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# Evaluation of the Antioxidant Potential of Fennel Seed Extract as Compared to the Synthetic Antioxidants in Margarine under Accelerated Storage Condition

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ABSTRACT: The oxidative stability of margarine supplemented with different concentrations of fennel (*Foeniculum vulgare*) seed extract (FSE) was compared to the synthetic antioxidants; butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) at legal concentrations. The experiment was carried out under accelerated storage conditions for 4 weeks employing peroxide value (PV), p-anisidine value (AV) and TOTOX value at constant time intervals of 7 days. The induction period measuring the stability of the oil was determined by Metrohm Rancimat. The extraction yield from fennel seeds was 12.92% w/w. It was concluded that the antioxidant effeciency of the extract was concentration dependent and by increasing the concentration from 80 to 100 mg $kg^{-1}$, the antioxidant activity of the extract was increased. After 4 weeks of storage at 90°C, margarine containing 100 $mgkg^{-1}$ FSE showed lower peroxide values, p-anisidine values and TOTOX values and higher induction periods as compared to the synthetic antioxidants, BHA, BHT and the control. These results illustrate that FSE might be used as a potential source of natural antioxidant to retard lipid oxidation in margarine.

**Keywords**: *Antioxidants, Fennel Seed Extract, Margarine, Oxidative Stability*.

## Introduction

Oxidative deterioration of fats and oils during processing and storage produces off-flavour, that affect their acceptability and consequently their marketability. Furthermore, compounds such as aldehydes, ketones and organic acids are produced through oxidation process that might have adverse effects namely cardiovascular diseases, mutagenesis and carcinogenesis (Kulisic *et al*., 2004). This process is favored in emulsions system due to the large contact surface between the oxidizable lipid hydroperoxides in emulsion droplets and water-soluble prooxidants resulting in the propagation of oxidation reactions (Waraho *et al*., 2012). In the past synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), have been used extensively to inhibit the oxidation in foods. However, in recent times, epidemiological studies have pointed out the possible health risks associated with the consumption of synthetic antioxidants and strict regulations now limits their use in foods (Laguerre *et al*., 2007). Therefore there is a growing interest to study the natural additives as potential antioxidants (Ito *et al*., 1983; Zheng and Wang, 2001). Many sources of plant origin antioxidants have been studied in recent years, such as aromatic and medicinal plants that might have important roles in lipid oxidation (Kulisic *et al*., 2004). Fennel (*Foeniculum vulgare*) is a plant belonging to the Umbelliferae (*Apiaceae*)

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family and has a long history of herbal applications and is widely cultivated in countries surrounding the Mediterranean sea (Piccaglia and Marotti, 2001). The major constituents of fennel essential oil such as anethole and limonene are used for some medicinal purposes and as essence in cosmetics and perfumes industries (Stuart, 1982; Marotti *et al.*, 1993). The antioxidant activity of water and ethanol extracts of fennel seeds was evaluated by various methods, consisting of total antioxidants, free radical scavenging, superoxide anion radical scavenging, hydrogen peroxide scavenging, metal chelating activities and reducing power. The various antioxidant activities were compared to some antioxidants such as BHA, BHT, and α-tocopherol. The water and ethanol extracts of fennel seeds showed strong antioxidant activity (Oktay *et al.*, 2003). Margarine is a water–in–oil emulsion made basically of vegetable oils and fats that must contain minimum of 80% fat and maximum of 16% water and about 4% additives (Codex Alimentarius Commission, 2001). In such emulsion (w/o type), oxygen diffuses from air directly to the continuous oil phase where the oxidation takes place (Pokorna *et al.*, 2004). The aim of the present work was to evaluate the efficiency of FSE as an alternative natural antioxidant to improve the oxidative stability of margarine and compare it to the synthetic antioxidants, BHA and BHT under accelerated storage conditions.

**Materials and Methods**

- *Materials*

Seeds of *Foeniculum vulgare* were purchased from the local market. Margarine without synthetic antioxidants was graciously provided by the "margarine vegetable oil" company (Tehran, Iran). All reagents and solvents were of analytical grade. BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoluene), were purchased from Sigma-Aldrich Co.

- *Samples preparation*

The fatty substrates (Margarine) were packed in 250g polyethylene cups and stored in the freezer (-18°C) for 48 hours inorder to complete the crystallization. Fennel seeds were dried at 40°C for 24 h in a drying oven (ULM500, Memmert, Germany) to a final moisture content of 7.6 %, then ground using a grinder (A11, IKA, Germany) and passed through the sieve with aperture size of 0.5 mm.

- *Extraction*

The dried powdered samples were subjected to soxhlet extraction using ethanol-water mixture at the ratio of 4:1 v/v as solvent. The extraction yield was 12.92% w/w. The solvents were evaporated under reduced pressure using a rotary evaporator (Rotavapor B-169, Büchi Labortechnik AG, Switzerland) until sticky extracts were obtained. The sticky extracts obtained were vacuum dried (Vacuum oven, VT570, Ehret, Germany) at 40°C for 1 day to remove excessive moisture. The extract was kept at -18°C until required for use.

- *Accelerated storage*

Dried FSE were added to margarine at the concentrations of 80, 90 and 100 $mgkg^{-1}$. Synthetic antioxidants (BHA and BHT) were applied at their legal limits of 75 $mgkg^{-1}$ as reference. All the samples (250 mL) were placed in dark brown colour reagent bottles with narrow necks and mixed for 30 min at 90°C. All the samples (250 mL) were then stored in an oven (ULM500, Memmert, Germany) at a fixed temperature of 90°C to accelerate the deterioration of the oil. Control samples without antioxidants were also placed under the same storage conditions. The required amounts (ml) of the samples were withdrawn at 0, 7, 14, 21 and 28 days after storage in the oven and were analyzed for peroxide value (PV), p-anisidine value (AV) and TOTOX value and

the induction period (IP) of the substrates were measured in order to monitor the oxidation rates.The measurements were carried out in triplicate orders.

Peroxide values (PV) of the samples measuring the primary oxidation products were carried out according to AOAC Official Method 965.33 (AOAC, 1999).

P-Anisidine values (AV) of the samples measuring the secondary oxidation products were carried out according to AOCS Official Method CD 18-90 (AOCS, 1999).

Totox values of the samples defined as 2PV+ AV were calculated according to Shahidi and Wanasundara (2008):

The Induction period (IP) measuring the secondary oxidation products or the susceptibility of margarine samples to oxidation was measured using Metrohm Rancimat apparatus model 743 (Läubli and Bruttel, 1986) by measuring the induction period at 110±1°C with an airflow rate of 18-20 $dm^3h^{-1}$.

- *Statistical analysis*

All experiments were performed in triplicate orders and the results were represented as mean ± standard deviation. Statistical data analysis was conducted using one-way analysis of variance (ANOVA) (Minitab Version 16.0) to determine the significant differences ($P<0.05$). Comparison between standard and extracts means differences were conducted using Duncan's multiple range test at $P<0.05$.

**Results and Discussion**

Peroxide value is a measure of the concentration of peroxides and hydroperoxides formed in the initial stages of lipid oxidation. They are odorless and colorless, but are labile species that can undergo both enzymatic and non-enzymatic degradation to produce a complex array of secondary products. High temperature and light are two well-known factors promoting peroxide formation (Gharby *et al.*, 2011). Peroxide value is one of the most widely used tests for oxidative rancidity in oils and fats. The effect of FSE and synthetic antioxidants on PV of margarine samples over 28 days of storage period under accelerated storage conditions was shown in Table 1. The results showed that by increasing the heating period the peroxide value of all the samples are increased. Margarine samples without the antioxidant (control) exhibited the highest PV throughout the storage period and reached the maximum concentration after 28 days of storage. A significant difference ($P<0.05$) in PV was observed between the control and margarine sample containing FSE and the synthetic antioxidants that slowed down the rate of peroxide formation. The PVs of margarine sample with FSE (80 $mgkg^{-1}$), FSE (90 $mgkg^{-1}$), FSE (100 $mgkg^{-1}$), BHA and BHT were (301.0±0.38) $meqkg^{-1}$, (292.0±0.63) $meqkg^{-1}$, (202.0±0.31) $meqkg^{-1}$, (235.0 ± 0.25) $meqkg^{-1}$, and (254 ± 0.29) $meqkg^{-1}$, respectively. The corresponding inhibition rates were 18.6%, 21.1%, 45.4%, 36.5% and 31.4%, respectively after 28 days under accelerated storage condition as compared with the control sample. Among all samples FSE with 100 $mgkg^{-1}$ concentration showed the lowest PV throughout the storage period followed by BHA at 75 $mgkg^{-1}$ content.

During lipid oxidation, hydroperoxides, the primary reaction products are decomposed to produce secondary oxidation products (aliphatic aldehydes, ketones, alcohols, acids and hydrocarbons) that are more stable during the heating process and are responsible for off-flavors and off-odors of edible oils. In order to ensure a better monitoring of lipid oxidation process under accelerated storage conditions due to the fact that peroxide value might not be reliable all the times particularly at elevated temperature, the simultaneous detection of primary and secondary lipid oxidation products is necessary. AV is a reliable

measurement of the amount of secondary oxidation products (De Abreu *et al.*, 2010; Zhang *et al.* 2010). The results of AV measurements are very similar to results of PV values measurements (Table 2). Generally, AV for all the samples increased significantly throughout the storage time. Addition of BHA, BHT and various levels of FSE resulted in significant decreases in AV ($p<0.05$) relative to the control sample during 28 days of storage.

The use of PV and AV together provides a comprehensive overview of the oxidation process in fats and oils based food products. This is a mathematical prediction of oxidative stability and the value is calculated as Totox value. Totox value was used as an indication of overall oxidative stability and was correlated with the extent of oil deterioration (De Abreu *et al.*, 2010). The effect of FSE and synthetic antioxidants on Totox value of margarine samples over 28 days of storage period under accelerated storage conditions were shown in Figure 1.

Totox value for all samples increased with increasing heating time. Totox values for samples mixed with BHA, BHT and FSE were significantly lower than the value registered for control sample ($p<0.05$). After 28 days of storage period, samples with various doses of FSE resulted in decreases of Totox values in the range 19–46% relative to the control sample. The highest level of FSE had the best inhibitory effect on oil oxidation in the heating time. At any stage of storage period under accelerated storage conditions, the lowest Totox values by supplementation with FSE to a level of 100 $mgkg^{-1}$ were recorded.

The susceptibility of margarine samples to oxidation was measured by Rancimat apparatus and the results were expressed by the induction period. The induction period represents the time needed for decomposition of hydroperoxides produced by oil oxidation (Läubli and Bruttel, 1986). The induction periods for margarine subjected to accelerated oxidation conditions without or with added antioxidants are presented in Figure 2.

Table 1. Peroxide value of margarine with added extracts (T=90°C)

| Sample | Peroxide value ($meqkg^{-1}$) Mean Value ± SD n=3 | | | |
|---|---|---|---|---|
| | 7 (d) | 14 (d) | 21 (d) | 28 (d) |
| Margarine | 17.2 ± 0.72 | 103.0 ± 0.39 | 218.0 ± 0.23 | 370.0 ± 0.53 |
| Margarine + ethanolextract 80 $mgkg^{-1}$ | 17.2 ± 0.18 | 100.0 ± 0.76 | 205.0 ± 0.77 | 301.0 ± 0.38 |
| Margarine + ethanolextract 90 $mgkg^{-1}$ | 16.5 ± 0.32 | 91.0 ± 0.23 | 190.0 ± 0.85 | 292.0 ± 0.63 |
| Margarine + ethanolextract 100 $mgkg^{-1}$ | 15.3 ± 0.09 | 70.0 ± 0.85 | 130.0 ± 0.82 | 202.0 ± 0.31 |
| BHA 75 mg kg-1 | 15.5 ± 0.08 | 73.0 ± 0.53 | 152.0 ± 0.28 | 235.0 ± 0.25 |
| BHT 75 mg kg-1 | 16.3 ± 0.02 | 84.0 ± 0.84 | 177.0 ± 0.25 | 254.0 ± 0.29 |

Table 2. P-anisidine value of margarine with added extracts (T=90°C)

| sample | P-anisidine value Mean Value ± SD n=3 | | | |
|---|---|---|---|---|
| | 7 (d) | 14 (d) | 21 (d) | 28 (d) |
| Margarine | 7.0 ± 0.45 | 16.0 ± 0.84 | 24.0 ± 0.48 | 37.0 ± 0.25 |
| Margarine + ethanolextract 80 $mgkg^{-1}$ | 6.5 ± 0.31 | 15.6 ± 0.58 | 22.0 ± 0.34 | 31.2 ± 0.28 |
| Margarine + ethanolextract 90 $mgkg^{-1}$ | 6.0 ± 0.37 | 15.5 ± 0.16 | 20.5 ± 0.39 | 29.1 ± 0.77 |
| Margarine + ethanolextract 100 $mgkg^{-1}$ | 4.5 ± 0.67 | 10.5 ± 0.30 | 13.0 ± 0.27 | 16.0 ± 0.36 |
| BHA 75 $mgkg^{-1}$ | 5.0 ± 0.29 | 11.0 ± 0.23 | 15.0 ± 0.25 | 20.3 ± 0.27 |
| BHT 75 $mgkg^{-1}$ | 5.4 ± 0.15 | 13.0 ± 0.69 | 18.0 ± 0.16 | 23.1 ± 0.73 |

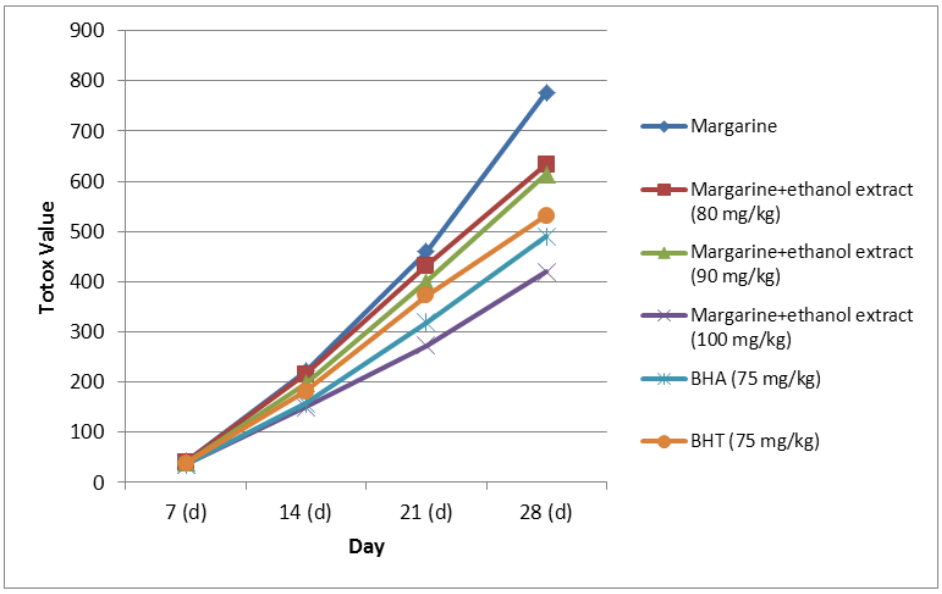


Fig. 1. Totox value of margarine with added extracts (T=90°C)

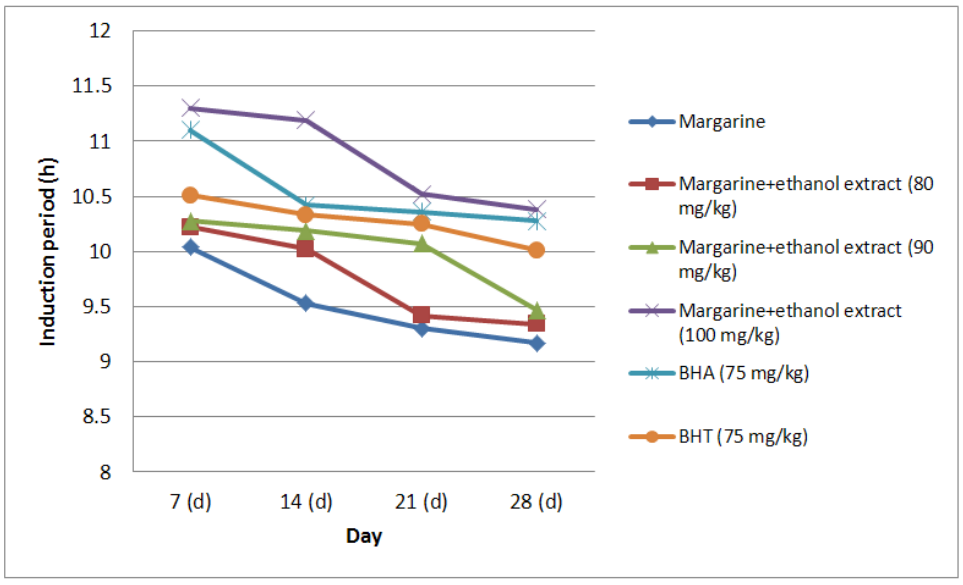


Fig 2. Induction period at 100°C of margarine with added extracts

It is evident that the induction period decreases with the time of storage. The antioxidant potential decreased according to the following sequence; FSE (100 $mgkg^{-1}$) >BHA (75 $mgkg^{-1}$) >BHT (75 $mgkg^{-1}$) >FSE (90 $mgkg^{-1}$) >FSE (80 $mgkg^{-1}$) >control after 28 days. FSE (100 $mgkg^{-1}$) increased the induction period of margarine 13.2% relative to the control sample. FSE at concentration of 100 $mgkg^{-1}$ was considered a good protector against the oxidation with increased induction period of 10.38 h.

## Conclusion

The results of the present study indicated that the efficiency of FSE to enhance the oxidative stability of margarine during storage period under accelerated storage conditions was increased by increasing the antioxidant concentrations in the studied

range (80–100 $mgkg^{-1}$). After 4 weeks of storage at 90°C, margarine containing FSE (100 $mgkg^{-1}$) showed lower peroxide values (202.0 ± 0.31 $meqkg^{-1}$), p-anisidine values (16.0 ± 0.36), Totox values (420.0 ± 0.85) and higher induction periods (10.38 ± 0.12) as compared to the synthetic antioxidants; BHA and BHT and the control. Among all the samples, ones treated with FSE (100 $mgkg^{-1}$) showed the highest antioxidant activity throughout storage period but one has to consider that margarine is made of partially hardened vegetable oils and different fatty acids constitutes the fatty acid composition of the oil and some are more liable than others to oxidation chain reactions. Therefore vegetable oils even hardened and refined contain considerable concentrations of natural antioxidants such as tocopherols that might affect the true role and true activity of FSE in the substrate.

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