Journal of Chemical Health Risks 3(2):53-61, 2013
ISSN:2251-6719

Evaluation of Antioxidant Activity of Fennel (*Foeniculum vulgare*) Seed Extract on Oxidative Stability of Olive Oil

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(Received: 15 April 2013 Accepted: 20 May 2013)

Abstract: Lipid oxidation has adverse effect on food deterioration and human health. The antioxidant activity of fennel seed extracts (FSE) was evaluated by synthetic antioxidant. Its oxidative stability was compared in olive oils in concentrations of BHA (75 ppm), BHT(75 ppm) and 1:1 BHA to BHT ratio. Peroxide (PV), P-anisidine (AV) and TOTOX values were evaluated to assess the extent of oil deterioration. During 28 days of storage, a compromise was accomplished based on the results assessed by PV, TOTOX, at which the antioxidant activity of FSE was higher than BHA (75 ppm), BHT (75 ppm) and BHA to BHT ratio of 1:1 at the concentration of 150 ppm. Among them, concentration of 150 ppm showed the best antioxidant activity. The results of present experiments suggest that FSE has potential source of natural antioxidant for the application in food industry to prevent lipid oxidation.

Keywords: Lipid oxidation, P-anisidine, TOTOX, BHA, BHT, antioxidant, fennel seed extract

INTRODUCTION

Among animal and vegetable fats, olive oils are particularly recommended for human nutrition because of their health benefits. It is considered a functional food for its favorable fatty acid profile and presence of minor components with asserted antioxidant power. Oleic acid constitutes about 70% of its total fatty acids; furthermore there are essential fatty acids and minor components such as α-tocopherol, phenolic compounds and carotenoids which prevent the formation of free radicals.

Lipid oxidation leads to the development of off-flavours and unpleasant tastes which result in shelf life reduction [1]. Lipid oxidation in fats and fatty foods not only deteriorates their quality and brings about chemical spoilage, but also generates free radicals and reactive oxygen species which are implicated in carcinogenesis, mutagenesis, inflammation, aging and cardiovascular diseases [2, 3, 4]. Oxidative reactions limit the shelf-life of fresh and processed foodstuffs which are of great concern in the vegetable oil and fat industry. The strongly oxidized oils could have toxic effects on human health and, therefore, these oils are not suitable for nutritive purposes because of reaction products [5, 6]. With the concern of the adverse effects of lipid oxidation on food deterioration and human health, there is a need to develop the exogenous antioxidants to prevent not only the presumed deleterious effects of free radical in the human body, but also the deterioration of fats and other constituents of food stuffs [5].

Traditionally, chemically synthesized compounds, such as butylated hydroxyanisole (BHA),

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butylated hydroxytoluene (BHT) and ter-butyl hydroquinone (TBHQ), are widely used as antioxidants in oil products [7]. However, these synthetic antioxidants (BHT, BHA and TBHQ) are known to have toxic and carcinogenic effects on human health. For example, BHT causes liver expansion [8]. Therefore, the search for natural antioxidants are gaining importance day-by-day. Natural antioxidants are constituents of many fruits and vegetables, which have attracted a great deal of public and scientific attention due to their anti-carcinogenic potential and other health promoting effects. Recent epidemiological studies have indicated that diets rich in fruits and vegetables and those of selected natural antioxidants such as plant polyphenols, vitamin C and flavonoids are correlated with reduced incidence of cardiovascular and chronic diseases and certain cancers [9, 10, 11, 4, 12]. Nowadays, because consumers are quite cautious about the quality of their diet and its chemical additives, there is an increasing interest on the part of the food industry and preventive medicine to replace synthetic antioxidants with those of safer, more natural origins. This has prompted the investigation and characterization of active natural antioxidant compounds in various plant-derived foods [12]. The fact that various antioxidants occur naturally in plants is well proven [13, 14, 15, 2, 16]. A number of *in vitro* and *in vivo* assays have been developed to measure the antioxidant activity of plant extracts [14, 16]. In this area, the assessment of the effectiveness of potential antioxidants, using vegetable oils and fats as oxidation substrates, has been the focus of intense research.

Economou and his colleagues (1991) reported the antioxidant activity of some plant extracts of the *Labiatae* family, stored at 75 ^{o}C[17]. Metha and his colleagues (1994) studied the antioxidant activity of the extract of ajowan seeds in an accelerated storage test using soybean oil [18]. Bandoniene and his colleagues (2000) evaluated the antioxidant activity of some selected plant extracts in rapeseed oil [13]. The antioxidant effects of thyme in rapeseed oil, at 40 ^{o}C, have also been reported by Wyen and his colleagues (2000) [19]. Abdalla and Roozen (2001) investigated the antioxidant activity of sage and oregano in salad dressing [20]. Jeong and his colleagues (2004) studied the effects of heat treatment on the antioxidant activity of extracts from citrus peel [21]. The antioxidant effects of some natural compounds have also been reported under microwave heating of vegetable oils [22]. A group of researchers at Iowa State University has examined the antioxidant capacity of oat grout and hull extracts to inhibit the oxidation of vegetable oils at elevated temperatures [15].

Fennel (*Foeniculum vulgare*) is a member of *Apiaceae* family and commonly known as "Fennel" is a small genus of annual, biennial or perennial herbs. It is cultivated mainly in central Europe and Mediterranean region due to its unique aroma [23].

The objectives of this study were to determine antioxidant activity of ethanolic extract of Fennel (*Foeniculum vulgare*) by comparing its oxidative stability in olive oils with that of synthetic antioxidant, BHA (75 ppm), BHT (75 ppm) and 1:1 BHA to BHT ratio.

MATERIALS AND METHODS

Synthetic antioxidant 2, 6-di-tert-butyl-4-methylphenol (BHT) butylated hydroxyanisole (BHA), were purchased from Sigma Chemical Co. Ethanol, chloroform, acetic acid, potassium iodide, sodium carbonate, sodium thiosulfate, potassium iodide, starch reagent and isooctane. All others un-labeled chemicals and reagent were used analytical grade.

Preparation of ethanolic extract of Foeniculum vulgare

For ethanolic extraction, 100 g of Fennel (*Foeniculum vulgare*) powder was extracted by stirring with 400 ml of 96 % ethanol at 30-40 ◦C for 5 h in soxhelt apparatus. Subsequently, the extracts were filtered through a Whatman No. 1 filter paper. The combined filtrate was concentrated in a rotary evaporator at 30-40 ◦C and 50 rpm until the 5% of first volume.

Introduction of extracts into the oil

Calculated amounts of the extracts (varied from 0 to 500 ppm) were mixed with 4 ml of absolute ethanol and added to the 250 g of olive oil. According to our experiments, it was the optimal and the lowest amount of alcohol needed for the uniform distribution of the extracts in oil. The additive was mixed into the oil with a magnetic stirrer for 10 min at ambient temperature. Synthetic antioxidant BHT, BHA and 1:1 proportion of them (BHA+BHT) were used as reference substances for comparative purposes. Synthetic antioxidants were prepared in the same way as all other plant extracts used in the present study. Ethanol was removed from the oil in a vacuum oven during 12 h at 35◦C. All oil samples were prepared in duplicate. The oils were then stored in an oven at 90 °C for 28 days to accelerate the deterioration of the oil. Approximately 30 mL of the oils were withdrawn and pipetted into a 50 mL Scott bottle at day 7, 14, 21 and 28 after storage in oven for further analyses.

ANALYTICAL METHODS

Peroxide values (PV)

Peroxide values (PV) of oil samples were according to AOCS (AOCS, 1984).

p-Anisidine values (AV)

P-Anisidine value assay was carried out according to the procedure IUPAC 2.504 *(*IUPAC, 1987).

Total oxidation (TOTOX) values

Total oxidation (TOTOX) values of oil samples were determined using the following equation according to Shahidi and Bhanger [34]:

Total oxidation (TOTOX) value = 2 × PV + AV

Statistical analysis

The data were analyzed using one-way analysis of variance at $p < 0.01$ significance with SAS software (ver. 9.1, 2002-2003 by SAS Institute Inc., Cary, NC, USA.). Duncan's multiple range tests were conducted to determine the statistical differences among different means.

RESULTS AND DISCUSSION

Peroxide values (PV)

The effects of Synthetic antioxidant BHT, BHA and 1:1 proportion of them (BHA+BHT) and different concentration of Fennel seeds extract added into the olive oils on Peroxide values , shown in Table 1.

All extracts (from 50 to 500 ppm) showed more antioxidant activity in comparison with control sample after 7, 14, 21 and 28 days ($P<0.001$). The peroxide values of the oils containing Fennel ethanolic (150 ppm) in the whole of storage time was less than synthetic antioxidant ($P<0.001$).

There were regular increases of PVs for all the samples over the storage period. The total increase in PV was followed in descending order by 0 ppm (control) > 500 ppm > 450 > 400 > 350> 300> 50> 100> 250> 200> BHT> BHA> BHT+ BHA> 150 which achieved a maximum PV of 78.38±0.06, 66.88±0.10, 64.77±0.09, 62.48±0.08, 61.86±0.08, 60.44±0.07, 60.11±0.09, 57.46±0.10, 57.22±0.09, 56.79±0.09, 55.46±0.07, 54.24±0.06, 53.63±0.09 and 51.11±0.09 meq O_2/ kg of oil, respectively in 28th day. The results showed that the PV of concentration of 150 ppm was lower than synthetic antioxidant BHT, BHA and 1:1 proportion of them (BHA+BHT), revealed the antioxidant efficacy in stabilizing the oils by delaying the hydroperoxides formation. As can be

seen in Table 1 and Figure 1, the difference between peroxide values of the oils containing Fennel ethanolic extract and synthetic antioxidants during the storage time was significant (P>0.01) and increased regularly over the time.

Table 1: Changes in peroxide values (PV) of treated olive oil samples under accelerated storage which stored in the dark condition at 90ºC.

	Storage days[1]			
	7	**14**	**21**	**28**
0	18.14 ± 0.81^{Ad}	23.65 ± 0.7^{Ac}	33.55 ± 0.7^{Ab}	78.38 ± 0.6^{Aa}
50	13.38 ± 0.13^{Fd}	19.68 ± 0.7^{Cc}	28.66 ± 0.11^{Eb}	60.11 ± 0.9^{Ga}
100	12.36 ± 0.73^{Gd}	16.76 ± 0.7^{Ec}	27.86 ± 0.8^{Fb}	57.46 ± 1^{Ha}
150	7.13 ± 0.71^{Ld}	8.11 ± 0.8^{Mc}	10.23 ± 0.7^{Nb}	51.11 ± 0.9^{Na}
200	9.24 ± 0.72^{Jd}	9.8 ± 0.6^{Kc}	24.26 ± 1.1^{Jb}	56.79 ± 0.9^{Ja}
250	9.91 ± 0.87^{Id}	12.57 ± 0.8^{Ic}	25.61 ± 1.1^{Ib}	57.22 ± 0.9^{Ia}
300	13.31 ± 0.12^{Fd}	14.75 ± 1.1^{Gc}	26.45 ± 0.9^{Hb}	60.44 ± 0.7^{Fa}
350	14.67 ± 0.73^{Ed}	15.73 ± 0.9^{Fc}	27.14 ± 0.6^{Gb}	61.86 ± 0.8^{Ea}
400	14.9 ± 0.85^{Dd}	16.76 ± 0.7^{Ec}	28.87 ± 0.7^{Db}	62.48 ± 0.8^{Da}
450	16.83 ± 0.79^{Cd}	18.59 ± 0.8^{Dc}	29.1 ± 0.4^{Cb}	64.77 ± 0.9^{Ca}
500	17.53 ± 0.63^{Bd}	20.45 ± 0.6^{Bc}	31.78 ± 0.8^{Bb}	66.88 ± 1.1^{Ba}
BHA	8.59 ± 0.11^{Kd}	10.71 ± 1.1^{Jc}	19.63 ± 0.5^{Lb}	54.24 ± 0.6^{La}
BHT	11.65 ± 0.11^{Hd}	12.82 ± 0.8^{Hc}	20.49 ± 0.8^{Kb}	55.46 ± 0.7^{Ka}
BHA+BHT	9.17 ± 0.13^{Jd}	9.42 ± 0.9^{Lc}	18.23 ± 0.5^{Mb}	53.63 ± 0.9^{Ma}

[1] Data represent the mean value of two biological replicate and three statistical replicate ± standard deviation. Different lowercase letters within each row and capital letters within each column are significantly different ($p < 0.01$), Duncan's test.

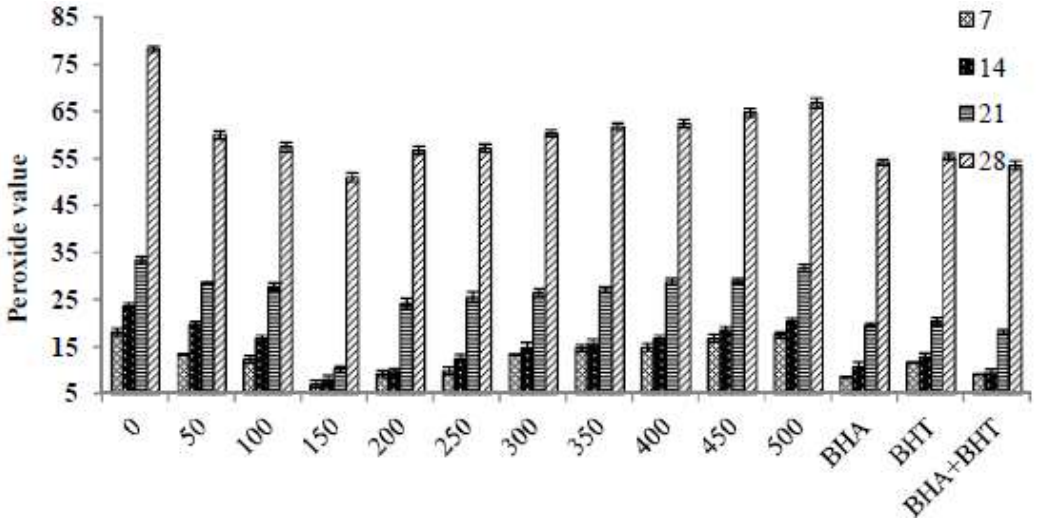

Figure 1: Changes in peroxide values (PV) of treated olive oil samples at different storage time (7, 14, 21 and 28 days of storage)

P-Anisidine values (AV)

Generally, there were increases of AV for all the samples with irregular patterns (Table 2). Under accelerated storage for 28 days, the total increased of AVs were in the following sequence: 0 ppm (control) > 500 ppm > 450 > 400 > 350> 300>50> 250> 200> 100> BHT> BHA> BHT+ BHA> 150 with maximum values of 24.85±0.08, 22.16±0.07, 21.48±0.08, 20.88±0.10, 18.87±0.09, 17.25±0.08, 16.89±0.11, 16.8±0.10, 15.86±0.07, 15.28±0.06, 13.75±0.09, 12.14±0.08, 11.44±0.09 and 10.11±0.08 respectively in 28th day. The higher increased of AV in lower and higher concentration of extracts compared to BHA, BHT and 1:1 proportion of them might suggest the reduced capability of extracts in chelating metal ions. Besides, this could be explained that BHA, BHT and 1:1 proportion of them are more effective in retarding the formation of secondary oxidation products [24], compared to primary

oxidation products. The AV contents of olive oil supplemented with 150 ppm of ethanolic extracts of Fennel were far less than those of in the olive oil supplemented with lower and higher concentration of Fennel ethanolic extracts and synthetic antioxidants [25]. Therefore, the 150 ppm of Fennel ethanolic extracts can be recommended as a potent source of antioxidants for the stabilization of food systems, especially unsaturated vegetable oils. The AV of all the samples increased in a regular pattern over the storage time (Fig 2).

Table 2: Changes in P-anisidin value (AV) of treated olive oil samples under accelerated storage

	Storage days[1]			
	7	**14**	**21**	**28**
0	8.74 ± 0.1^{Ad}	13.29 ± 0.1^{Ac}	18.62 ± 0.8^{Ab}	24.85 ± 0.8^{Aa}
50	6.57 ± 0.5^{Ed}	11.78 ± 0.1^{Dc}	14.83 ± 0.4^{Fb}	16.89 ± 0.1^{Ga}
100	6.29 ± 0.7^{Fd}	9.82 ± 0.8^{Hc}	12.83 ± 0.8^{Jb}	15.28 ± 0.6^{Ia}
150	5.23 ± 0.9^{Id}	7.87 ± 0.7^{Mc}	8.54 ± 0.1^{Nb}	10.11 ± 0.8^{Ma}
200	5.73 ± 0.7^{Hd}	8.26 ± 0.9^{Kc}	13.35 ± 0.1^{Ib}	15.86 ± 0.7^{Ha}
250	6.10 ± 0.9^{Gd}	9.33 ± 0.7^{Ic}	13.76 ± 0.7^{Hb}	16.8 ± 0.1^{Ga}
300	6.28 ± 0.1^{Fd}	10.62 ± 0.6^{Gc}	14.34 ± 0.6^{Gb}	17.25 ± 0.8^{Fa}
350	6.65 ± 0.7^{Ed}	10.82 ± 0.7^{Fc}	15.36 ± 0.9^{Eb}	18.87 ± 0.9^{Ea}
400	6.88 ± 0.7^{Dd}	11.56 ± 0.7^{Ec}	16.13 ± 0.6^{Db}	20.88 ± 0.1^{Da}
450	7.12 ± 0.7^{Cd}	12.55 ± 0.9^{Cc}	16.85 ± 0.1^{Cb}	21.48 ± 0.8^{Ca}
500	7.54 ± 0.8^{Bd}	12.9 ± 0.4^{Bc}	17.74 ± 0.6^{Bb}	22.16 ± 0.7^{Ba}
BHA	5.79 ± 0.1^{Hd}	8.45 ± 0.8^{Jc}	11.09 ± 0.6^{Lb}	12.14 ± 0.8^{Ka}
BHT	6.14 ± 0.9^{Gd}	11.76 ± 0.7^{Dc}	12.4 ± 0.18^{Kb}	13.75 ± 0.9^{Ja}
BHA+BHT	5.68 ± 0.9^{Hd}	8.1 ± 0.8^{Lc}	10.19 ± 0.5^{Mb}	11.44 ± 0.9^{La}

[1] Data represent the mean value of two biological replicate and three statistical replicate ± standard deviation. Different lowercase letters within each row and capital letters within each column are significantly different ($p < 0.01$), Duncan's test.

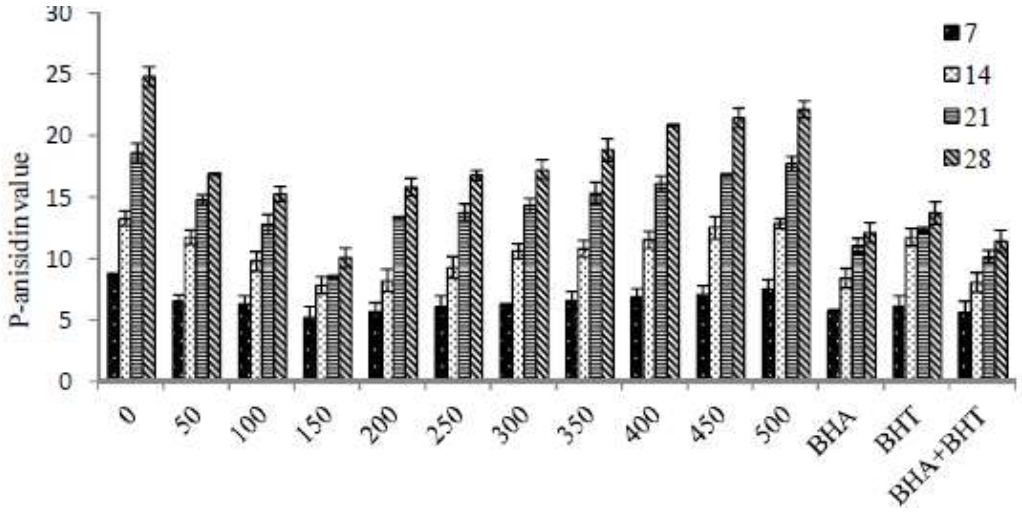

Figure 2**:** Changes in P-anisidin value (AV) of treated olive oil samples at different storage time (7, 14, 21 and 28 days of storage)

Total oxidation (TOTOX) values

As can be seen in Table 3 and Figure 3, the TOTOX values of all the samples increased in a regular pattern over the storage time. Changes in TOTOX values among the samples were in the following orders: 0 ppm (control) > 500 ppm > 450 > 400 > 350> 300 > 50 > 250 > 100 > 200 > BHT > BHA > BHT+ BHA > 150 with maximum values of 181.61±0.11, 155.99±0.13, 151.01±0.21, 145.83±0.25, 142.6±0.19, 138.13±0.14, 137.12±0.13, 131.25±0.21, 130.21±0.24, 129.44±0.19, 124.66±0.22, 120.62±0.14, 118.69±0.10 and 112.32±0.20 respectively in 28th day. Table 1 demonstrated the higher antioxidant activity of 150 ppm of Fennel ethanolic extract rather than synthetic antioxidant. According to obtained results it can be concluded that optimum concentration of Fennel ethanolic extracts have

better preventing effects on retarding oil degradation in compared with synthetic antioxidants.

Table 3**:** Changes in Total oxidation (TOTOX) values of treated olive oil samples under accelerated storage

	Storage days[1]			
	7	**14**	**21**	**28**
0	45.01 ± 0.54^{Ad}	60.6 ± 0.10^{Ac}	85.72 ± 0.6^{Ab}	181.61 ± 1.1^{Aa}
50	33.32 ± 0.23^{Fd}	51.14 ± 1.1^{Cc}	72.21 ± 2.1^{Eb}	137.12 ± 1.3^{Ga}
100	31.01 ± 0.76^{Hd}	43.35 ± 1.2^{Fc}	68.54 ± 1.3^{Gb}	130.21 ± 2.4^{Ia}
150	19.49 ± 0.10^{Md}	24.09 ± 1.7^{Nc}	40.99 ± 2.3^{Nb}	112.32 ± 0.20^{Na}
200	24.2 ± 0.21^{Kd}	28.05 ± 2^{Lc}	61.87±2.7Jb	129.44 ± 1.9^{Ja}
250	25.91 ± 0.81^{Jd}	34.47 ± 2.2^{Jc}	64.98±2.7Ib	131.25 ± 2.1^{Ha}
300	32.89 ± 0.21^{Gd}	40.12 ± 1.5^{Hc}	67.24±1.7Hb	138.13 ± 1.4^{Fa}
350	35.98 ± 0.13^{Ed}	42.29 ± 1.7^{Gc}	69.65±1.9Fb	142.6 ± 1.9^{Ea}
400	36.67 ± 0.24^{Dd}	45.08 ± 1.9^{Ec}	73.87±0.8Db	145.83 ± 2.5^{Da}
450	40.77 ± 0.23^{Cd}	49.74 ± 0.20^{Dc}	75.04±0.15Cb	151.01 ± 0.21^{Ca}
500	42.61 ± 0.20^{Bd}	53.81 ± 1.3^{Bc}	81.31±1.1Bb	155.99 ± 1.3^{Ba}
BHA	22.98 ± 0.13^{Ld}	29.87 ± 1.7^{Kc}	50.34±0.6Lb	120.62 ± 1.4^{La}
BHT	29.45 ± 0.24^{Id}	37.41 ± 2.3^{Ic}	53.38±1.8Kb	124.66 ± 2.2^{Ka}
BHA+BHT	24.02 ± 0.20^{Kd}	26.94 ± 2.3^{Mc}	46.65±1.7Mb	118.69 ± 1.1^{Ma}

[1] Data represent the mean value of two biological replicate and three statistical replicate ± standard deviation. Different lowercase letters within each row and capital letters within each column are significantly different ($p < 0.01$), Duncan's test.

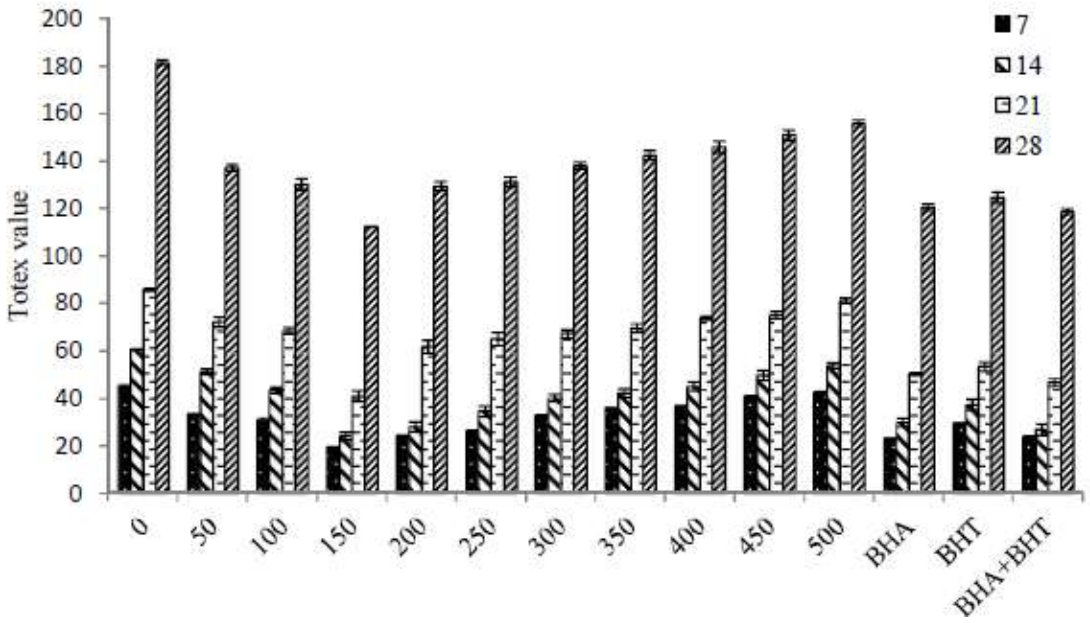

Figure 3**:** Changes in Total oxidation (TOTOX) values of treated olive oil samples at different storage time (7, 14, 21 and 28 days of storage).

The antioxidant activities of extracts are well known and can exert their antioxidant activity by various mechanisms, for example, by scavenging radicals, which initiate lipid per oxidation and lipid peroxide radicals, by binding metal ions, and by inhibiting enzymatic systems responsible for free radical generation. The antioxidant properties of many herbs and spices are reported to be effective in retarding the development of rancidity in oils and fatty foods. It is known that a number of natural extracts from herbs, spices and some vegetables are stable to autoxidation due to the presence of some natural components e.g. phenollic compounds. The antioxidant activity of extracts depends on the type and polarity of extraction solvent, the isolation procedures, purity

and identity of antioxidant active components from the raw materials. The use of synergistic mixtures of antioxidants allows a reduction in the concentration of each substrate and also increases the antioxidative effectiveness as compared with the activity of each separate compound [26]. It is possible that natural antioxidants exhibit complex interfacial affinities between air-oil interfaces that significantly affect their relative activities in different lipid systems [27].

CONCLUSION

Plants and their products have been used for many years for human health. There are still many plants which have various medicinal values but still not explored and used. Plants contain many novel compounds with medicinal values which need scientific exploration. The free radicals are produced in aerobic cells due to consumption of oxygen in cell growth [30, 31]. Free radicals cause decrease in membrane fluidity, loss of enzyme receptor activity and damage to membrane protein leading to death [15]. These free radicals are involved in different disorders like ageing, cancer, cardiovascular disease, diabetes, rheumatoid arthritis, epilepsy and degradation of essential fatty acids [30, 32, 31]. Antioxidant helps in treatment of above disorders. As ethanol extract of this plant showed the dose dependent antioxidant activity comparable to ascorbic acid, antioxidant agent might be developed from this plant for the treatment of above disorders associated with free radicals. Phenolic compounds containing free hydrogen are largely responsible for antioxidant activity [27, 28] thus the phenolic compounds of *F. vulgare* can be referred to be responsible for the antioxidant activity.

Our findings suggest that there are still many plants in Iran which are not traditionally used but possess medicinal values. So, scientific studies also need to be focused on plants which are not traditionally used. As *F. vulgare* showed the antioxidant and antibacterial activities, a detailed biological & phytochemical study is needed to find out the chemical constituent responsible for their activities.

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