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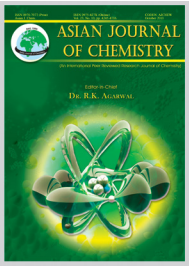

Antioxidant Efficacy of Methanolic and Ethanolic Extracts of Sesame Seed Using Differential Scanning Calorimetry

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Antioxidant activity of ethanolic and methanolic extracts of sesame cake was evaluated in soybean oil using peroxide value method and free radical scavenging assay using 2,2-diphenyl-1-picryl hydrazyl radical ($DPPH^{\cdot}$) and differential scanning calorimetry analysis. Results of peroxide value showed that the ethanolic extracts of sesame cake at concentrations of 1500 and 2000 ppm and methanolic extracts of sesame cake at concentration of 2000 ppm in vegetable oils, could significantly ($P < 0.05$) lower the peroxide value of oils during storage accelerated. The IC_{50} values for antioxidants butylated hydroxy toluene, ethanolic and methanolic extract of sesame cake were 63.57, 1762 and 2075 µg/mL. In differential scanning calorimetry analysis there are no differences between methanolic extracts of sesame cake, ethanolic extracts of sesame cake and butylated hydroxy toluene (as control). This is indicative of antioxidant activities are similar to each other. The sesame cake extracts can serve as natural antioxidant in food applications, much more effective than butylated hydroxy toluene.

Key Words: Antioxidants, Butylated hydroxy toluene, Differential scanning calorimetry analysis, Schaal oven method, Sesame cake extract.

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[1]. 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 are widely used in the food industry because they are effective and less expensive than natural antioxidants[2,3]. Their safety, how ever, has been questioned[4]. Butylated hydroxy toluene and butylated hydroxy anisole are banned in Japan and certain European countries and they are reported to be carcinogenic[5]. 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[6] in foods and vegetable oils. Some other sources , such as oat extract and peanut hull extract, have also been reported to offer antioxidant protectionto vegetable oils during storage[2,3,7].

Sesame (*Sesamum indicum* L) seed and oil contain abundant lignans, including sesamol, sesamin, sesamolin and lignin glycosides. The oil shows remarkable stability. Sesame seeds are used in confectionery and are considered, in the orient, to be a health food[8]. Sesame is one of the most important oilseed crops, cultivated in India, China and Iran, it is used in some traditional foods in Iran also.

EXPERIMENTAL

Seeds from Iranian variety (Darab)were obtained from local markets in Boshehr. They were sealed in a bottle and stored at 4 ºC until used. Refined, bleached, deodorized (RBD) soybean oil, without added antioxidant was supplied by Behshahr industrial company. Butylated hydroxy toluene was purchased from Jan Dekler company. All chemicals used were obtained from Sigma and Merck chemical company.

Extraction: Sesame seed was dried and powdered. The sample (100 g) was initially extracted with hexane (twice with a total of 300 mL) at room temperature. The defatted residue was washed with distilled water (two times with a total of 300 mL of water) to remove soluble sugars and proteins followed by drying at temperatures below 70 ºC. 10 g of the above purified residue was extracted with 150 mL ethanol and methanol. The extract was filtered and the solvent removed under vacuum/N_2 flow to dryness, weighed and stored in a refrigerator until analyzed[9].

Oil storage study: Schaal oven test was conducted to evaluate the effect of antioxidants against oxidation during

the accelerated storage (65 ºC for 24 days) of oils. The storage test were carried out on soybean oil. Refined, bleached, deodorized soybean oil, without any added synthetic antioxidant supplied by soybean oil manufacture, was used for storage study. Oil samples were stored at 60 ºC for a definite period (24 days) in oven. Sesame cake extracts at different concentrations (500, 1000, 1500, 2000 ppm based on extract weight) were partly dried under nitrogen and then added to 50 g of antioxidant free oil. Experiments were also carried out with synthetic antioxidants such as butylated hydroxy toluene at 75 ppm level and a control sample. The samples were analyzed after 3, 6, 9, 12, 15, 18, 21, 24 days for peroxide value to follow the oxidative changes[10].

DPPH assay: 2,2-Diphenyl-1-picryl hydrazyl (DPPH) is a stable free radical with a dark violet colour. It has maximum absorption at 517 nm. The absorption of the DPPH radical is decreased in the presence of an antioxidant. To evaluate the free radical scavenging activity, the extracts were allowed to react with a stable free radical. Each antioxidant was tested at different concentration (expressed as ppm or g/L or µg/mL). One millilitre of antioxidant solution in methanol was added to 1 mL of 90 µM methanol. DPPH˙ solution and the final volume adjusted to 4 mL with methanol. Methanol (3 mL) with DPPH solution (1 mL) was used as blank. Butylated hydroxy toluene antioxidant was used for comparison. The mixture was shaken vigorously and kept in the dark for 1 h. The absorption of the resulting solution was measured spectrophotometrically at 517 nm. The radical scavenging activity (RSA) was calculated as follows:

$$\% \text{ RSA} = [(\text{control absorbance} - \text{sample absorbance})/(\text{control absorbance})] \times 100$$

The % RSA was plotted against concentration and IC_{50} value was calculated. IC_{50} value is defined as the amount of antioxidant necessary to decrease the initial DPPH˙ concentration by 50 % (as extrapolated from the dose response curve).

Differential scanning calorimetry analysis: Differential scanning calorimetry (DSC) was applied to evaluate the oxidative stability of edible oils. This technique is used for studing various heat-related phenomena in materials by monitoring associated changes in enthalpy[11,12]. Oxidation is an exothermic process and the heat of reaction involved makes it possible to employ differential scanning calorimetry for the evaluation of oxidative stability of oils. Differential scanning calorimetry analysis was carried out in a Mettler Toledo (822E) instrument. The oil without additives was first studied under a dynamic heating regime from 90 to 200 ºC and the temperature of onset of oxidative changes was noted from the differential scanning calorimetry curve. Both the sample and reference are maintained at nearly the same temperature throughout the experiment. The samples were analyzed isothermally at the temperature 50º below the onset temperature. Oxidative stability was evaluated under isothermal oxidation at 110 ºC. Purified oxygen was passed through the sample at 40 mL/min. 8-10 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 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[7].

Statistical analysis: Data were analyzed using SPSS software (IBM SPSS statistics version 19). Significant differences between means ($P < 0.05$) were determined by Wilcoxon Signed Rank Test (NPar Tests).

RESULTS AND DISCUSSION

Peroxide value: Peroxide value was determined to know the extent of lipids oxidation. The primary products of lipid oxidation are hydroperoxides, which are generally referred to as peroxides. The peroxide value was determined by means of an automatic potentiometric titrator (Metrohm 751 GPD). The oxidative stability of ethanolic extracts of sesame cake and methanolic extracts of sesame cake were studied by the accelerated storage method of schall (at 65 ºC for 24 days). Figs. 1 and 2 show change in peroxide value during storage of soybean oil added with various concentrations of methanolic extracts of sesame cake and ethanolic extracts of sesame cake. Soybean oil without the antioxidant (control) reached registered a peroxide value of 72.77 meq/kg at 24th day of storage; the value was highest amongst all samples. A significant difference ($P < 0.05$) in peroxide value was observed between the control, soybean oil containing methanolic extracts of sesame cake and ethanolic extracts of sesame cake and the one containing butylated hydroxy toluene. The peroxide value of 24 days old soybean oil samples with 500, 1000, 1500, 2000 ppm of methanolic extracts of sesame cake and ethanolic extracts of sesame cake were 54.38 and 67.44, 53.92 and 48.23, 58.86 and 40.15 and 43.30 and 37.13 meq/kg respectively. The soybean oil containing 75 ppm of butylated hydroxy toluene had peroxide value of 65.43 meg/kg at 24 day. These results indicate that the antioxidant effects of methanolic extracts of sesame cake and ethanolic extracts of sesame cake at 1000-2000 ppm (even at 1000 ppm of methanolic extracts of sesame cake or ethanolic extracts of sesame cake the peroxide value was lower than with butylated hydroxy toluene) were better compared to that of butylated hydroxy toluene at 75 ppm.

DPPH analysis: DPPH, a stable free radical with a characteristic absorption at 515 nm, was used to study the radical scavenging effects of extracts. As antioxidants donate protons to this radical, the absorption decreases, the decrease absorption

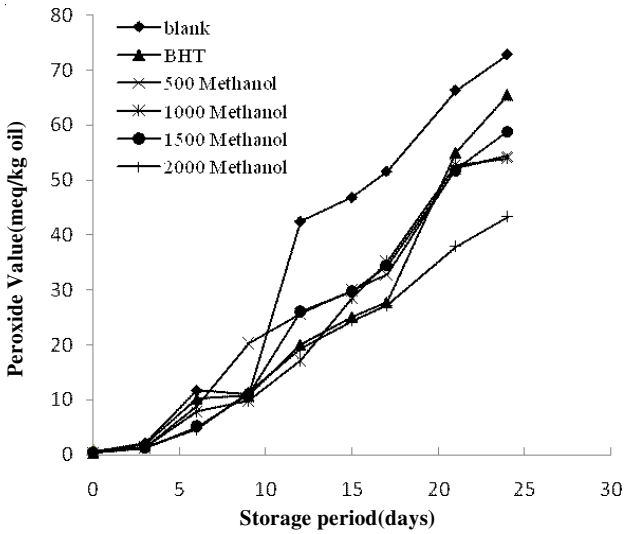

Fig. 1. Changes in peroxide value of soybean oil containing methanolic extract of sesame cake during accelerated storage

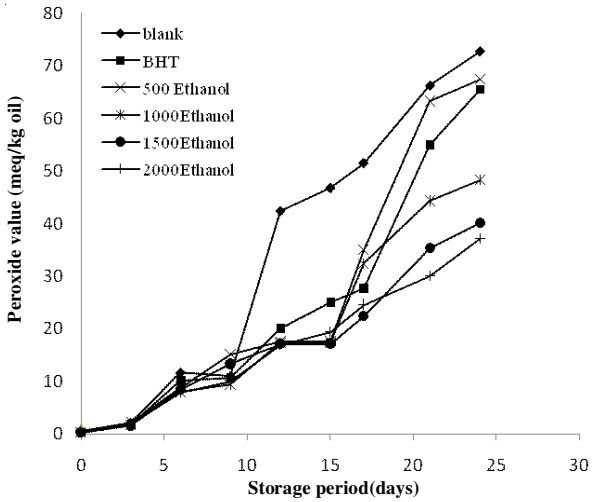

Fig. 2. Changes in peroxide value of soybean oil containing ethanolic extract of sesame cake during accelerated storage

is taken as a measure of the extent of radical scavenging. The EC_{50} value for butylated hydroxy toluene, ethanolic extracts of sesame cake and methanolic extract of sesame cake were 63.57, 1762 and 2075 µg/mL respectively. These results show antioxidant activity in ethanolic extracts of sesame cake and methanolic extract of sesame cake. We applied this analysis to confirm the presence of antioxidants at applied concentrations[13,14].

Differential scanning calorimetry results: Differential scanning calorimetry analysis can be adopted as a fast and reliable method for evaluation of oil stability. The differential scanning calorimetry profiles are shown in Figs. 3 and 4 when heated at 110 ºC for 400 min. Samples with methanolic extracts of sesame cake at concentration of 2000 ppm , ethanolic extracts of sesame cake at concentration of 2000 ppm and butylated hydroxy toluene at 75 ppm showed induction period 301.85, 297.57 and 281.77 respectively. These results indicate that, even at higher temperatures, sesame extract at 2000 ppm is capable of protecting the oil to butylated hydroxy toluene at the 75 ppm and they have the same oxidative stabilities[15]. Oil samples, which require 24 days, using the schaal oven method, could be evaluated for their oxidative stabilities in less than 1 day by the differential scanning calorimetry analysis. The results of differential scanning calorimetry analysis support the results obtained by the shaal oven method.

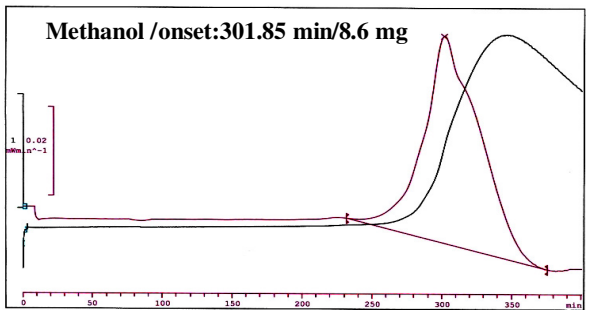

Fig. 3. DSC profile of methanolic extract of sesame cake

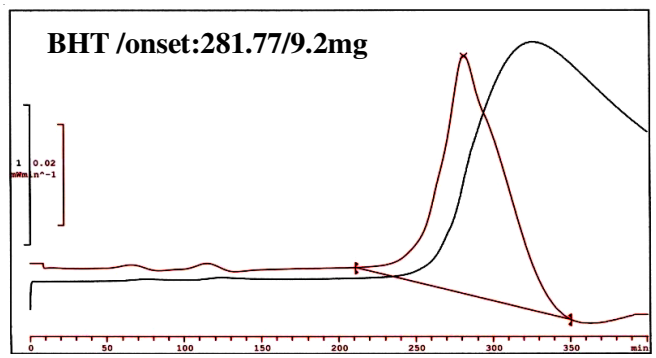

Fig. 4. DSC profile of ethanolic extract of sesame cake

Conclusion

In this study we investigated the effects of extracted lignin compounds on soybean oil. Sesame cake was extracted with two solvents (methanol and ethanol) to obtain a purified antioxidant with higher antioxidant content and better activity. Ethanol was found to be more efficient than methanol in extracting these antioxidant compounds, retaining its property.

The natural sesame extracts used at 1500-2000 ppm concentration gave antioxidant effect comparable to the one exerted by butylated hydroxy toluene at 75 ppm concentration. The study demonstrated that sesame cake extract could be used as a natural antioxidant to substitute synthetic antioxidants to protect vegetable oils against oxidative deterioration. The natural sesame extract when used as antioxidant may not pose hazard to human health at the concentrations recommended.

ACKNOWLEDGEMENTS

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