroxide value measurement

The peroxide value was determined according to Yang et al. (2016). Oil samples (5 g) were accurately weighed and dissolved in 30 ml of acetic acid/chloroform (3:2 v/v) (18 ml of acetic acid and 12 ml of chloroform), followed by the addition of 0.5 ml of saturated potassium iodide, the solution was mixed thoroughly and placed in the dark for one minute. After that, 30 ml of distilled water was added, and the mixture was then titrated with the thiocylglycerate 0.01 Normal to get colorless. The absorbance was measured at 560 nm using the spectrophotometer.

2.7. Thiobarbituric acid-reactive substances

TBA was determined weekly. Sesame oil (1000 mg) was dissolved in 8 ml tri-chloroacetate (TCA) 5% (w/v) and centrifuged (3600) for 20 min. Then, 1 ml, 2-TBA (0.01 M) with 5 ml of aqueous phase solution was mixed and heated for 40 min in boiling water. After cooling, the absorption was determined at 532 nm. The value of TBA is expressed as mg of malondialdehyde (Kamkar et al., 2014).

2.8. Statistical analysis

Oxidation experiments were carried out in triplicate. The findings were averaged and statistically analyzed with one-way ANOVA using SPSS 22. Also, the Duncan's as a post hoc test was used to measure specific differences between pairs of means.

3. Results and Discussion

3.1. Total phenolic content (TPC)

The total phenolic content of rosemary ethanol extract (RE) was measured on day 1. The TPC of RE was 70.51 ± 1.24 mg GAE/g extract (mean \pm SD, n=3).

3.2. Antioxidant activity based on DPPH assay

The free radical scavenging ability of RE was determined using the DPPH assay, a standard method for evaluating antioxidant activity. The scavenging activity of RE and the reference antioxidant (BHA, 200 μ g/mL) are shown in Fig. 1. The percentage of scavenging activity in RE ($72.6 \pm 2.3\%$) was significantly higher than the control group ($12.4 \pm 1.1\%$, $p < 0.05$), but lower than that

of BHA ($89.2 \pm 1.7\%$, $p < 0.05$). Fig. 1 shows antioxidant activity of rosemary ethanol extract (RE) measured by DPPH radical scavenging assay compared with BHA and control (mean \pm SD, n=3).

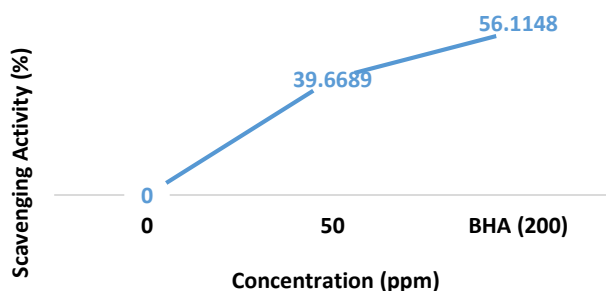


Fig. 1. The antioxidant properties of extracts measured based on DPPH activity.

3.3. Antioxidant activity of RE in sesame oil

The antioxidant effect of different concentrations of RE (50, 100, and 200 μ g/mL) on refined sesame oil was evaluated by measuring peroxide value (PV), thiobarbituric acid (TBA), and Rancimat induction period (IP) at 60 $^{\circ}$ C over 30 days of storage.

3.3.1. Peroxide value (PV)

On day 1, PV was equal across all groups (2.93 ± 0.04 meq/kg). As shown in Figs. 2 and 3, PV increased during storage but was significantly reduced in samples treated with higher concentrations of RE or BHA compared to the control ($p \leq 0.05$).

- Control group: PV reached 17.4 ± 0.3 , 19.46 ± 0.3 , and 21.59 ± 0.11 meq/kg at days 10, 20, and 30, respectively.
- BHA (200 μ g/mL): PV remained lowest at 13.0 ± 0.32 , 15.28 ± 0.27 , and 16.42 ± 0.02 meq/kg at days 10, 20, and 30, respectively.
- RE (100 μ g/mL): PV values were not significantly different from BHA ($p > 0.05$).
- RE (50 μ g/mL): PV values were significantly higher than BHA and RE 100 μ g/mL ($p \leq 0.05$).

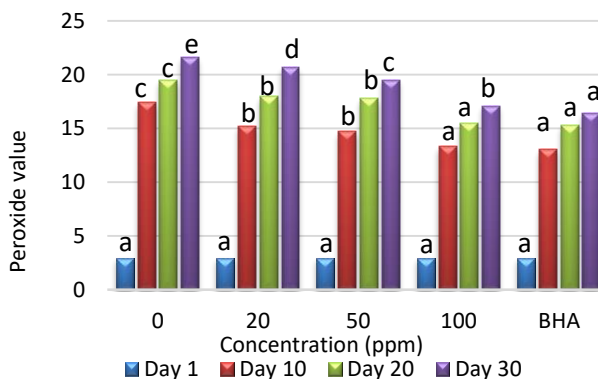


Fig. 2. Changes in peroxide value (PV) of sesame oil treated with rosemary ethanol extract (RE) at different concentrations compared with BHA and control (mean \pm SD, n=3).

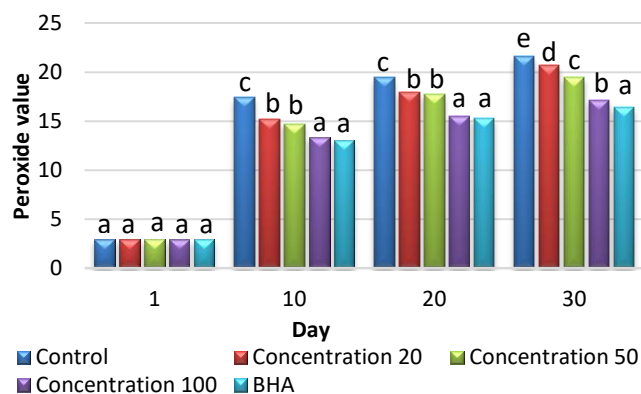


Fig. 3. Time-dependent increase in peroxide value (PV) during 30 days of storage at 60 °C (mean \pm SD, n=3).

3.3.2. Thiobarbituric acid (TBA) value

The thiobarbituric acid (TBA) values of sesame oil samples are shown in Figs. 4 and 5. On day 1, TBA values were equal across all treatments ($1.51 \pm 0.03 \mu\text{mol/g}$, mean \pm SD, n=3). During storage, TBA values increased in all groups but were significantly reduced in samples treated with RE or BHA compared to the control ($p \leq 0.05$).

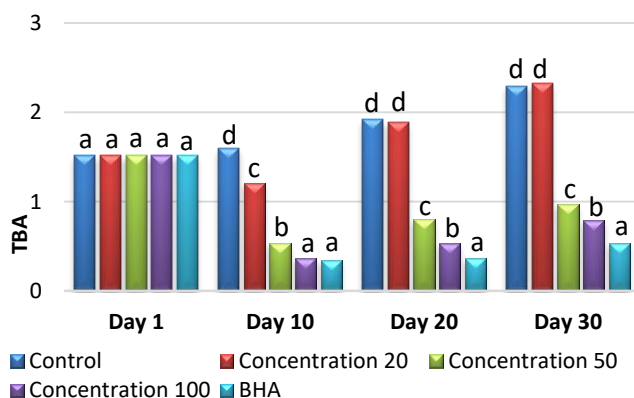


Fig. 4. Changes thiobarbituric acid (TBA) values of sesame oil treated with rosemary ethanol extract (RE) compared with BHA and control (mean \pm SD, n=3).

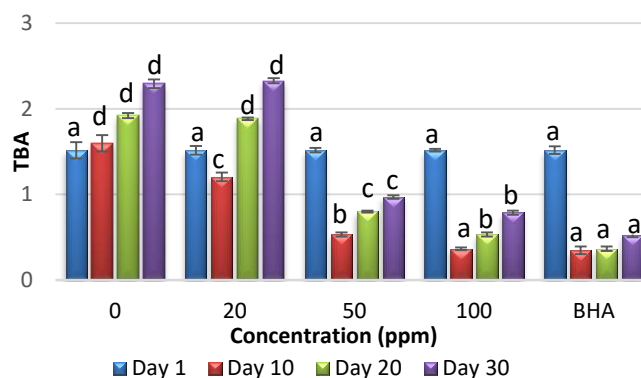


Fig. 5. Time-dependent increase in thiobarbituric acid (TBA) values during 30 days of storage at 60 °C (mean \pm SD, n=3).

- Control group: TBA values reached 1.59 ± 0.10 , 1.92 ± 0.03 , and $2.29 \pm 0.05 \mu\text{mol/g}$ at days 10, 20, and 30, respectively.
- RE treatments (50 and 100 $\mu\text{g/mL}$): Showed significantly lower TBA values compared to control, with reductions more pronounced at higher concentrations ($p \leq 0.05$).
- BHA (200 $\mu\text{g/mL}$): Produced the lowest TBA values throughout storage, comparable to RE at 100 $\mu\text{g/mL}$.

3.3.3. Rancimat analysis

The oxidative stability of refined sesame oil treated with RE and BHA was evaluated using the Rancimat method (Fig. 6). Significant differences were observed among treatments ($p \leq 0.05$).

- Control group (no antioxidant): Induction period (IP) was the lowest (8.46 ± 0.14 h).
- RE treatment (200 $\mu\text{g/mL}$): IP increased to 9.41 ± 0.30 h, indicating improved oxidative stability compared to control.
- BHA (200 $\mu\text{g/mL}$): Showed the highest IP (11.28 ± 0.25 h), confirming its superior antioxidant efficacy.

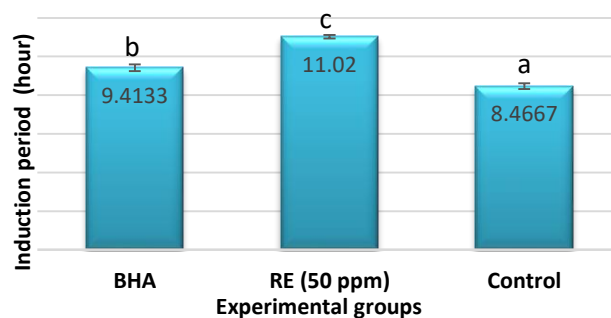


Fig. 6. Induction period (IP) of sesame oil treated with rosemary ethanol extract (RE) compared with BHA and control, measured by Rancimat analysis (mean \pm SD, n=3).

3.4. Discussion

In recent years, herbs have been evaluated as a source of various phytochemicals, many of which possess powerful antioxidant activity (Shashi et al., 2014; Alok et al., 2014). Therefore, the present study was aimed at assessing the antioxidant activity of ethanol rosemary extract on the refined sesame oil at 60 °C during the time. Phenolic compounds are biological components, greatly diffused in plants, which act as radical scavengers or heavy metal chelators and inhibit lipid oxidation. Rosemary extract includes a high concentration of phenolic compound which is responsible for the rosemary antioxidant's efficacy. Phenolic diterpenes such as carnosol, carnosic acid, rosmanol, epirosmanol and isorosmanol are the main phenolic ingredients of rosemary extract (Gad & Sayd, 2015). The total phenolic content of rosemary extract was measured on day 1 of the experiment and was $70.51 \text{ mg GAE/g extract}$ (Yang et al., 2016) expressed that phenolic compounds of the oils enriched by rosemary extract on day 1 were higher than the control group, also, the phenolic content of the treatments decreased with increasing storage time. Antioxidant efficacy of the plant extracts is dependent on the phenolic compounds concentration in the plants (Heimet al. 2002; Pourghanbari et al. 2021).

The main ingredients of ethanol rosemary extract were camphor, phytol, borneol, caryophyllene oxide (Wafaa et al., 2016). However,

it has been reported that the main components of the rosemary essential oil were camphor (23.17%), α -Pinene (18.56%), and 1,8-Cineole (11.89%) (Raiesi et al., 2016). Besides, a study was conducted to evaluate the rosemary essential oil properties which obtained 1,8-Cineole, α -Pinene, camphor, camphene and β -Pinene as major components of rosemary essential oil (Jiang et al., 2011).

Rancimat analysis was done to measure the induction period (IP) by detecting the formed volatile acids during oil oxidation (Mathäus, 1996). According to Fig. 6, Induction period was observed to be significantly larger in the sesame oils that were enriched by rosemary extract or synthetic antioxidant agent than the control group ($p < 0.05$). Yang et al. (2016) showed the IP values of the three types of vegetable oils inoculated with rosemary extract were significantly higher than the oil samples that received synthetic antioxidants (Yang et al., 2016). Gharby et al. (2017) showed that the induction time of sesame oil was 28.5 ± 1 h at 110 °C; also, the IP at the same temperature was found to be 31, 27, 17, and 7 h for argan, olive, nigella, and cactus oils, respectively (Suja et al. 2004).

The scavenging ability of the RE was measured by using the DPPH method, which is commonly used for evaluation of antioxidant activity. Scavenging capability of RE was significantly higher ($P < 0.05$) than that of control group but less than the sesame oil containing BHA as synthetic antioxidant. The antioxidant efficacy of plant extracts is mainly attributed to the concentration of the phenolic compounds of the plants (Heim et al., 2002; Zhou & Yu, 2004; Zhang et al. 2022) reported DPPH radical scavenging activity increased as the concentration of phenolic compounds increased. Furthermore, Carnosic acid with two O-phenolic hydroxyl groups located at the benzene ring were shown as the main antioxidant compound in rosemary extract (Erkan et al. 2008; Terpinc et al. 2009).

In this study, oxidation rate on refined sesame oil samples was evaluated by measuring Peroxide value and Thiobarbituric acid value in the absence and presence of natural and synthetic antioxidants at 60 °C for 30 days. According to the Iranian National Standard No. 4179, the acceptable PV is 20 meq/kg. In accordance with the results, the PV was increased linearly with storage time and reduction of RE concentration in all treatment groups, the blank oil without any antioxidant component showed maximum PV. Peroxide components are the most important primary products of oil oxidation, and they can be measured by using the peroxide value (PV) (Malheiro et al., 2013). Oxidative stability can be diminished by rising peroxide value (Naghshineh et al. 2010; Yang et al., 2016). The rosemary extract at 100 $\mu\text{g/ml}$ and BHA exhibited the same ability to postpone the PV values of the sesame oil. However, the antioxidant activity of the rosemary extract under the 100 $\mu\text{g/ml}$ was observed to be significantly lower than BHA. Erdmann et al. (2017) observed the PV in the sausages containing 50 mg/kg encapsulated rosemary extract was decreased by more than 90% at day 21 compared to control group. Surprisingly, they observed that the PV of the primary oxidation products increased in the RE inoculated groups from day 21-35, while PV in the model pork sausages without antioxidant had a diminutive pattern during the same time. Rosemary ethanol extract can effectively reduce the peroxide value of palm oil, also under frying situation, the oil with rosemary ethanol extract showed an enhanced stability compared to the oil with synthetic antioxidants (Guo Qing et al., 2016). Oxidation of unsaturated aldehydes led to malonaldehyde formation, which is the foundation for the thiobarbituric acid (TBA) method that is generally used for measuring lipid oxidation. Shahidi et al., (2010) expressed the TBA test has been used nearly as often as the peroxide values. the TBA value illustrates the aldehyde level in the lipid fraction of oxidized

foods. Therefore, TBA value is an indicative parameter for the secondary oxidation step.

Based on the finding, the ethanol extracts of rosemary are able to inhibit both primary and secondary oxidation of sesame oil during storage time, as shown in Fig. 2. While the PV increased over time, TBA levels of the sesame oil showed a different pattern, so that TBA in all treatment groups was observed to follow a decreasing pattern in comparison with the control group until day 10, after that, interestingly, it increased slowly so that the value of the group containing 20 $\mu\text{g/ml}$ RE was shown to be equal to the TBA value of control at days 20 and 30. Nevertheless, TBA value of all groups containing RE was lower than blank group at all test days. During the secondary stage of the autoxidation procedure aldehydes such as alkanals, 2- alkenals, dienals are produced which react with TBA (Guillen-Sans & Guzman-Chozas, 1998). However, seems to be responsible for decreasing the TBA value during the storage time. In a research study on the pork sausage by (Erdmann et al., 2017), rosemary extract was able to reduce production of the TBARS at day 21 of experiment in comparison with control group, but the secondary oxidation products like as TBARS, propanal and hexanal were increased in the treatment group from day 21-35, whereas the TBA measurement was reduced in control group. Kamkar et al., (2014) showed the ethanol and methanol extracts of *S. hortensis* can be restrained primary and secondary oxidation of soybean oil during storage. From this point of view, the PVs and TBA values of the soybean oil which is enriched by the extract were lower as compared with the control group. Moreover, based on the findings, the sesame oil containing synthetic antioxidant (BHA) was shown to be more efficient than RE as the PV and TBA levels of sesame oil containing BHA (200 $\mu\text{g/ml}$) were lower than the groups that were treated by rosemary extract. The synthetic antioxidants like BHT and BHA were observed to release more antioxidant activity than plant extract (Anwar et al. 2007).

4. Conclusion

In this study antioxidant efficacy of the ethanol rosemary extract on the refined sesame oil during accelerated storage time in comparison with the synthetic antioxidant (BHA) was evaluated. The findings of the present study showed that different concentrations of the rosemary extract are able to inhibit oxidative rancidity of refined sesame oil when compared with synthetic antioxidants. Therefore, now the antioxidant activity of the rosemary extract was detected and it could be advised as an effective and natural antioxidant component. However, further research is needed to elucidate the antioxidant mechanisms of rosemary extract and its derivatives.

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Conflict of interest

The authors declare that there is no conflict of interest.

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