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Antioxidant efficacy of sesame cake extract in vegetable oil protection

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Abstract

Antioxidant activity of methanolic extract of sesame cake was evaluated in soybean, sunflower, and safflower oils, using the Schaal oven method and differential scanning calorimetry (DSC) analysis. Results showed that sesame cake extract (SCE), at concentrations of 5, 10, 50 and 100 ppm in vegetable oils, could significantly ($P<0.05$) lower the peroxide value, diene value and *p*-anisidine value of oils during storage at 60 °C. The study also indicated a better antioxidant effect for sesame cake extract than BHT at 200 ppm. The DSC analysis produced results comparable to the Schaal oven method. Lower concentrations of sesame cake extract were effective in protecting vegetable oils, irrespective of their unsaturation and vitamin E content.

Keywords: Antioxidants; BHT; DSC analysis; Safflower oil; Schaal oven method; Sesame cake extract; Soybean oil; Sunflower oil; TBHQ

1. Introduction

Lipid oxidation lowers quality and nutritional value of foods. The products of lipid oxidation are known to be health hazards since they are associated with aging, membrane damage, heart disease and cancer (Cosgrove, Church, & Pryor, 1987). The addition of antioxidants is effective in retarding the oxidation of lipids and lipid containing foods. Synthetic antioxidants, such as butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT) and *tert*-butyl hydroquinone (TBHQ), are widely used in the food industry because they are effective and less expensive than natural antioxidants (Pin-Der Duh & Gow-Chin Yen, 1997). Their safety, however, has been questioned (Labuza, 1971). TBHQ is banned in Japan and certain European countries (Shahidi, 1997) and BHA and BHT are reported to be carcinogenic (Ito, Hagiwara, Shibata, Ogiso, & Fukushima, 1982). Hence, research for a safer and effective natural antioxidant is underway and several natural sources are being examined. Some of the antioxidant extracts from natural sources, such as Rosemary have been widely studied for their protection efficiency (Joyeux et al., 1998) in foods and vegetable oils. Some other sources, such as oat extract and peanut hull extract, have also been reported to offer antioxidant protection to vegetable oils during storage (Pin-Der Duh & Gow-Chin Yen, 1997; Tian & White, 1994). In the present study, a byproduct of an oil seed source, namely sesame cake, containing unique antioxidant compounds, such as lignans, is studied as an antioxidant extract that can be used in the place of synthetic antioxidants in vegetable oils.

Sesame (*Sesamum indicum* L) is an important source of edible oil. The oil shows remarkable stability despite being is highly unsaturated. Sesame seeds are used in confectionery and are considered, in the orient, to be a health food (Namiki, 1995). Many studies were conducted to investigate the health-promoting effects of sesame (Kang, Kawai, Naito, & Osawa, 1999; Sugano et al., 1990). It shows a hypocholesterolemic effect, suppressive effect on chemically induced cancer and anti-aging properties. (Kang et al., 1999; Sugano et al., 1990, Yamashita, Lizuka, Imai, & Namiki, 1995). Lignans and lignan glycosides, present in sesame seed, oil and cake, are responsible for the important properties of sesame. From literature reports, it can be seen that antioxidant source such as sesame or its byproducts, especially cake, have not been systematically investigated as a source of natural antioxidants to substitute

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for synthetic antioxidants for vegetable oil protection. Even though the antioxidant properties of sesame seed and oil have been studied, the antioxidant potential of sesame cake extracts has not been explored. Preliminary studies showed that an appreciable amount of antioxidants was still present in sesame cake, which is used as a cattle feed. One of the objectives of the present study was to examine the antioxidant efficacy of extracts from sesame cake for protecting vegetable oils from oxidation, thus resulting in better byproduct utilization.

2. Materials and methods

2.1. Materials

Sesame cake was purchased from local markets. Sesamol standard was obtained from Sigma Chemical Company (USA). Sesamin and sesamolin were isolated from sesame oil by the method of Soliman (Soliman, EI-Sawy, Fadel & Osman, 1985). High performance liquid chromatography (HPLC) grade solvents were purchased from Merck India Ltd. All other reagents were of analytical grade. Refined, bleached, deodorized (RBD) soybean oil, without the addition of antioxidants, was supplied by Soybean oil Industry (M/s Sakti Soya's, Pollachi, Tamil Nadu, India). Similar RBD samples of sunflower and safflower oils, without added antioxidants, were obtained from M/s Marico Industries, Mumbai, India.

2.2. Extraction

A commercial sample of sesame cake was dried and well powdered. 100 g of sample were initially defatted with hexane (500 ml×3 times), at room temperature. The defatted residue was water-washed using distilled water (500 ml×3times) and dried below 70 °C. 10 g of the above residue were extracted with 150 ml methanol for 16 h in a Soxhlet extractor. Extract was filtered, solvent removed under vacuum/N_2 flow to dryness, weighed and the residue (0.5 g) redissolved in 100 ml of methanol to give an antioxidant solution of known concentration and stored in refrigerator until further experiments (US Patent Application No. 60/404.004).

2.3. Composition analysis of extract

Sesame cake extract was analyzed for contents of fat, protein, sugar and ash according to AOAC methods (1984).

2.4. HPLC analysis of sesame cake extract

HPLC analysis was carried out in a Shimadzu make binary system with LC-10 AD model pump, a 7125 model Rheodyne injector fitted with a 20 μl sample loop, a SPD-10 A UV-Visible detector, and with a C-R7Ae plus integrator for data acquisition, analysis and display. The analysis was carried out using a Waters μ-bondapak C_{18} column (4.6 mm ID × 25 cm) in the reversed phase with a guard column of C_{18} (Supelco). The mobile phase used was methanol: water (70:30) with a flow rate of 1 ml/min. The UV-Visible detector was set at 290 nm, with a detector sensitivity of 0.005 AUFS.

The extracts were filtered through PTFE membrane before injection into the HPLC. The Quantitation was done with respect to the response factor for standard sesamol. The ε-values of sesamin and sesamolin are comparable with that of sesamol according to Bailey (Bailey's Industrial Oil & fat Products, 1996).

2.5. Oil storage studies

Schaal oven test (Fennema, 1976) was conducted to evaluate the effect of antioxidants against oxidation during the accelerated storage of oils. The storage tests were carried out on three different vegetable oils like (soybean, sunflower and safflower).

Refined, bleached, deodorized soybean oil, without any added synthetic antioxidant, such as TBHQ, supplied by soybean oil manufacturer, was used for storage studies. Oil samples were stored in uniform glass containers at 60 °C for a definite period in an incubator. The following sets of samples were included in the study. Sesame cake extracts, at different concentrations (5, 10, 50 and 100 ppm, based on extract weight), were partly dried under nitrogen and then added to 15 g of oil. Experiments were also carried out with synthetic antioxidants, such as TBHQ and BHT at 200 ppm level, and a control set without added antioxidants. There were separate 15 g sample duplicate sets for each time point studied. Samples were analyzed after 3, 6, 9, 12 and 15 days for peroxide value (AOCS Method, 1990), diene value (Mahinda Wettasinghe & Shahidi, 1999), and *p*-anisidine value (Jirusova, 1975) to follow the oxidative changes. The percentage inhibition of oil oxidation is given by,

$$100 - \left[\left(\text{PV increase of sample}/\text{PV increase of control}\right) \times 100\right]$$

All experiments were conducted with duplicate sets, and analyses of samples were run in triplicate and averaged.

The above experiments were repeated with sunflower and safflower oils.

2.6. DSC analysis

DSC analysis was carried out in a Mettler Toledo instrument. This technique is used for studying various

heat-related phenomena in materials by monitoring associated changes in enthalpy (Hassel, 1976; Simon, Kolman, Niklova, & Schmidt, 2000). Oxidation is an exothermic process and the heat of reaction involved makes it possible to employ DSC for the evaluation of oxidative stability of oils. The measurement was carried out on a macroscopic quantity of sample. The oil without additives was first studied under a dynamic heating regime from 90 to 200 °C, and the temperature of onset of oxidative changes was noted from the DSC curve as the point of inflection. The samples were then analyzed isothermally at a temperature 10 degrees below the onset temperature. Oxidative stability of vegetable oils was evaluated under isothermal oxidation at 150 °C under a stream of oxygen at 40 ml/min. Heat of reaction was measured. An aliquot (7–10 mg) of sample was kept in the aluminium sample cell and another pan without sample was kept as reference. The flow of nitrogen was 200 ml/min. The vegetable oil samples, containing synthetic antioxidants and sesame cake, extracts were analyzed isothermally at the temperature. The time at which the onset of oxidation occurred was noted and this induction period was taken as indicative of the oxidative stability of the oil.

2.7. Statistical analysis

Analysis of variance was performed by (ANOVA) procedures. Significant differences between means ($P < 0.05$) were determined by Duncan's multiple range test.

3. Results and discussions

3.1. General

Compositional analysis of sesame cake extract showed 52.0% fat, 20.8% sugar, 5.9% protein, 3.8% ash and a total of 15.4% lignans (lignans and lignan glucosides). The lignan content of the extract was analyzed by HPLC. It contained sesamol (22,677 ppm), sesamin (105,893 ppm) and sesamolin (12,504 ppm). Lignan glucosides included sesaminol diglucoside (6506 ppm) and sesaminol triglucoside (6792 ppm). During preliminary studies, crude extract, obtained by extraction of sesame cake with methanol, was used and it was found that 200 ppm of crude extract was effective and the results comparable with BHT at 200 ppm. By purification, the contents of antioxidants were increased and the activity enhanced as will be discussed below. The total lignan content of the crude extract was 0.8–1% while that of purified extract was 12–15%. For further studies, purified extracts were used and these results are detailed here.

The oxidative stability studies were carried out at 60 °C in an incubator. Determination of peroxide value (PV) of oils oxidized at 100 °C is unreliable because hydroperoxides decompose at elevated temperature (Frankel, 1993). Therefore, the antioxidant efficacies of the sesame cake extracts in soybean oil, sunflower oil, and safflower oils were evaluated at 60 °C. The tocopherol contents of soybean, sunflower and safflower oils were analyzed by the standardized method (Renukadevi, Suja, Jayalekshmy, & Arumughan, 2000) using HPLC in a Shim-pack CLC-NH_2(M) column (4.6mm×25cm) with *n* hexane:isopropanol (96:4) at a flow rate of 1 ml/min. Wavelength of detection was 297 nm. Tocopherol/tocotrienol contents were 1099, 479 and 576 ppm for soybean oil, sunflower oil and safflower oils, respectively.

3.2. Soybean oil (SBO)

Fig. 1 shows the PV developments during the storage of SBO at 60 °C for 15 days with various concentrations of sesame cake extracts (SCE). Additional treatments included TBHQ and BHT at 200 ppm and a control containing no additives. SBO without the antioxidant (control) reached a maximum PV of 89.2 meq/kg after 15 days of storage. A significant difference ($P < 0.05$) in PV was observed between the control and SBO containing sesame cake extract (SCE), BHT and TBHQ which slowed the rate of peroxide formation. The PV of SBO with 5, 10, 50 and 100 ppm of SCE, 200 ppm BHT and 200 ppm TBHQ were 74.2, 65.7, 72.9, 69.4, 80 and 41.4 respectively. The corresponding inhibition rates were 16.7, 26.3, 18.2, 19.8, 10.3 and 53.6% after 15 days

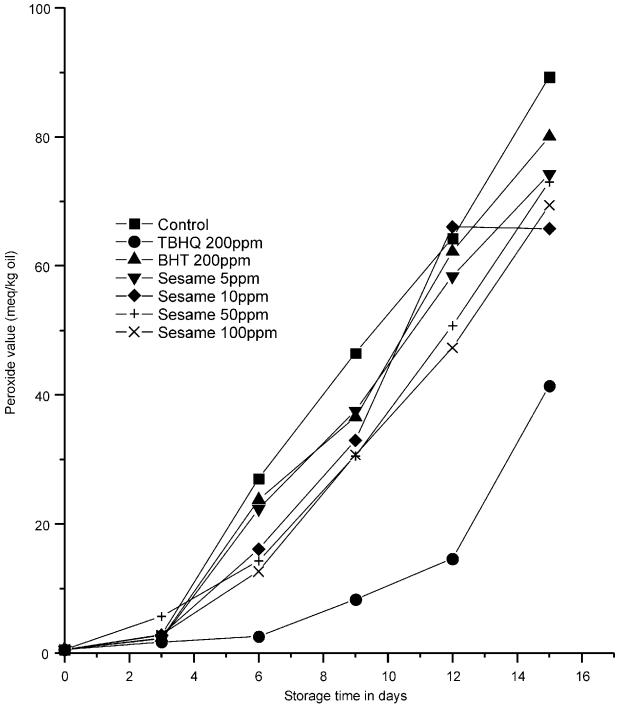

Fig. 1. Peroxide value (mequiv. O_2/kg) of soybean oil stored at 60 °C.

of storage compared with the control. Although the effects of four different SCE concentrations varied during the incubation periods, the total effect is more or less equal. These results indicated that SCE inhibited soybean oil oxidation. Further, the antioxidant effect of SCE at 5, 10, 50 and 100 ppm was better than BHT at 200 ppm. During incubation, TBHQ maintained a significantly lower PV than all other treatments. Sesame cake extract was tried at 200 ppm concentration during initial trials. The protective effect offered at 200 ppm was comparable to that at 100 ppm concentration. Hence, for final set of experiments, 200 ppm was not included.

Diene value also represents the formation of hydroperoxides. Fig. 2 represents the diene value of the experimental sets. The diene value of the control reached 11.9 from an initial value of 2.9 after 15 days of storage. The values for cake extracts at 5, 10, 50, 100 ppm, 200 ppm BHT and 200 ppm TBHQ were 10.2, 9.9, 10.1, 9.8, 10.0 and 7.1, respectively. The diene values of all treatments were significantly lower than that of the control.

Changes in *p*-anisidine value, which represent the secondary oxidation products produced during the oxidative degradation of oil, are shown in Fig. 3. The formation of secondary oxidation products also increased during storage. The *p*-anisidine value of control reached a maximum of 226 from an initial value of 12.4 after 15 days of storage. The values for SCE at 5, 10, 50 and 100 ppm, BHT at 200 ppm and TBHQ at 200 ppm were 183, 166, 176, 174, 202 and 128, respectively. A sudden increase in *p*-anisidine value was noted after 12 days of storage. A significant difference was noted between the values for control and experimental samples.

Antioxidants are mainly used in lipids to delay the accumulation of primary oxidation products and thus to improve the oxidative stability. The primary products of lipid peroxidation are hydroperoxides, which are generally referred to as peroxides. Therefore the results of peroxide value estimation give a clear indication of lipid autoxidation. For further confirmation of these results, other oxidation parameters, such as diene value and *p*-anisidine values were also measured. Thus PV, diene value and *p*-anisidine value of soybean oil that contained the extract were significantly lower than that of the control, which clearly showed the marked antioxidant effect of the sesame cake extract in soybean oil protection.

3.3. Sunflower oil (SUFO)

The PV developments of SUFO with sesame cake extract at 5, 10, 50, 100 ppm, BHT 200 ppm, TBHQ 200 ppm and of the control sample are shown in Fig. 4 PV of control increased from an initial value of 1.5 to 84.9. The values for SCE at 5, 10, 50 and 100 ppm, BHT 200 ppm and TBHQ 200 ppm were 56.4, 57.4, 61.4, 57.9, 79.0 and 15.8, respectively. The percentage inhibition for TBHQ was very high. The antioxidant activities were calculated (for BHT 200, SCE 5, 10, 50 and 100 ppm) as 6.9, 33.5, 32.4, 27.8 and 31.8% respectively, and significant differences were found between these

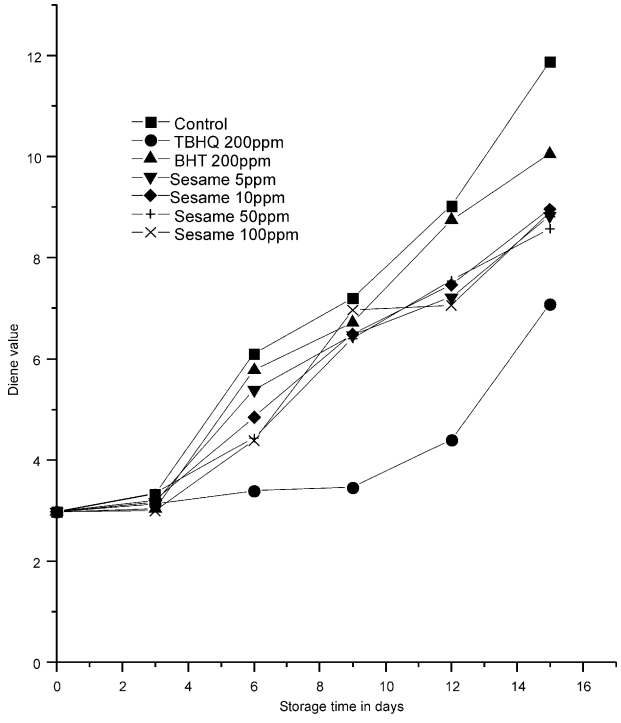

Fig. 2. Diene value of soybean oil stored at 60 °C.

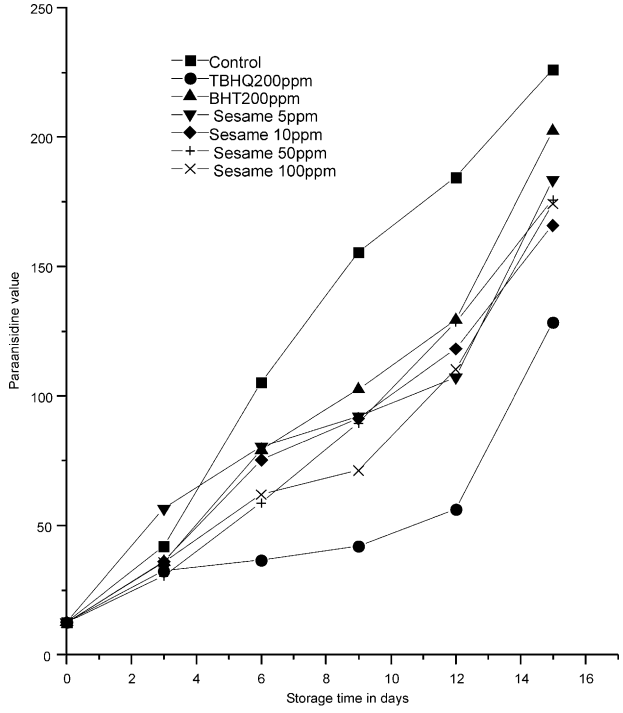

Fig. 3. *p*-Anisidine value of soybean oil stored at 60 °C.

values, indicating that SCE exhibited an inhibitory effect on sunflower oil oxidation. Moreover, SCE was significantly more effective than BHT at 200 ppm.

The diene value of SUFO, without added antioxidant, increased to 13.9 (initial value 4.4) after 15 days of storage (Fig. 5). The diene values of SUFO with 5, 10, 50 and 100 ppm SCE, 200 ppm BHT or 200 ppm TBHQ were 11.2, 10.2, 10.0, 6.9, 12.5 and 4.5 respectively. These values are lower than that of the control stored for 15 days.

The *p*-anisidiene value of SUFO (Fig. 6) without added antioxidant (control) increased from an initial value of 18.8 to 247 after 15 days. The *p*-anisidine value of SUFO with 5, 10, 50, 100 ppm SCE, 200ppm BHT or 200 ppm TBHQ were 222, 167, 167, 153, 222 and 84.9, respectively. These results confirmed the protective action of sesame extracts against the oxidative changes of sunflower oil.

3.4. Safflower oil

The peroxide value developments for safflower, oil with and without added antioxidants, are shown in Fig. 7. The peroxide formation for control was 85.7 (initial value = 1.5) after 15 days. The PVs for SCE at 5, 10, 50, 100, BHT at 200 ppm or TBHQ at 200 ppm were 72.1, 74.7, 74.7, 56.9, 78.2 and 19.2 meq of oxygen/kg, respectively. There is a significant difference between the control and other treatments. SCE 5, 10 and 50 ppm showed more or less equal antioxidant activities while SCE (100 ppm) showed higher activity.

The diene values and *p*-anisidiene values for the experimental samples have been shown if Figs. 8 and 9. The diene value of the control reached 12.7 from an initial value of 3.2 while those of SCE at 5, 10, 50, 100,

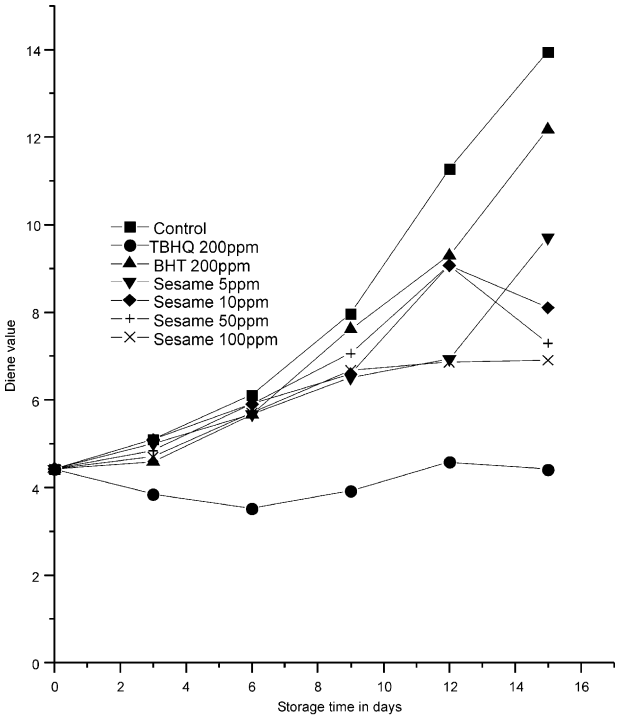

Fig. 5. Diene value of sunflower oil stored at 60 °C.

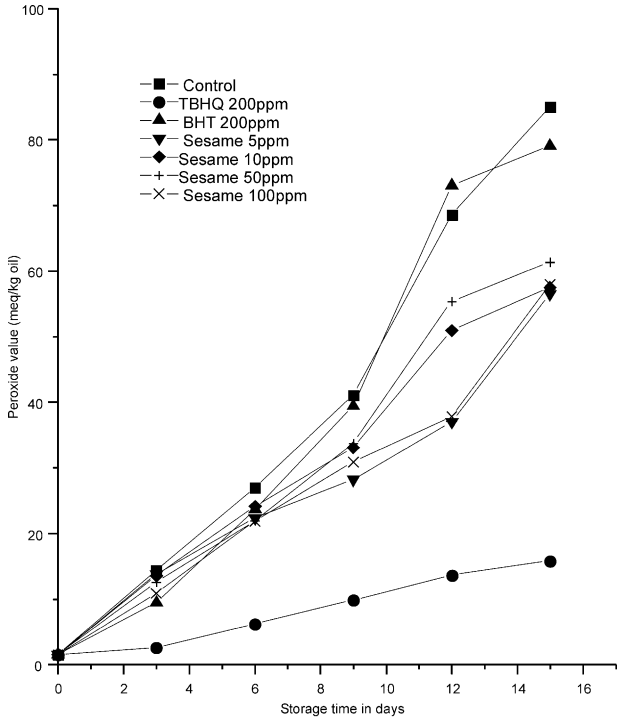

Fig. 4. Peroxide value of sunflower oil (mequiv. O_2/kg) stored at 60 °C.

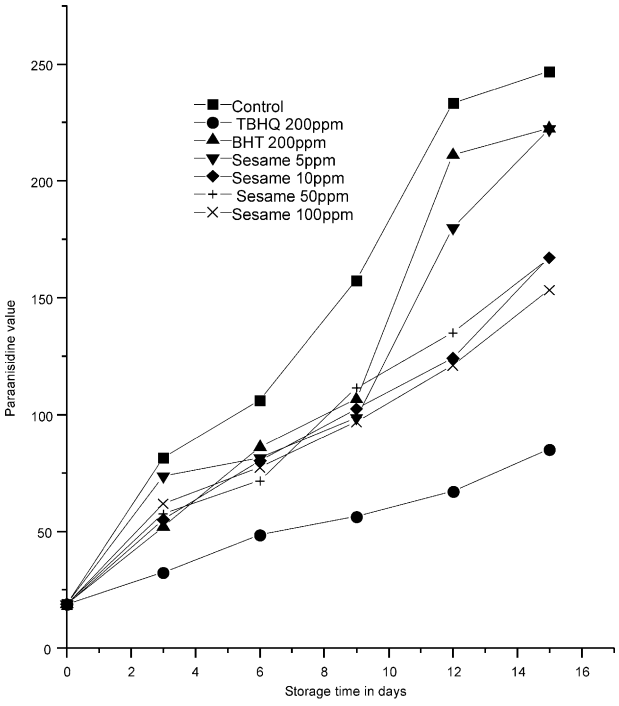

Fig. 6. *p*-Anisidine value of sunflower oil stored at 60 °C.

BHT 200 or TBHQ 200 ppm levels were 10.2, 10.1, 9.8, 10.0, 10.7 and 4.7, respectively, after 15 days. The *p*-anisidine value of control was 189 (initial value = 20.7). The SCE at 5, 10, 50, 100, BHT 200, or TBHQ at 200 ppm showed *p*-anisidine values as 153, 141, 112, 150, 153 and 59.9, respectively. There were significant differences between the diene and *p*-anisidine values of control and experimental samples. The antioxidative effects of SCE were comparable and even better than that of BHT in safflower oil oxidation.

The DSC profile of sunflower oil is shown in Fig. 10. When heated at 150 °C for 45 min, the onset of oxidation of control was at 3.58 min. However, in the case of oil containing SCE at 100 ppm and 50 ppm and BHT at 200 ppm, the induction periods were 6.93, 6.98 and 6.34, respectively. Samples with TBHQ showed an induction period of 8.71 min. These results indicate that, even at higher temperatures, sesame extract is capable of protecting the oil to the same extent as BHT at the 200 ppm level (Fig. 11). Moreover, in soybean and safflower oils, sesame extract was more effective in protecting vegetable oils at lower concentrations than BHT. DSC analysis can be adopted as a fast and reliable method for evaluation of oil stability. Oil samples, which require 15 days, using the Schaal oven method, could be evaluated for their oxidative stabilities in less than 1 h by the DSC method as demonstrated here. The results of DSC analysis support the results obtained by the Schaal oven method.

There are reports about the antioxidant activity of natural extracts in vegetable oil during storage. Tian and White (1994) conducted a series of experiments to evaluate the antioxidant activity of oat extract in soybean and cottonseed oil. The added antioxidant concentration was based on total phenolic content and they used higher concentration of 200 and 300 ppm.

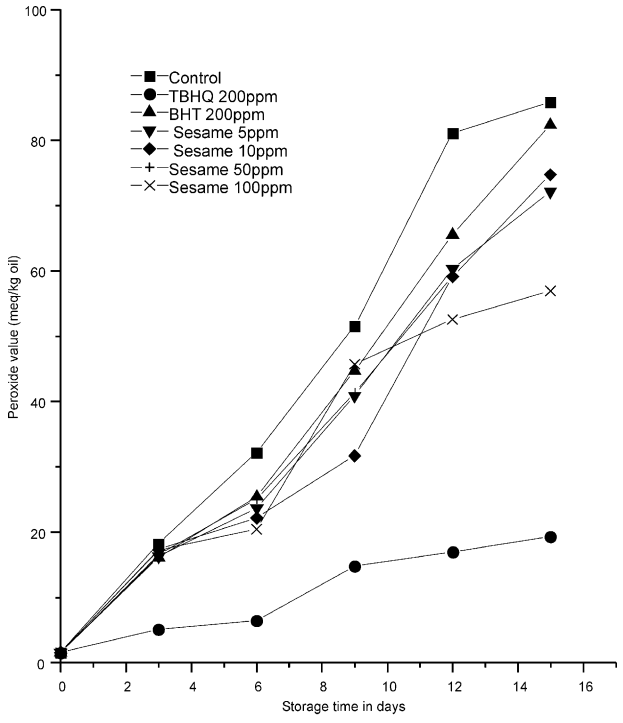

Fig. 7. Peroxide value of safflower oil (mequiv. O_2/kg) stored at 60 °C.

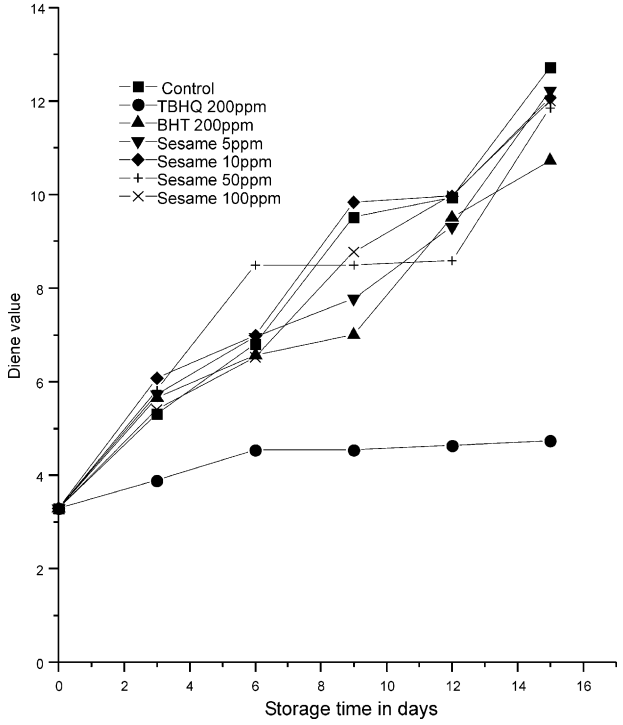

Fig. 8. Diene value of safflower oil stored at 60 °C.

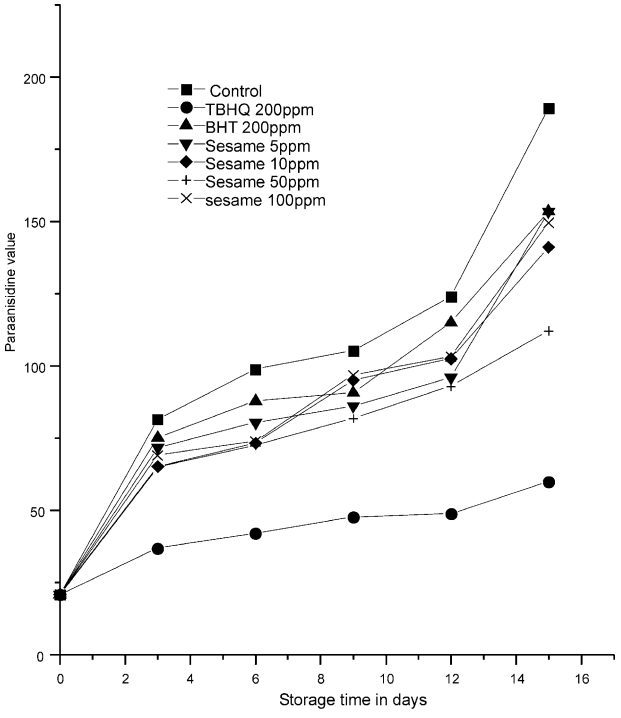

Fig. 9. *p*-Anisidine value of safflower oil stored at 60 °C.

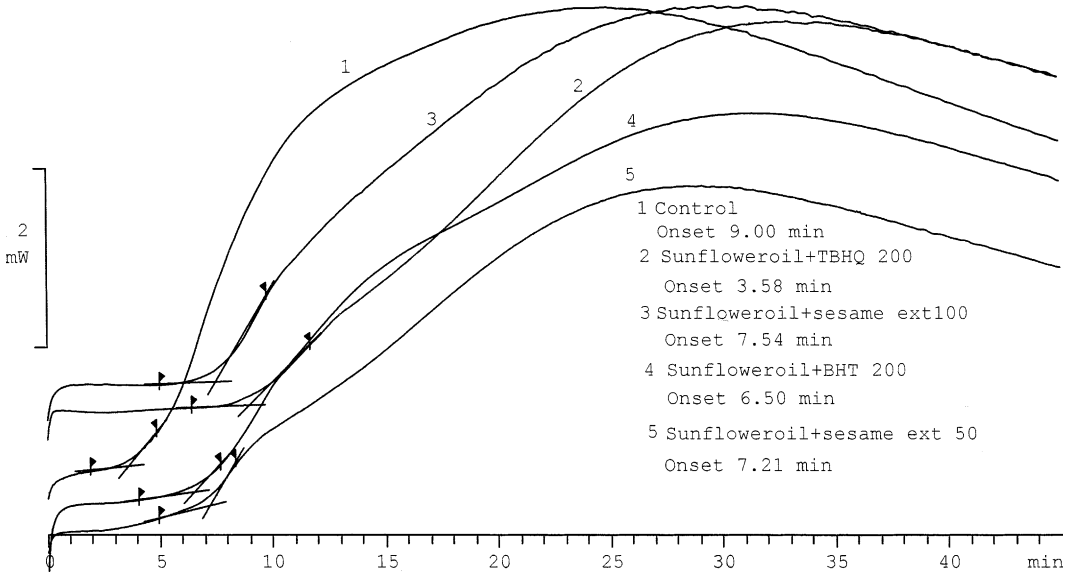

Fig. 10. DSC profile of oxidative stability of sunflower oil.

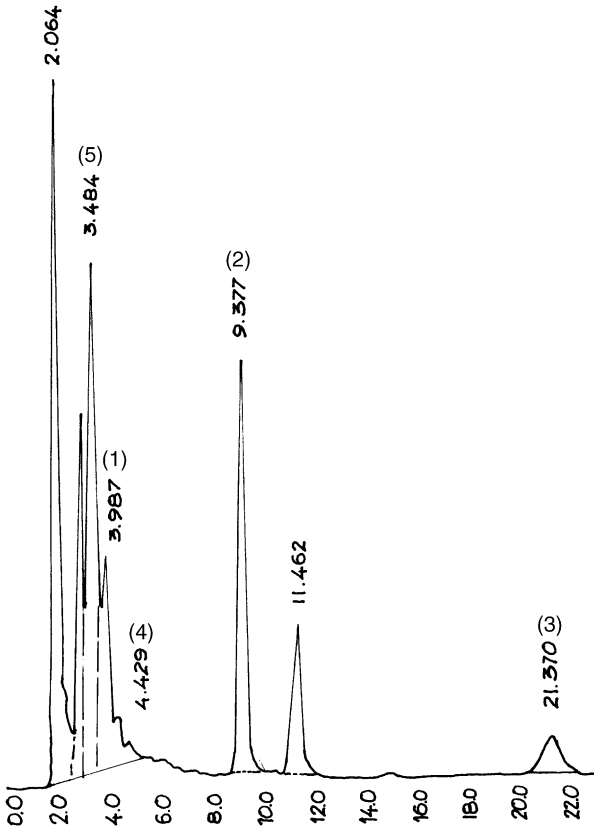

Fig. 11. HPLC profile of sesame cake extract: 1. Sesamol, 2. Sesamin, 3. Sesamolin, 4. Sesaminol diglucoside, 5. Sesamol triglucoside (for conditions refer to text).

Duh, Yen, Du, and Yen (1997) examined the antioxidant activity of mung bean hulls in soybean oil oxidation. They compared the activity with those of BHA and tocopherol and the activity was less than either of BHA and tocopherol. Antioxidant efficacy of peanut hull extracts was evaluated in soybean and peanut oil by Duh and Yen (1997). They used higher concentrations of extract and obtained results comparable with that of BHA.

The results of our study demonstrated that sesame cake extract could be used as a substitute for synthetic antioxidant to protect vegetable oils, such as soybean, sunflower and safflower oil, which have different levels of unsaturation and tocopherol. The protection offered by the sesame cake extract is comparable with, or in some cases better than, that of the widely used synthetic antioxidant BHT. The sesame extracts are effective at lower concentrations than synthetic or other available natural antioxidants.

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