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Antioxidant Efficacy of Sesame (*Sesamum indicum* L.) Cake Extract on Stability of Refined Sesame Oil during Storage Time

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HIGHLIGHTS:

- Radical-scavenging capability of sesame cake was high.
- Sesame cake is a rich source of phenolic contents.
- Sesame cake's extract reduced thiobarbituric acid and peroxide value indexes in refined sesame oil.

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Acronyms and abbreviations
BHA=Butylated Hydroxyanisole
BHT=Butylated Hydroxytoluene
DPPH=2,2-Diphenyl-1-Picrylhydrazyl
GAE=Gallic Acid Equivalents
IP=Induction Period
PV=Peroxide Value
SCEE=Sesame Cake Ethanol Extract
TBA=Thiobarbituric Acid
TBHQ=Tertbutyl Hydroquinone
TPC=Total Phenolic Content

ABSTRACT

Background: Sesame cake extract is the by-product left behind after sesame oil extraction including almost 30% of protein and phytochemicals, which possess free radical scavenging activity. In this study, antioxidant activity of sesame cake extract on the quality of refined sesame oil has been evaluated under 60 °C during storage time.

Methods: After extraction of ethanolic extract, sesame oil was treated with concentrations of 0, 20, 50, and 100 ppm of sesame cake extract and stored at 60 °C for 30 days. The sesame cake and refined sesame oil were taken from Yazd province and extracted with ethanol. Total Phenolic Content (TPC) (at the beginning of the study) and 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) radical scavenging capacity (30 days after inoculation) were evaluated. The antioxidant activity of various dilution of the Sesame Cake Ethanol Extract (SCEE) was assessed by measuring, rancimat analysis, Peroxide Value (PV), and Thiobarbituric Acid (TBA) assay, and also evaluation of the TPC. Butylated Hydroxyanisole (BHA) as a synthetic antioxidant was used. Experiments were carried out in triplicates and data were processed with ANOVA test by SPSS Software.

Results: Radical-scavenging capabilities of SCEE were significantly larger than the group without SCEE but it was detected to be lower than the BHA group. The induction period (IP) of sesame oil raised as the concentration of SCEE increased. All concentrations of SCEE were able to decline the PV and TBA value. Also, the group containing BHA and 100 µg/ml of SCEE significantly showed the same antioxidant activity ($p \leq 0.05$).

Conclusion: The SCEE as a natural substance can prevent lipid oxidation of the refined sesame oil like synthetic antioxidants.

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Introduction

Sesame oil (*Sesamum indicum* L., *Pedaliaceae*) is one of the most significant oils used for many years and the ingredients such as lipid, carbohydrate, fiber, and protein are the main compositions of the sesame seed (Mohdaly et al., 2011; Wei et al., 2022). The efficacies of the sesame seed and derivatives on health problems were evaluated in different studies (Dossou et al., 2023; Hussain et al., 2018). In addition to the antioxidant activity, hypocholesterolemic effect, anticancer, and anti-aging properties of the sesame have been detected (Majdalawieh and Mansour, 2019; Tachibana et al., 2020). Sesame oil has some special and strong antioxidant substances such as sesamin, sesamolin, sesaminol, sesamol, and α-tocopherol, and polyphenols. These substances have the properties to inhibit free radical formation and activity of the phenolic compounds is linked to their ability to chelate metals, inhibit lipoxygenase, and scavenge free radicals (Taghvaei and Jafari, 2015; Wei et al., 2022). The main problem of oils which may lead to health hazard and act as a detrimental factors for safety and health of organisms such as humans and animals, is lipid oxidation of oils with high contents of unsaturated fatty acids, generation of peroxides, and decomposition to secondary lipid oxidation by-products such as aldehydes, ketones, and malondialdehydeis (Kamkar et al., 2014; Mohdaly et al., 2011; Musakhanian et al., 2022; Wang et al., 2023). Sesame cake is the by-product left behind after sesame oil extraction including almost 30% of protein and phytochemicals and possesses free radical scavenging activities. Generally, the sesame oil industry waste is utilized as animal feed or fertilizer in its production areas (Suja et al., 2004). This by-product is also rich in minerals. On the other hand, its health benefits can be attributed to its phytochemicals, possessing free radical scavenging activities. The major active ingredient in Sesame Cake Ethanol Extract (SCEE) is sesaminol glucoside. Additionally, lignans including sesamin, sesamolin, and sesaminol from the decomposition of sesame fiber are present (Almasi and Fathi, 2021).

Antioxidants are the components that are able to inhibit free radical formation, scavenge them, and promote their destruction. Two basic groups of them have been defined till now. Butylated Hydroxyl Anisole (BHA), Butylatedhydroxyl Toluene (BHT), propyl gallate, and Tertbutyl Hydroquinone (TBHQ) were used as the most common synthetic types (Rašković et al., 2014). Many studies have approved that synthetic antioxidants which are commonly used in industrial products have many beneficial effects such as anticarcinogenic and antimutagenic properties (Majdalawieh and Mansour, 2019; Musakhanian et al., 2022; Tachibana et al., 2020; Taghvaei and Jafari, 2015; Wang et al., 2023). Nevertheless the safety of synthetic antioxidants on the health of humans has been doubted (Ammar, 2016; Chen et al., 2014; Taghvaei and Jafari, 2015). Although sesame has a strong oxidation prevention capacity, the sesame oil contains high amounts of nearly 85% unsaturated fatty acids like oleic acid and linoleic acid with percentages of 39.09 and 40.39%, respectively, which are prone to oxidation (Ehsani et al., 2018; Heidari-Soureshjani et al., 2017; Lourenço et al., 2019).

The present study was designed to evaluate the antioxidant potential of SCEE on the satiability and oxidation properties of the refined sesame oil under high temperature condition during different exposure times.

Materials and methods

Materials

Sesame cake and sesame oil (*S. indicum* L, with Herbarium voucher number 903 at Faculty of Natural Resources, Yazd University) was obtained from Barsam Ardakan sesame products company (October 2016). The instruments used included spectrophotometer (UV-Visible S-2,150 UNICO model, USA), Rancimat 892 (Metrohm, Herisau, Switzerland), centrifuge (HB110, Behsan Company, Iran), and Soxhlet extractor Pyrex® Soxhlet extraction apparatus. BHA, acetic acid, chloroform, potassium iodide, and sodium thiosulfate were purchased from Sigma Chemical Co. (St. Louis, MO, USA). 1,1-Diphenyl-2-picrylhydrazyl (DPPH) was obtained from Fluka (Buchs, Switzerland). Folin-Ciocalteau reagent, Ethanol, Methanol, tri-chloroacetate, Thiobarbituric Acid (TBA), and sodium carbonate were purchased from Merck, Germany. All the chemical reagents and solvents were of highest analytical grade.

Experiments

In this study, firstly, the antioxidant properties of sesame cake extracts were investigated by measuring DPPH free radical inhibitory rate and total phenolic compounds of the extract. Also, it was designed to evaluate the antioxidant effects of the ethanolic extract of SCEE on refined sesame oil stored under high temperature conditions and over time. After preparing the ethanolic extract from SCEE with concentrations of 0, 20, 50, and 100 µg/ml, the extract was added to the refined sesame oil in three replicates (within the concentration range of 20-100 ppm). Then, they were kept at 60 °C for 30 days (aggravating circumstances) (Guo et al., 2016). On days 0, 10, 20, and 30 after inoculation of the extract into sesame oil, the TBA and Peroxide Value (PV) were measured.

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Extraction

First, the cakes were washed, dried at 40 °C, and ground into powder. Then, the sesame cake was dried and well powdered. One hundred g of the 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 below 70 °C. Ten 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 removed under vacuum/N2 flow to dryness, weighed, and the residue (0.5 g) redissolved in 100 ml of methanol to obtain an antioxidant solution of known concentration and stored in refrigerator until further experiments (Hussain et al., 2018).

Total Phenolic Content (TPC)

Folin–Ciocalteau method was used to evaluate the TPC of the sesame cake extract (Mohamed et al., 2016). The dilution 1:200 of ethanol extract was prepared in distilled water, and then 100 mg aliquot of the sample was mixed with the concentrated Folin–Ciocalteau reagent (2 ml). After that, 2 ml of sodium carbonate (Na_2CO_3) was inoculated into the mixture followed by shaking the mixture for 30 s. Next, 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 findings were expressed as mg of Gallic Acid Equivalents (GAE) per 1 g of powdered extract using standard curve prepared from gallic acid (0.1 mg/ml) solution.

Rancimat analysis

The rancimat analysis was done using Rancimat 892. Sesame cake 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 their legal limit of 200 mg/kg as positive control. Oils without added antioxidants were considered as blank control. The air flow and heating changed the electrical conductivity of the solution by producing oxidative substance and then it was evaluated and displayed in a curve. The inflection point of the curve was the highest resistance value of the oil samples. The results are shown as Induction Period (IP) (Ban et al., 2016).

DPPH radical scavenging capacity

Antioxidant properties of the extracts were measured based on activity of DPPH by spectrophotometry in 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 µl (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 distilled water was used instead of sample. The absorbance of the solution was measured by using a spectrophotometer in a 517 nm wavelength. The scavenging percentage is calculated according to the following equation (Sui and Zhou, 2014).

PV

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 adding 0.5 ml of saturated potassium iodide and it was mixed thoroughly and placed in the dark for 1 min. After that, 30 ml of distilled water was added and titrated by sodium thiosulfate 0.01 N to get colorless. The absorbance was detected at 560 nm using the spectrophotometer (Yang et al., 2016).

TBA

Sesame oil (1,000 mg) was solved in 8 ml tri-chloroacetate 5% (w/v) and centrifuged (3,600 rpm for 20 min). Then, 1 ml of 2-TBA (0.01 M) with 5 ml of aqueous phase of the tube was blended and heated for 40 min in boiling water. After cooling, the absorption was measured with spectrophotometer at 532 nm. TBA value is presented as mg of malondialdehyde (Kamkar et al., 2014).

Statistical analysis

Oxidation experiments were performed in triplicates. The results were averaged and statistically analyzed with one-way ANOVA using SPSS 22. Also, the Duncan's as a Post hoc test was used to evaluate specific differences between pairs of means ($p<0.05$).

Results

Total phenolic compounds

The TPC of SCEE was evaluated when the study started on the first day, total phenolic compound of SCEE was obtained to be 55.48 mg GAE/g extract.

DPPH radical scavenging activity

Inhibition of free radicals' formation and scavenging properties of SCEE were significantly higher than control group (without any SCEE) but the index at the group giving BHA was observed to be significantly ($p\leq0.05$) higher than the other treatment groups.

DPPH (%)=(1− absorbance of control)×100

PV

The PV on day one of the study was 2.93±0.04 meq/kg. Figure 1 shows that PV declined linearly by increasing concentrations, but it rose during storage time. Sesame oils samples which were not supported by SCEE or synthetic antioxidant (control) represented the highest PV on different days of the study, so that the maximum PV on

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days 10, 20, and 30 in control experiments was 17.4±0.3, 19.46±0.3, and 21.59±0.11 meq/kg, respectively. The lowest PV on days 10, 20, and 30 were 12.6±0.16, 13.56±0.35, and 14.82±0.08 meq/kg, belonging to the sesame oils enriched by 100 µg/ml SCEE. There was a significant difference between the group containing 100 µg/ml SCEE and BHA, but the treatments receiving lower concentrations of SCEE obtained a significant higher PV than the two groups (100 µg/ml SCEE and BHA) ($p \leq 0.05$).

TBA value

As represented at Figure 2, TBA value of the treatment samples at the starting point of the study was 1.51±0.028 µmol/g. The maximum TBA value was shown in the control groups, in fact the groups without any SCEE at different times of the study. Accordingly, TBA values on days 10, 20, and 30 in the control groups were 1.59±0.095, 1.92±0.03, and 2.29±0.05 µmol/g, respectively. On the base of the TBA findings, this value significantly increased in the groups containing different concentrations of SCEE over time, but there was no significant difference between the treatments taking 100 µg/ml SCEE and BHA ($p \leq 0.05$).

Rancimat analysis

The experiments showed a significant difference in rancimat analysis. The group containing 100 µg/ml SCEE and BHA with 8.7±0.43 and 8.46±0.25 h illustrated the largest induction period, in which there was no difference between them. The sesame oil without SCEE and BHA showed the highest IP with 11.02±0.16 h ($p \leq 0.05$).

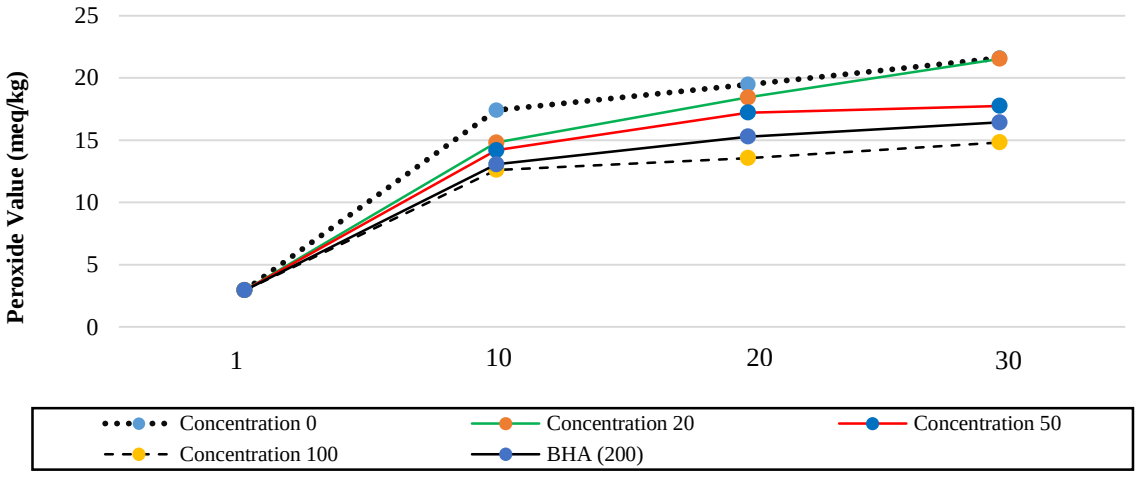

Figure 1: The Peroxide Value (PV) of the refined sesame oil incorporated with different concentrations (µg/ml) of sesame cake extract during different time (the concentrations were comprised at each day). BHA=Butylated Hydroxyanisole

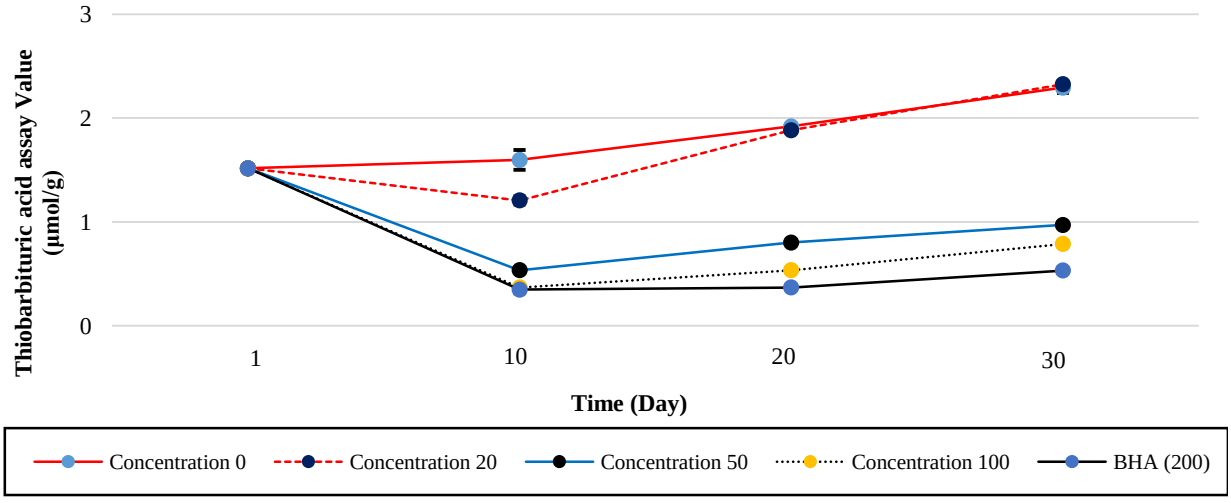

Figure 2: The Thiobarbituric Acid (TBA) value of the refined sesame oil incorporated with different concentrations (µg/ml) of sesame cake extract was shown during different time (the concentrations were comprised at each day). BHA=Butylated Hydroxyanisole

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Discussion

TPC can improve oxidative stability of oils and act as radical scavenger and inhibitor of lipid oxidation. Antioxidant efficacy of plant extracts depends on the concentration of the phenolic compounds in plants (Pisoschi et al., 2016; Zhang et al., 2022). SCEE containing high amounts of phenolic substance that could be accountable for SCEE antioxidant activity (Bopitiya and Madhujith, 2013; Dossou et al., 2023), whereas the TPC of SCEE was evaluated on day one of the study, which was 55.48 mg GAE/g extract. Bopitiya and Madhujith (2013) reported that sesame seed oil extracts contain 26.00±0.14 (mg GAE/g of extract) phenolic components. Zhou et al. (2016) reported that TPC in black sesame varieties ranged from 4.54 to 7.32 g GAE/kg, whereas white sesame seed displayed higher TPC of 4.04±0.13 g GAE/kg. A total of 112 metabolites were characterized in sesame cake, 86 metabolites of them were phenolic compounds such as sesamol and lignans, also C-glycosides and other phenolic compounds could contribute to antioxidant activity (Mekky et al., 2019). The total contents of phenolic compounds observed in the present study were lower than previous studies. This difference might due to the diversity of the methodology used for phenolic analysis in the current study.

Rancimat method measures the stability of natural fats and oils against oxidation. For this reason, by accelerating the aging process of oils by increasing the temperature and applying heat along with increasing the volume of air blown into the sample and the time required to oxidize it as induction time or oxidation stability index will be measured. Based on the results, IP was found to be higher in group containing different dilutions of SCEE and BHA compared to the control group (p<0.05). Gharby et al. (2017) indicated 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. It showed that flaxseed oil was susceptible to oxidation with IP of 1.95 h, whereas the IP of sesame oil was 4.88 h (Figueiredo et al., 2017). Induction period is one of the methods used to examine the degree of oil to resist oxidation at elevated temperatures. Airflow rate, oil sample weight, and temperature are the operational parameters that can be adjusted easily in the rancimat method and may affect the determination of the IP (García-Moreno et al., 2013). Induction period can be expressed at various temperatures varying from 100 °C to 130 °C. In the case of palm olein, the induction period measured at 130, 110, and 100 °C were 13.7, 24.2, and 44.0 h, respectively. It appears that the induction period is more or less doubles for every increment of 10 °C (Tarmizi et al., 2016).

It can be concluded that the SCEE can act as a potent antioxidant component and diversity between the results of different studies can be related to the parameters such as temperature and time used in the experiment.

The oxidation capacity of oil is related to the content of unsaturated fatty acids, so that sesame oil had high polyunsaturated fatty acid content (63.78%). It has been reported that antioxidant effect ethanolic and methanolic extracts of sesame meal at concentrations of 100 and 200 mg/kg are not much different from BHT at 50 and 100 mg/kg concentrations (Soodbar et al., 2016). In the present study, scavenging capability (DPPH) of the treatment containing 50 µg/ml SCEE was significantly higher (p<0.05) in comparison with control group, but less than the sesame oil containing 200 µg/ml BHA and the sesame cake extract displayed a concentration-dependent scavenging activity. Moreover, it was shown that scavenging activities of SCEE were significantly higher (p<0.05) than that of BHT and BHA but less than that of TBHQ (Mohdaly et al., 2011). The results of the present study and other studies indicated that there is a direct relation between phenolic content of SCEE and antioxidant activity.

PV is one of the most widely used tests for the measurement of oxidative activity in oils and fats. PV is the amount of peroxide and hydroperoxide formed in the initial stage of primary oxidation of oils and acts as an indicator for the primary oxidation and rancidity of fats and oils (Hussain et al., 2018). The present study revealed that PV increased linearly through time and a gradual and continuous loss of value was observed as the concentration increased. In the control group, where SCEE was absent, the PV was at maximum (Figure 1). Moreover, a direct relationship was observed between oxidative stability and PV raising in different studies (Naghshineh et al., 2010; Yang et al., 2016). In a study, the PV index increased during the storage of refined, bleached, deodorized soybean oils at 60 °C for 15 days with various concentrations of sesame cake extracts (5, 10, 50, and 100 ppm, based on extract weight). Soybean oils without antioxidant (control) reached a maximum PV of 89.2 meq/kg after 15 days of storage. The PVs of soybean oils with 5, 10, 50, and 100 ppm of sesame cake extract, 200 ppm BHT, and 200 ppm TBHQ were 74.2, 65.7, 72.9, 69.4, 80, and 41.4, respectively (Suja et al., 2004).

It was shown that the PV of sunflower oil enriched with sesame cake extract (5-200 ppm) increased linearly with storage time and its increase was accelerated after 32 h, and the maximum PV was demonstrated in control group after 72 h of storage. The PVs of the oil samples with BHA (200 ppm), BHT (200 ppm), and TBHQ (200 ppm) were lower than other treatments (Mohdaly et al., 2011).

It was reported that PVs of catfish fat samples treated with the black and white sesame cake extract (50, 100, 200,

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400 ppm) reduced at concentrations of 400 and 200 ppm BHT, when stored at both room temperature and 60 °C up to 42 days. PV indices of all samples containing black and white sesame cake extract at concentrations of 50, 100, and 200 ppm increased during the first four weeks of storage quickly. However, both white and black sesame cake ethanol showed a strong effect on retarding lipid oxidation in catfish fat with a PV lower than 16 meq/kg fat, in comparison with BHT at 200 ppm (Lieu and Dang, 2015).

Nevertheless, antioxidant efficacy of the SCEE under the concentration of 100 µg/ml was shown to be significantly lower than the treatments receiving BHA. In the present study, primary and secondary oxidation of sesame oil giving SCEE decreased during storage time. TBA value at all treatment groups showed a decrease compared to the control group until day 10 and then increased over time, but the index in all groups was evaluated to be lower than the control group (Figure 2). The reaction of TBA with the secondary oxidation step products such as aldehydes may lead to observe the pattern (Guillén-sans and Guzmán-chozas, 1998). In a research study, it was shown that ethanol and methanol extracts of Satureja hortensis can inhibit both primary and secondary oxidation of soybean oil during storage. From this point of view, PVs and TBA values of soybean oil enriched by the extract was lower than the control group (Kamkar et al., 2014). It has been indicated that the insertion of sesame seed extract (500, 750, 1,000 µl/100 ml of the sunflower oil) and BHT (200 ppm) into the sunflower oil resulted in significant inhibition of TBA production during storage time, so that the lowest TBA was observed in the group containing 1,000 µl of sesame seed extract (0.23 µM/g on 30th day of storage), whereas the highest TBA was obtained in the control group (0.351 µM/g on 30th day of storage). Also, TBA value of sunflower oil, sesame oil, and their blends enriched with 5 µl/g tocotrienol rich fraction extracted from crude rice bran oil, increased gradually with respect to time of exposure during storage at 60 °C for 42 days due to accumulation of secondary oxidation products mainly malonaldehyde (Bardhan et al., 2014). TBA data of the mentioned studies are in accordance with findings of the present study, so that TBA increased over time and reduced by increasing the extracts concentrations.

Conclusion

The PVs and TBA values were affected by storage time and high temperature condition. Also, different concentrations of sesame cake extract can inhibit oxidative rancidity of refined sesame oil compared to synthetic antioxidants. Therefore, it can be used as an effective and natural antioxidant component. However, further research is necessary to determine antioxidant mechanisms activity of sesame cake extract and its derivatives.

Author contributions

A.S.A. wrote the manuscript; H.M is doctoral supervisor and designed the study; H.K is doctoral advisor; S.A.Y.A. is doctoral advisor; G.H.P.M. analyzed the data. All authors read and approved the final manuscript.

Conflicts of interest

There is no conflict of interest.

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