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Effect of Nigella Seed Extract on Oxidative Stability of Refined Sunflower Oil

MUSTAFA KIRALAN*, ALI BAYRAK and EDA ÇALIKOGLU
Department of Food Engineering, Faculty of Engineering
Ankara University, Diskapi, 06110 Ankara, Turkey
Fax: (90)(312)3178711; Tel: (90)(312)5961433
E-mail: mustafakiralan@yahoo.com

Alcohol extracts from nigella seed were added to refined sunflower oil which was oxidized at various temperatures (ambient temperature, 60 and 110 °C). Oxidation was followed by active oxygen method, Schaal Oven test and specific absorptivity at 232 nm. All of nigella extracts (500, 1000, 2000 ppm) showed antioxidant activity with regard to active oxygen method results. While nigella seed extract of 500 and 1000 ppm have shown antioxidant activity, 2000 ppm application showed prooxidant activity in Schaal oven test. At ambient temperature, oxidation was followed by peroxide value and 1000 ppm nigella seed extract showed strong antioxidant activity during 12 weeks. The study showed that nigella seed extract retarded oxidation of sunflower oil and may be used as a good natural antioxidant source.

Key Words: Oxidation, Sunflower oil, Nigella, Extract.

INTRODUCTION

Marketing of refined sunflower oil is widespread in Turkey as most countries around the world. It is rather unstable during the storage due to the high content of linoleic acid (between 60 and 70 %) and relatively low content of δ- and γ-tocopherols, which are more active antioxidants in oil than α-tocopherol-the main tocopherol in sunflower oil[1].

Oxidation is an important deterioration process for oil and fats. The retardation of this process has been developed with different methods. An effective method of them is using antioxidants obtained from natural and synthetic sources. Natural antioxidants are more effective than synthetic antioxidants in some cases. Moreover, synthetic antioxidants may cause health problems[2-4]. Therefore, there is an increasing interest in natural antioxidants which show strong antioxidant activity.

Nigella (*Nigella sativa*), a member of Ranunculaceae is used to decorate bakery products, flavouring ingredients in food recipes since ancient times and also in cosmetics and pharmacology[5]. Salem[6] reviewed nigella's antioxidant, antihistaminic, antiinflammatory, antimicrobial and antitumor properties.

Extracts of many herbs and spices have strong antioxidant activity in oils due to their phenolic compounds. The antioxidant activity of these extracts depends on the isolation procedures, polarity of solvent, type of active components of extracts and purity[1].

The aim of this work is to evaluate effect of alcohol extracts from nigella seed on the oxidation of refined sunflower oil. For this purpose, the procedures of Schaal Oven test, active oxygen method (AOM) and UV absorption characteristic (K_{232}) were used.

EXPERIMENTAL

Dried plant seeds were purchased from spice-sellers in Mugla and Ankara in Turkey. The seeds were stored at room temperature in dark until analysis. Refined sunflower oil was supplied from a local market in Ankara. Ethyl alcohol (96 %), chloroform, acetic acid were of pro analysis purity and purchased from Dizdarer Chemical Lim (Ankara, Turkey). Potassium iodide, starch, sodium thiosulphate, hexane were obtained from Merck (Darmstadt, Germany).

Preparation of nigella extract: The seeds (20 g) were ground and added with 180 mL of ethyl alcohol (96 %). The mixture was kept for 24 h at room temperature without shaking. The solvent was then evaporated at 35 ºC under vacuum in rotary evaporator (Buchi R110, Switzerland) and the extracts were stored at -18 ºC until further use.

Evaluation of antioxidative activity: Sunflower oil was used as a lipid substrate to evaluate antioxidant activity of nigella seed extract because of its prone to oxidation and widespread consumption in Turkey. Nigella seed extracts were added directly to sunflower oil as 500, 1000 and 2000 ppm and then mixed with vortex to get uniform distribution of extract in oil. Blank samples were prepared under the same conditions without any additives.

Accelerated oxidation test was performed using Rancimat method (Metrohm model 743, Herisan, Switzerland) at 110 ºC with the airflow rate of 20 L/h according to AOCS Official Method Cd 12-57[7]. The oxidative stability was expressed as induction period (h).

Oxidation of sunflower oils with and without the extract was carried out under Schaal oven test at 60 ± 1 °C. After addition of the extracts, the bottles were filled half with oil and covered with cellophane paper. Oxidation was accelerated in a forced-draft air oven set at 60 °C for up to 12-16 d. During storage, the samples were withdrawn for analysis and the progress of the oxidative deterioration was followed by peroxide value[8] and UV absorption characteristic (K_{232})[9]. PV is expressed as milliequivalents of active oxygen per kilogram of oil (meq O_2/kg). K_{232} were calculated from absorption at 232 nm, using a UV-Visible spectrophotometer.

Specific extinction at 232 wavelength as follows:

$$K_{232} = A_{232} / c \times l$$

where, K_{232} = specific extinction at wavelength 232; A_{232} = extinction (absorbance) measured at wavelength 232; c = concentration of the solution in g/100 mL; l = light path of the cuvette in cm.

The inhibition percentage of oil was calculated according to formula below[10]:

100 – [(PV increase of sample/PV increase of control) × 100]

where, PV = peroxide value.

Pre-determined dose (1000 ppm) of nigella seed extract by the results of Schaal oven were added to sunflower oil and the samples were stored at ambient temperature and absence of ambient light for 12 weeks and oxidation was followed by peroxide value (PV).

Statistical analysis: Peroxide value and K_{232} extinction coefficient were performed in duplicate with all samples. A correlation procedure (pearson correlation coefficient) was performed to evaluate the relationship between peroxide value and K_{232}. p values of less than 0.01 were regarded as significant.

RESULTS AND DISCUSSION

Table-1 shows induction period of sunflower oils with and without the extract. It was observed that concentration has linear relationship with the antioxidant activity. Stabilization factor (F) is a measure of effectiveness and was calculated according to formula below[11]:

$$F = IP_{inh}/IP_o$$

where, IP_{inh} = Induction period in the presence of an inhibitor, IP_o = Induction period of non-inhibited system.

TABLE-1
INDUCTION TIME (h) OF SUNFLOWER OILS WITH AND WITHOUT NIGELLA SEED EXTRACT

Samples	Induction time (h)*
Sunflower oil (no additive)	5.15
Sunflower oil + 500 ppm alcohol extract of nigella seed	5.69
Sunflower oil + 1000 ppm alcohol extract of nigella seed	5.96
Sunflower oil + 2000 ppm alcohol extract of nigella seed	6.84

*Values represent means (Induction time) (n = 2). In all cases relative error was below 10 %.

Stabilization factors of 500, 1000 and 2000 ppm nigella seed extracts were calculated as 1.10, 1.15, 1.32, respectively. The positive correlation between extract concentration and oxidation stabilization of oil.

Peroxide value (PV) of all samples is presented in Fig. 1. Sunflower oil having 500 and 1000 ppm of nigella seed extracts indicated higher antioxidant activity than the one without added extract. However, sunflower oil with 2000 ppm nigella seed's alcohol extracts has shown higher prooxidant activity when compared to sunflower oil without extract (Fig. 1). After 12 d, peroxide value of the control sample and sunflower oil containing 500 and 1000 ppm nigella seed extracts reached to 305.89, 209.44 and 166.45 meq O_2/kg oil, respectively. Using these results, inhibition rate of 500 and 1000 ppm nigella extracts was calculated as 31.53 and 45.58 %, respectively.

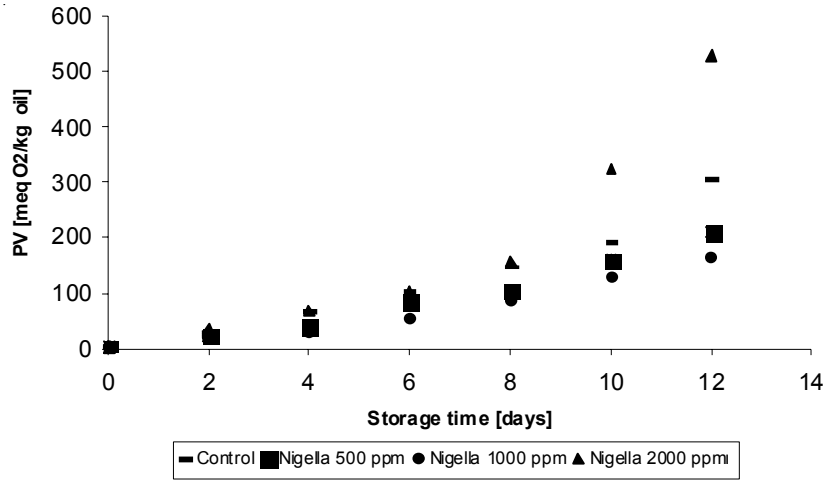

Fig. 1. Effect of nigella extract on the formation of peroxide value (PV) (meq O_2/kg) of sunflower oil stored at 60 ºC

A significant positive correlation between peroxide value and K_{232} was determined at 60 °C ($r = 0.90$) ($p > 0.01$).

Antioxidant activity may be affected by many factors such as concentration, temperature, light, type of substrate, physical state of the system, micro components acting as prooxidants or synergists as well as structural features of antioxidant[12]. The present findings also contributed that concentration is a factor for antioxidant activity. For instance, 500 and 1000 ppm nigella seed extract showed antioxidant activity, hovewer, 2000 ppm showed pro-oxidant activity. Similar behaviour was observed for α-tocopherol showing prooxidative effect at concentration higher than 0.01 % in lard[13]. Furthermore, phenolic compounds can show either antioxidant or prooxidant activity with regard to oxidizing target and conditions used in the test system[14]. Pro-oxidant activity in present study may be originated of phenolic compounds, oxidizing target (sunflower oil) and temperature used in test.

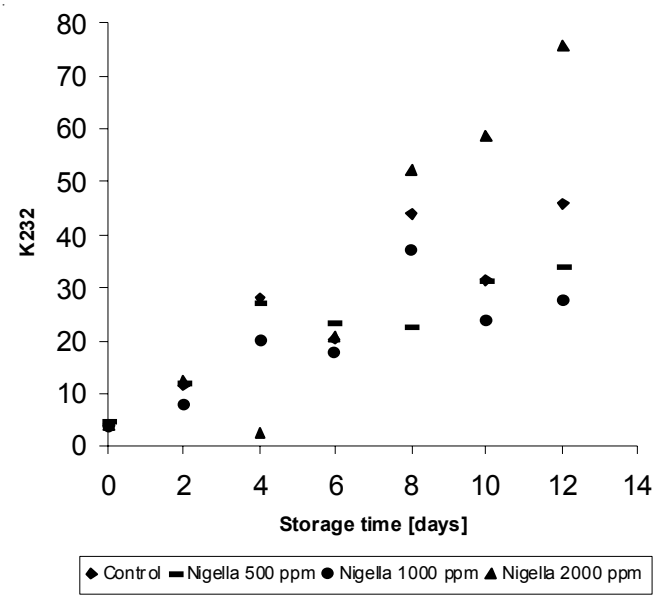

Fig. 2. Effect of nigella seed extract on the formation of conjugated dienes (%) of sunflower oil stored at 60 ºC

As seen in Fig. 3, during 12 weeks, 1000 ppm nigella seed extract showed strong antioxidant activity comparing with control sample. After 12 weeks, peroxide value of control sample and sample with 1000 ppm nigella extract reached to 94.05 and 46.14 meq O_2/kg oil, respectively. Inhibition rate of this extract was calculated as 50.94 %. The results of two oxidation tests (Schaal oven and active oxygen method) were comparable with each other.

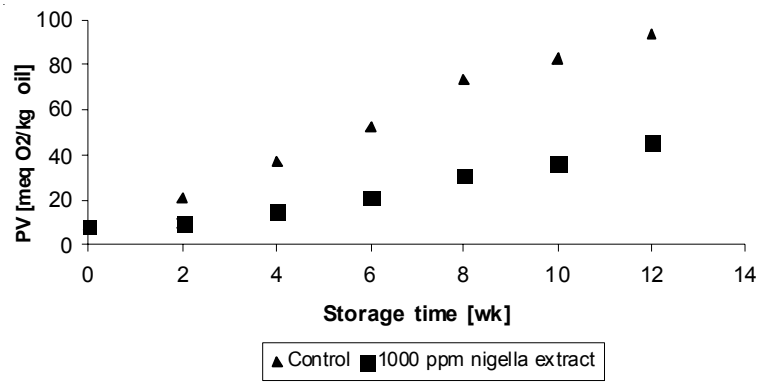

Fig. 3. Effect of nigella seed extract on peroxide value (PV) (meq O_2/kg) of sunflower oil stored at ambient

Active oxygen method, Schaal oven test and storage at ambient temperature indicated that nigella seed extract has strong antioxidant activity. Studies[15-18] generally state that antioxidant activity of nigella originates from essential oils. However a few studies[19,20] reported that nigella extract has shown antioxidant activity.

Conclusion

Nigella seed extract showed antioxidant activity in sunflower oil at 60, 110 ºC and ambient temperature which might be originated of phenolic compounds. These results suggest that the use of nigella seed extract may be possible in industrial scale. In the use of this extract, concentration and temperature are very important because they have great effects on the oxidative stability. Shelf-life of the oils can be prolonged depending on the conditions.

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