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ORIGINAL ARTICLE

A comparative study on the in vitro antioxidant activity of tocopherol and extracts from rosemary and *Ferulago angulata* on oil oxidation during deep frying of potato slices

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Abstract There is a tendency towards the use of natural antioxidative substances due to toxic effects of synthetic antioxidants. The purpose of this research was to evaluate the effects of tocopherol and extracts from rosemary and ferulago on oxidation in a mixture (1:1, *w/w*) of sunflower seed oil and palm olein, during deep frying of potato slices. Besides the control groups, tertiary butylhydroquinone (TBHQ) served as a standard for comparison. The DPPH radical scavenging activities of extracts followed the order of rosemary10% > rosemary1% > rosemary0.1 % > ferulago 10 % > ferulago 1 % > ferulago 0.1 %. Frying performance of antioxidants were tested with regards to primary (peroxide value (PV)) and secondary (anisidine value (AnV), free fatty acids (FFA), total polar compounds (TPC) and volatile oxidation compounds such as hexanal and heptanal) oxidation products and by sensory evaluation. After frying process, rosemary extract treatment exhibited PV (1.2 meq O_2/kg), FFA content (0.124 %), TPC (12.2 %), hexanal concentration (62.4 ng/g) and heptanal concentration (73.8 ng/g), which were significantly equal or sometimes less than those of TBHQ treatment ((1.23 meq O_2/kg), (0.123 %), (12.6 %), (64.4 ng/g) and (74.1 ng/g), respectively). Whilst the ferulago extract showed higher PV (2 meq O_2/kg) than the control sample, it was efficient in delaying secondary oxidative changes. Tocopherol markedly retarded primary stage but its activity fell down toward secondary oxidation products. The overall results of this research suggest that rosemary extract may be a good natural alternative to TBHQ, but further studies are necessary to investigate the use of ferulago extract.

Keywords Deep fat frying · Natural antioxidant · Ferulago extract · Rosemary extract · Tocopherol

Research highlights
- Rosemary extract possesses higher DPPH radical-scavenging ability.
- Rosemary extract inhibits both primary and secondary oxidation changes, as same as TBHQ.
- Tocopherol is more effective in controlling the primary stage of oxidation.
- Ferulago extract shows strong protective effect toward the secondary oxidation products.
- Rosemary extract can be recommended as a good alternative to TBHQ.

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Introduction

Frying is one of the most common cooking techniques used in domestic and industrial food preparation. Deep fat frying can be defined as a process of immersing food in hot oil with a contact among oil, air, and food at a high temperature of 150 to 190 °C. During frying, oils and fats are exposed to high temperature, atmospheric oxygen and water content of food stuff. This results in a series of reactions namely hydrolysis, oxidation and polymerization(Choe and Min 2007; Katragadda et al. 2010). These reactions produce rancid odors, undesirable flavors and discoloration that considerably influence the functional, sensory and nutritional quality of oils. The rate at which these degradation reactions occur, is affected by several factors such as time, temperature, food and oil composition, food to oil ratio, kind of fryer and antioxidants (Choe and Min 2007). Antioxidant is a molecule that inhibits the oxidation of other molecules. Antioxidants can be used to avoid or delay frying oil deterioration. These compounds act by inhibiting formation of free radicals or by interrupting propagation of

the free radicals by one (or more) of several mechanisms (Choe and Min 2009). The use of synthetic antioxidants such as butylated hydroxytoluene (BHT), Butylated hydroxyanisole (BHA), TBHQ in food processing has raised questions regarding toxicity health risks(Samotyja and Małecka 2010; Zhang et al. 2010; Chen et al. 2014). Apart from detrimental effects on human health, these antioxidants provide poor protection, especially under frying conditions. This low efficiency or availability is attributed to their loss because of thermal degradation, steam distillation, evaporation and adsorption by the fried food (Choe and Min 2007). Consequently, to overcome these shortcomings, several studies have been conducted, in order to find natural antioxidants and the food industry has been motivated to use natural alternatives. A number of spices and herbs containing antioxidant compounds have been concentrated as extracts, essential oils, or resins. Among them, rosemary (*Rosmarinus officinalis L.*) extract has gained considerable attention as a spice with one of the most powerful antioxidant potential (Zhang et al. 2010; Chen et al. 2014). The antioxidant properties of rosemary extract has contributed to the presence of phenolic diterpene compounds, such as carnosic acid, carnosol and rosmanol, rosmariquinone and rosmaridiphenol, which break free radical chain reactions by hydrogen atom donation and chelating metal ions (Erkan et al. 2008). *Ferulago angulate* from Apiaceae family is another plant that received considerable attention, possessing potential antioxidant and antimicrobial properties. The *F. angulata* known in Iran as Chavir is an endemic plant used to extend shelf life in western parts of the country. This plant is added to dairy and oil ghee as a flavoring agent and strong preservative (Khanahmadi and Janfeshan 2006). *Ferulago* has also been used traditionally, for the treatment of digestive pains, aphrodisiac properties, hemorrhoids and headache. Some studies have already been performed on the investigation of chemical composition and biological activity of essential oil and extract of some species from the genus ferulago (Khanahmadi and Janfeshan 2006; Azarbani et al. 2014). Antibacterial and antifungal activities of *F. angulata* have been examined and inhibitory effects for microorganism have been observed (Taran et al., 2010). Based on literature survey, the essential oil of this plant has low antioxidant activity and can be used as a flavoring agent, while extract obtained from it possess high antioxidant potential(Khanahmadi and Janfeshan 2006). It seems that the considerable antioxidant activity of *F. angulata* extract is due to the presence of phenolic and polyphenolic compounds (Azarbani et al. 2014).

Tocopherol is the other natural component used in this study. Tocopherols are monophenolic compounds and derivatives of the 6-chromanol ring having a saturated side chain. These compounds are mainly present in oil seeds, oils, meats and green parts of higher plants (Seppanen et al. 2010). The antioxidant capacity of these fat-soluble carotenoids has been studied extensively. Tocopherols exhibit antioxidant activity by donating phenolic hydrogen to lipid free radicals, thereby retarding autocatalytic lipid peroxidation processes (Choe and Min 2009). Tocopherols are thermo stable, especially when a mixture of the four tocopherols is added (Barrera-Arellano et al. 2002).

Considering the complex changes that occur to oil during frying, the purpose of this research is two-fold: firstly, to evaluate oil oxidation by more than one assay because different methods measure different characteristics of the oxidation. The second objective is to investigate the antioxidant effects of rosemary extract, ferulago extract and tocopherol, and compare their antioxidant activity with that of TBHQ as a very strong synthetic antioxidant used by industry.

Materials and methods

Materials

Refined, bleached, and deodorised (RBD) frying oil with no added antioxidant was purchased from Behshahr company (of Iran) and was kept at −20 °C until use. Rosemary extract containing 24–26 % total antioxidative phenolic diterpenes with >16 % of carnosic acid, essential oil 1–4 %, water <1 %, alcohol <2 %, sunflower oil (organic), cuticular waxes, was obtained from Flavex GmbH. Mixed tocopherol concentrate was supplied by Vitae Natural. All chemicals and solvents applied in this study were of analytical reagent grade and obtained from Merck and Sigma chemical companies.

Preparation of ferulago extract

Fresh ferulago was collected from the western parts of Iran (Kermanshah). The best branches were chosen and exposed to air in a well shady ventilated area at room temperature, until the moisture evaporates (10 days). Then, dried branches were crushed into powder. Maceration method was applied in order to obtain plant extract. Extraction was carried out with 100 g plant powder dissolved in a mixture of ethanol and water (80:20v:v). The mixture was shaken for 24 h at room temperature and then filtered with a filter paper (chm, F2040; 125 mm in diameter; pore size, 7-9 μm). The extraction was repeated three times this same way and supernatants were added to the previous extract. Finally, ethanol was removed under reduced pressure in a vacuum rotary-evaporator at 40 °C and water was eliminated in a freeze-drier (model FDB5503) at −70 °C. The obtained powder extract was kept at −20 °C until use.

DPPH radical-scavenging assay

The ability of extracts to scavenge free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH•) was determined as procedure

described by Brand-Williams et al. (1995). For this mean extract solutions with concentration of 10, 1, 0.1 % (*w*/*v*) from each extract were prepared using methanol. Then 0.1 ml of each of these solutions was added to 3.9 ml of a DPPH methanolic solution. The mixtures were vortexed for 15 s and incubated at 25 °C for 60 min for the purpose of completing reaction. After this period the absorbances of all solutions and control sample were measured at 517 nm using a UV/VIS spectrophotometer (CE 7200). The free radical-scavenging activity of each solution was calculated applying the following formula:

$$\% \text{ RSA} = 100 \times [A_c - A_s]/A_c$$

Where A_c is absorbance of the control sample and A_s is the absorbance of the tested extract.

Frying process

A mixture (1:1, *w*/*w*) of sunflower seed oil and palm olein was used in this study. The following antioxidant treatments were prepared:

1- Frying oil with 500 ppm rosemary extract (FRE)
2- Frying oil with 1000 ppm ferulago extract (FCE)
3- Frying oil with 100 ppm TBHQ (FTQ)
4- Frying oil with 600 ppm tocopherol (FTC)
5- Frying oil with no antioxidant (FBL).

Potato slices were cut into pieces (70–80 × 10 × 10 mm). Deep frying was carried out using Delonghi house-hold fryer (model F 22310 CZ). 3 kg of each antioxidant treatment was placed in the fryer and heated to 180 °C. Six batches of potato slices, 300 g per batch, were consecutively introduced into hot oil and fried for 6 min at intervals of 1 h. Thus, the total frying time was 5 h. Samples of frying oils (100 g) and fried potatoes were taken after each frying operation and cooled to room temperature and frozen at −20 °C for further analyses. The fresh oil (zero time) was also stored at −20 °C for subsequent analyses.

Oxidative stability determination

Analysis of peroxide value

The peroxide value (PV) of all samples was measured based on the AOCS official method Ja 8–87(AOCS 2005).

Measurement of p-anisidine value

p-Anisidine value was determined for each of the samples following the AOCS Official Method Cd 18–90(AOCS 2005c).

Measurement of free fatty acids (FFA)

Free fatty acids, as oleic acid percentages in oil samples were measured according to AOCS Official Method Ca 5a-40(AOCS 2005).

Analysis of total polar compounds(TPC)

Total polar compounds of oils were evaluated using AOCS official method cd 20–91(AOCS 2005). Oil samples are separated by column chromatography into non polar and polar compounds, followed by the elution of the non polar compounds. The polar compounds are determined by calculating the difference between the weight of the sample added to the column and that of the non polar fraction eluted.

Headspace single-drop microextraction(HS-SDME)

A rapid headspace single-drop microextraction gas chromatography mass spectrometry (SDME-GC-MS) for the analysis of volatile oxidation compounds including hexanal and heptanal was applied under the optimal condition achieved by Enteshari et al. (2014). They demonstrated that higher yield of extracted analytes could be obtained under the following optimal conditions: extraction temperature of 45 °C, extraction time of 16 min, stirring rate at 700 rpm, and addition of 2 g NaCl.

HS-SDME procedure A 17-mL crimp top glass vials with PTEF-silicon septum containing 1 g of French fries samples, 2 g NaCl, 10 ml of distilled water and a 0.5 cm magnetic stirring bar were introduced into water bath which was maintained at 45 °C and placed on a magnetic hot-plate stirrer. After a period of sample equilibration (10 min), a 10 μl GC micro-syringe containing 3 μl of extraction solvent (n-dodecane) with 10 mg/l of the internal standard (2,5-Dimethylfuran) was pierced into the vial and fastened at the fixed position, while the tip of needle was 1 cm above the sample solution surface. The plunger was pushed down and microdrop was suspended at the tip of the micro-syringe needle and then exposed in the headspace above the sample. After extraction period (16 min), the drop of extraction solvent was drown back into the micro-syringe and consequently injected into GC.

GC-MS analysis All analyses were carried-out on a 7890A GC system purchased from Agilent Technologies (Palo Alto, CA, USA) equipped with a split/splitless injector and a 5975 inert MSD network mass selective detector with quadrupole analyzer. The column used was a HP-5 MS capillary column (30 m long ×250 μm inner diameter, 0.25 μm film thickness). The injection was performed under the split mode (1:50 ratio) and Helium (>99.999 % pure) was applied as the carrier gas at

a flow-rate of 0.8 ml/min. The column oven was initially set at 40 °C for 2 min, programmed to 80 °C at 5 °C/min (1 min hold) and finally to 280 °C at 30 °C/min, where it was held for 5 min. The temperatures of interface and injector were set at 280 °C. A SIM (Selected Ion Monitoring) program was constructed for GC–MS acquisition and quantification. Overall, quantification was based on the following target ions: the selected m/z were 56 and 57 for hexanal and 55 and 70 for heptanal.

Sensory analysis

Sensory properties of fried potatoes were evaluated by a 5-point hedonic scale. A panel of 9 trained and descriptive assessors was chosen from laboratory stuff. Samples were presented in plastic dishes and coded with three-digit number. Each panelist received a sample of each treatment at each frying period. The intensities of sensory attributes "flavor", "odor" and "color" were scored from low(1) to high(5) and at the end the fried potatoes samples were rated for overall acceptability on a 5-point using a numerical scale of 1 to 5 (1 = least acceptable, 5 = extremely good). Panelists were instructed to rinse their mouth with water between samples (Jaswir et al. 2000).

Results and discussion

Scavenging effect on DPPH radicals

The DPPH measures the ability of compounds to donate hydrogen to radicals. DPPH free radicals show maximum absorbance at 517 nm and are purple in color. The colour turns from purple to yellow as DPPH free radicals are reduced by an antioxidant. The resulting decolorization can be monitored spectrophotometrically as a measure of antioxidant activity. A more reduction in solution absorbance indicates a higher antioxidant activity. The DPPH radical-scavenging abilities of rosemary and ferulago extracts are given in Table 1. It can be seen that all tested extracts demonstrated a concentration-dependent scavenging activity. This is in line with the findings of Espín et al. (2000). As shown in Table 1, DPPH radical-scavenging abilities of extracts followed the order of rosemary10% > rosemary1% > rosemary0.1 % > ferulago 10 % > ferulago 1 % > ferulago 0.1 %. The higher antioxidant activity of rosemary extract is associated with the presence of high phenolic diterpenes content such as carnosol, rosmanol, rosmariquinone and principally carnosic acid; which is the most abundant constituent of rosemary extract, having the highest antioxidant power with two phenolic hydroxyl groups (Erkan et al. 2008). As reported by Zhang et al. (2010), DPPH radical-scavenging activities of rosemary extracts with three different content of carnosic acid, 24.9 % (CA25), 60.5 % (CA60) and 98.3 % (CA98), were significantly higher than BHT but less than TBHQ. Apart from the lower phenolic content, the less antioxidant activity of ferulago extract may also be attributed to the presence of impurities such as chlorophyll and solvent contaminant, that affect antioxidant power (Kamal-Eldin and Appelqvist 1996).

Table 1 Mean values for scavenging activity of extracts for DPPH radical; data are shown as % inhibition at different concentrations of extracts

Extract type	Inhibition (%)
Ferulago 10 %	85.00 ± 0.12[a]
Ferulago 1 %	43.33 ± 0.10[b]
Ferulago 0.1 %	29.32 ± 0.13[c]
Rosemary 10 %	99.00 ± 0.09[a]
Rosemary 1 %	98.01 ± 0.11[a]
Rosemary 0.1 %	97.61 ± 0.07[a]

*Means followed by the same lower case letter are not significantly ($P > 0.05$) different

*Data are given as means ± SD, $n = 3$

Effect on peroxide value(PV)

PV is one of the most widely-used methods in monitoring the initial stage of lipid oxidation and reflects the concentration of peroxides and hydroperoxides. As shown in Fig. 1, all the treatments showed three trends of evolution. Therefore, all PVs increased significantly at the end of the first frying period and then began to decrease and again followed the increasing trend. This is in agreement with the findings of Guillén et al. (2005). The reduction trend can be explained by the decomposition of unstable hydroperoxides to secondary oxidation products such as: hydrocarbons, alcohols, ketones and aldehydes. The second increase in PVs is due to acceleration of oxidation throughout the frying time (Guillén et al. 2005). The decreasing stage in PVs of oils containing rosemary extract continued until the fourth frying period and after this period, a clear and pronounced enhancement was produced. At the end

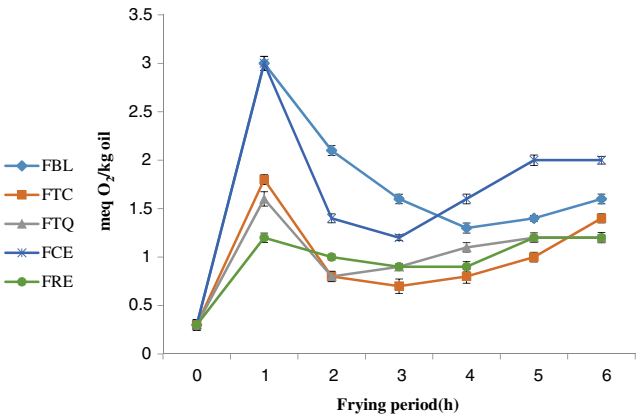

Fig. 1 Changes in PV (meq o2/kg oil) of oil samples during six frying periods

of frying operation, PV of this sample reached (1.2 meq O_2/kg) which was 1.4-fold less than that of control sample and additionally was 1.02-fold, 1.2-fold and 1.7-fold less than that of the sample treated with TBHQ, tocopherol and ferulago extract, respectively. These results showed that rosemary extract retarded the formation of hydroperoxides significantly ($p < 0.05$). Chen et al. (2014) also reported that the addition of 200 ppm rosemary extract to sunflower oil lowered the final peroxide value after 21 days of storage at 60 °C from (272 meq O_2/kg), as by control sample, to (75.7 meq O_2/kg). Tocopherol treatment exhibited a slight trend and lowered the final value from (1.62 meq O_2/kg), as by control sample, to (1.42 meq O_2/kg). This was likely due to the high reaction rate of tocopherol with peroxy radicals compared to the acyl group of lipids (Kamal-Eldin and Appelqvist 1996). Moreover, it was found that the antioxidant mechanism of tocopherol involved quenching singlet oxygen (Eitenmiller and Lee 2005). until the end of the fourth frying period, ferulago extract showed higher efficiency compared with the control sample. However, following this period, the inhibitory effect of the ferulago extract lowered significantly and reached the final PV of (2 meq O_2/kg) which was 1.2-fold higher than that of control sample. These results are not in accordance with the results of Khanahmadi and Janfeshan (2006) who noted that PVs of oil mixed with 0.5 % ferulago extract during incubation period at 63 °C, were markedly lower than those of oil samples treated with TBHQ.

Effect on p-anisidine value (AnV)

Anisidine analysis is based on secondary oxidation products of oil and is a measurement of the aldehyde content in oil, principally 2,4-dienals and 2-alkenals, produced from decomposition of hydroperoxide compounds. As presented in Table 2, AnVs of all analyzed oil increased significantly with increase in frying time. Adding all four antioxidants to oil caused a significant reduction in the AnVs, compared with those of the control sample but with different order. At the end of six frying periods, the AnV of control sample reached a maximum of 47.65 which was 1.33-fold, 1.30-fold, 1.2-fold and 1.14-fold higher than that of sample containing TBHQ, rosemary extract, ferulago extract and tocopherol, respectively. Thus the highest inhibitory effect on the propagation stage of oxidation was observed by the synthetic antioxidant TBHQ. This is in agreement with the results of Zhang et al. (2010), who found that inhibitory effects of three rosemary extracts were higher than BHA and BHT but less than TBHQ. The authors suggested that the superior antioxidant activity of TBHQ was due to its high hydrogen-donating ability, attributed to the two para-hydroxyl groups in its molecular structure. The next lowest amount of AnV belonged to rosemary extract treatment. Tocopherol treatment showed an intense variation trend for AnV and produced the highest final AnV of 41.85, compared to other antioxidants. According to the findings of Cuvelier et al. (2000), tocopherol is a primary antioxidant that directly donates hydrogen to lipid free radicals and prevent a new lipid from entering the oxidized chain. Since it has no chelator site in the structure, it is less effective in preventing the secondary stage of oxidation. The similar results were obtained by Samotyja and Małecka (2010), who reported that the addition of rosemary extracts to soybean oil stored at 60 °C for 312 h, decreased the final AnV from about 14, as by control sample, to about 8. They also mentioned that while the addition of tocopherol delayed the hydroperoxides formation 1.7 times more than control sample, it caused higher AnV and hexanal concentration. After the second frying period, a significant decrease was observed in the AnVs of oil treated with ferulago extract, compared to the control sample and lowered the final value from 47.65 to 39.90 at the end of frying process. These results confirmed the protective effect of ferulago extract on the secondary stage of oxidation while it exhibited a weak inhibitory effect on the primary stage of oxidation. This fact might be contributed to the presence of compounds that donate hydrogen to peroxy radicals, to form stable hydroperoxides. This resulted in slower decomposition rate of hydroperoxide and formation of fewer secondary products (Decker et al. 2005).

Effect on free fatty acids content (FFA)

The FFA content of oil is an important standard method for measuring the suitability of oils for human consumption and frying purposes. The content of FFA increased significantly throughout the frying time, which increased in acceleration after the fourth frying period. Free fatty acids are primarily a result of hydrolysis, although small amounts may be produced by oxidative reactions (Andres et al. 2005). This fact confirmed that adding antioxidants affected the rate of free fatty acids formation. As presented in Table 3, all tested antioxidant caused a significant reduction in FFA content, compared to the control sample. At the end of frying operation, the FFA content of oils treated with TBHQ, rosemary extract, ferulago extract and tocopherol reached maximum values of 0.123, 0.124, 0.141 and 0.141 %, respectively, which were significantly lower than that of control sample (0.164 %). It is evident from the results that among all treatments, the oils treated with rosemary extract and TBHQ showed significantly the slightest increase trend ($p < 0.05$). These results are in agreement with those of Zhang et al. (2010), who reported that after 21 days of storage at 60 °C, the FFA content of the sunflower oil control sample reached a maximum of (0.71 mg/g) which was 1.6-fold, 1.7-fold and 1.9-fold higher than that of the sample treated with CA25, CA60 and CA98, respectively. The development of FFA content in the oils treated with ferulago extract and tocopherol followed an intense trend.

Table 2 Mean values for p-anisidine value (AnV) of oil samples during six frying periods

Frying period (h)							
Treatment	0	1	2	3	4	5	6
FTQ	15.50 ± 0.71^{Aa}	16.80 ± 0.28^{Aa}	23.11 ± 0.15^{Bb}	23.55 ± 0.64^{Ab}	28.32 ± 0.11^{Ac}	29.85 ± 0.21^{Ac}	35.75 ± 0.07^{Ad}
FRE	15.50 ± 0.71^{Aa}	19.00 ± 0.28^{Bb}	22.65 ± 0.21^{Abc}	26.15 ± 0.21^{Bc}	30.50 ± 0.14^{Bd}	32.90 ± 0.14^{Bd}	36.70 ± 0.14^{Be}
FCE	15.50 ± 0.71^{Aa}	22.30 ± 0.14^{Eb}	24.90 ± 0.14^{Cbc}	28.95 ± 1.48^{Cc}	31.05 ± 0.07^{Cc}	33.40 ± 0.14^{Cc}	39.90 ± 0.14^{Cd}
FTC	15.50 ± 0.71^{Aa}	21.45 ± 0.20^{Db}	26.90 ± 0.14^{Dc}	31.10 ± 0.14^{Dcd}	35.20 ± 0.14^{Dd}	36.65 ± 0.07^{Dd}	41.85 ± 0.21^{De}
FBL	15.50 ± 0.71^{Aa}	19.95 ± 0.07^{Cb}	25.2 ± 0.14^{Cc}	32.05 ± 0.07^{Dd}	38.60 ± 0.14^{Ee}	41.55 ± 0.07^{Ee}	47.65 ± 0.21^{Ef}

*Means followed by the same lower case letters are not significantly ($P > 0.05$) different over time (rows). Means followed by the same upper case letter are not significantly ($P > 0.05$) different among treatments (columns)

*Data are given as means ± SD, $n = 3$

Effect on total polar compounds (TPC)

Total polar compounds (TPCs) produced during frying, are the most predominant indicators of oil deterioration. These nonvolatile compounds resulted from thermal, hydrolytic and oxidative alteration. The initial contents of total polar compounds in unused oil normally ranges between 0.4 and 6.4 %. The level of TPC in the fresh oil sample, in the present study, was within the limits published for refined oils. National legislation in several countries sets up a discarding level of total polar content of 24–27 % for frying oils (Farhoosh and Tavassoli-Kafrani 2010). As seen in Table 4, the amount of TPCs in all analyzed oils increased throughout the frying periods which increased in acceleration at the end of the third frying period. Similar findings were reported by Tabee et al. (2009), and Farhoosh and Tavassoli-Kafrani (2010), who reported a positive correlation between the formation of TPCs and frying time. In comparison with the control sample all antioxidant reduced the amount of TPCs during the frying periods significantly but with different levels of efficiency. After six frying periods, TPCs value of the control sample was 17.67 %, whereas values for oil treated with rosemary extract, TBHQ, tocopherol and ferulago extract were 12.22, 12.62, 13.17 and 15.25 %, respectively. The corresponding reduction levels were 30.8, 28.6, 25.5 and 13.7 %, respectively, compared with the control sample. These reduction levels indicated that the lowest amount of TPCs was dedicated to the oil sample treated with rosemary extract. Therefore, rosemary extract was more efficient than TBHQ, in delaying TPCs formation. The finding of this study were in accordance with those from the research conducted by Casarotti and Jorge (2014). They also noted that after 20 h heating of soybean oil at 180 °C, samples treated with 3000 ppm rosemary extract and 50 ppm TBHQ produced a reduction of 35.4 and 15.83 % in the level of TPCs, respectively, compared with control sample. The highest amount of polar compounds, among all tested antioxidant, was exhibited by the ferulago extract. With regarding the results demonstrated the higher antioxidant activity of ferulago extract(Khanahmadi and Janfeshan 2006), its lower effectiveness in present study may be attributed to negative effect of high temperature using in frying on antioxidant stability. At the end of frying process, TPCs values of all treatments were less than discarding level.

Effect on aldehydic compounds

Hydroperoxides formed during oxidative degradation of oils are rapidly transformed into a complex mixture of secondary oxidation products such as: hydrocarbons, alcohols, ketones

Table 3 Mean values for free fatty acid content (FFA) (%) of oil samples during six frying periods

Frying period (h)							
Treatment	0	1	2	3	4	5	6
FTQ	0.077 ± 0.003^{Aa}	0.087 ± 0.003^{Bb}	0.100 ± 0.000^{Cc}	0.112 ± 0.000^{Bd}	0.111 ± 0.001^{Ad}	0.113 ± 0.001^{Ad}	0.123 ± 0.000^{Ae}
FRE	0.077 ± 0.003^{Aa}	0.080 ± 0.000^{Aa}	0.094 ± 0.001^{Ab}	0.096 ± 0.001^{Ab}	0.113 ± 0.001^{Ac}	0.112 ± 0.001^{Ac}	0.124 ± 0.001^{Ad}
FCE	0.077 ± 0.003^{Aa}	0.090 ± 0.000^{Bb}	0.097 ± 0.003^{BCc}	0.097 ± 0.003^{Ac}	0.113 ± 0.001^{Ad}	0.134 ± 0.000^{Ce}	0.141 ± 0.002^{Bf}
FTC	0.077 ± 0.003^{Aa}	0.106 ± 0.000^{Cb}	0.112 ± 0.001^{Dc}	0.111 ± 0.001^{Bc}	0.124 ± 0.001^{Bd}	0.130 ± 0.000^{Be}	0.141 ± 0.002^{Bf}
FBL	0.077 ± 0.003^{Aa}	0.106 ± 0.000^{Cb}	0.113 ± 0.001^{Dc}	0.117 ± 0.001^{Bc}	0.124 ± 0.001^{Bd}	0.140 ± 0.000^{De}	0.164 ± 0.001^{Cf}

*Means followed by the same lower case letters are not significantly ($P > 0.05$) different over time (rows). Means followed by the same upper case letter are not significantly ($P > 0.05$) different among treatments (columns)

*Data are given as means ± SD, $n = 3$

Table 4 Mean values for total polar compounds (%) of oil samples during six frying periods

Frying period (h)							
Treatment	0	1	2	3	4	5	6
FTQ	3.55 ± 0.07^{Aa}	3.95 ± 0.07^{Bab}	5.05 ± 0.07^{Ab}	7.15 ± 0.07^{ABc}	8.95 ± 0.07^{Bd}	11.30 ± 0.14^{Be}	12.62 ± 0.08^{Bf}
FRE	3.55 ± 0.07^{Aa}	3.75 ± 0.07^{Aa}	5.02 ± 0.03^{Ab}	7.04 ± 0.21^{Ac}	8.75 ± 0.07^{Ad}	11.00 ± 0.00^{Ae}	12.22 ± 0.03^{Af}
FCE	3.55 ± 0.07^{Aa}	4.2 ± 0.000^{CDb}	5.62 ± 0.03^{Bc}	7.95 ± 0.07^{Cd}	10.95 ± 0.07^{De}	13.75 ± 0.07^{Df}	15.25 ± 0.07^{Dg}
FTC	3.55 ± 0.07^{Aa}	4.15 ± 0.07^{Cab}	5.08 ± 0.02^{Ab}	7.27 ± 0.03^{Bc}	9.20 ± 0.00^{Cd}	12.20 ± 0.14^{Ce}	13.17 ± 0.04^{Ce}
FBL	3.55 ± 0.07^{Aa}	4.35 ± 0.07^{Db}	5.85 ± 0.07^{Cc}	8.45 ± 0.07^{Dd}	11.22 ± 0.03^{Ee}	15.80 ± 0.14^{Ef}	17.67 ± 0.11^{Eg}

*Means followed by the same lower case letters are not significantly ($P > 0.05$) different over time (rows). Means followed by the same upper case letter are not significantly ($P > 0.05$) different among treatments (columns)

*Data are given as means ± SD, $n = 3$

and aldehydes. Most of these components are stable; however, some of them are unstable at high temperature. The volatile aldehydes hexanal and heptanal were used to monitor the oxidation of oils in previous studies (Guillén et al. 2005; Katragadda et al. 2010). Figure 2 showed a chromatogram resulted from HS-SDME/GC–MS method for a French fries sample based on optimum microextraction conditions. As seen in Figs. 3 and 4, the concentration of volatile compounds showed a similar evolution pattern to that observed for the peroxide values, such these three well differentiated regions presented in the curves were associated with different stages of oxidation, as previously commented (Katragadda et al. 2010). It was observed that until the second period of frying, the concentration of these aldehydes was low, which coincided with the first stage of oxidation and quick formation of hydroperoxides. This was complementary with the results obtained by the peroxide value. However, from the second to the fourth frying period, a sharp increase was produced and at the end of the fourth period, the concentration of volatile aldehydes reached maximum values. As an example, the hexanal concentration of French fries samples fried in control oil and those fried in oils treated with rosemary extract, TBHQ, ferulago extract and tocopherol increased, respectively, from 23.05, 13.98, 16.20, 18.35 and 17.14 ng/g after the first frying period to 120.93, 80.33, 79.64, 84.34 and 92.98 ng/g at the end of fourth frying period. This increase was due to the degradation of hydroperoxides to secondary oxidation products such as: aldehydes or ketones. After this period, a significant decrease in the concentration of volatile compounds resulted. At the end of fifth frying period, the former values of hexanal concentration in samples prepared with control oil and oils containing rosemary extract, TBHQ, ferulago extract and tocopherol lowered to 72.32, 39.30, 39.33, 45.10 and 58.01, respectively. This diminution stage was associated with the very advanced stages of oxidation. Throughout this period, most of the earlier produced hydroperoxides decomposed and the aldehyde compounds were oxidized to other compounds, such as oxygenated aldehydes and carboxylic acids. In addition, the polymerization of oil also took place in this stage (Guillén et al. 2005). According to the results of research conducted by Pignoli et al. (2009), the interaction between aldehyde compounds and amino groups of proteins, could be another reason for this diminution stage. Finally, on the sixth frying period, the second stage of increase occurred, as a consequence of the proceeding oxidation process. Katragadda et al. (2010) also found a similar pattern for the

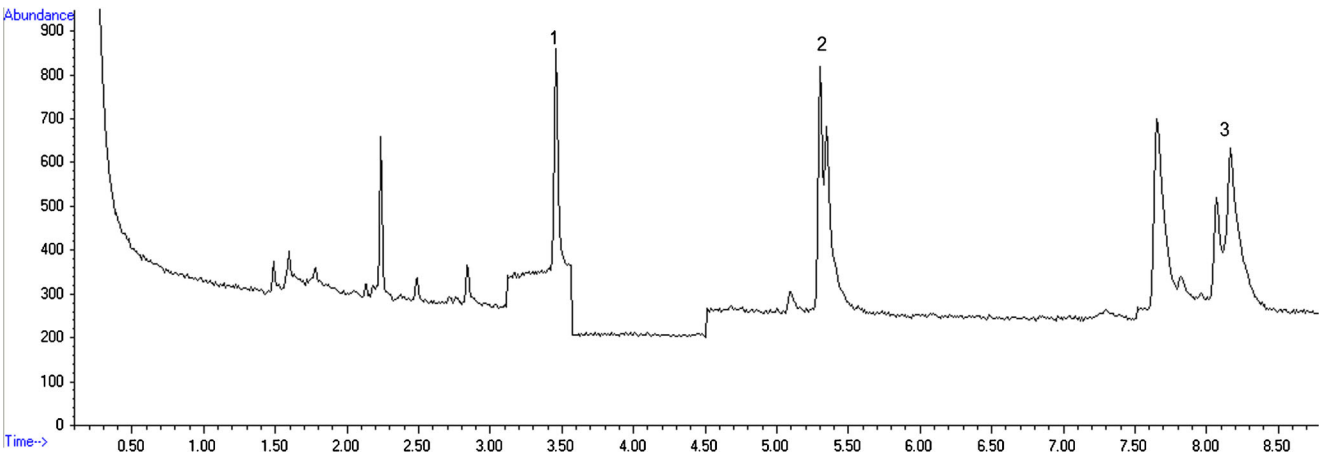

Fig. 2 A chromatogram obtained by HS-SDME/GC–MS method under SIM mode for a French fries sample based on optimum microextraction conditions. Peaks: 1 internal standard, 2 hexanal, 3 heptanal

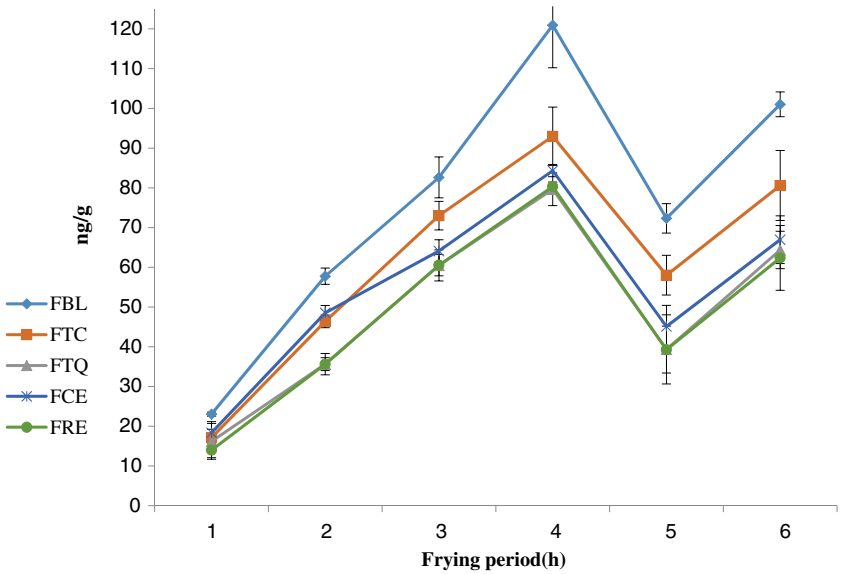

Fig. 3 Changes in Concentration of Hexanal (ng/g) of French fries prepared by antioxidant treatments during six frying periods

generation of aldehydes in coconut, safflower, canola and extra virgin olive oils during heating at 210 °C up to 6 h. As shown in Figs. 3 and 4, all antioxidants lowered the formation of aldehydes but with different order, due to the rate and magnitude of changes. It can be observed that among all treatments, the samples fried in oil treated with rosemary extract and TBHQ recorded the lowest increase in concentration of these compounds. At the end of frying operation, the samples fried in oil treated with rosemary extract revealed a reduction by 38.3 and 17.65 % in hexanal and heptanal concentration, respectively, compared to control sample. This is in accordance with findings of Samotyja and Małecka (2010), who reported that after 312 h of storage at 60 °C, the addition of 0.02 % oil-soluble rosemary extract in soybean oil lowered the hexanal concentration frome about 8 ppm, as by control sample, to about 5 ppm. Apart from control samples, those fried in oil treated with tocopherol had the highest level of aldehydic compounds. As explained formerly, this is due to the absence of a chelator site in the tocopherol structure and its function as a primary antioxidant (Cuvelier et al. 2000). Although the ferulago extract could not control the primary stage of oxidation, it presented a moderate increase in concentration of aldehydic compounds.

Sensory analysis

Among the volatile and semi volatile components formed throughout the oxidation process are the compounds responsible for typical rancid odor and flavor of oil and some of them have harmful effects on human health. As presented in Table 5, sensory scores showed a tendency to decrease as the length of frying time increased, due to oxidation. This diminution trend was in acceleration after the third frying period. This is in agreement with the findings of Lalas and Dourtoglou (2003), who found that clear and pronounced difference among the sensory score of treatments was observed

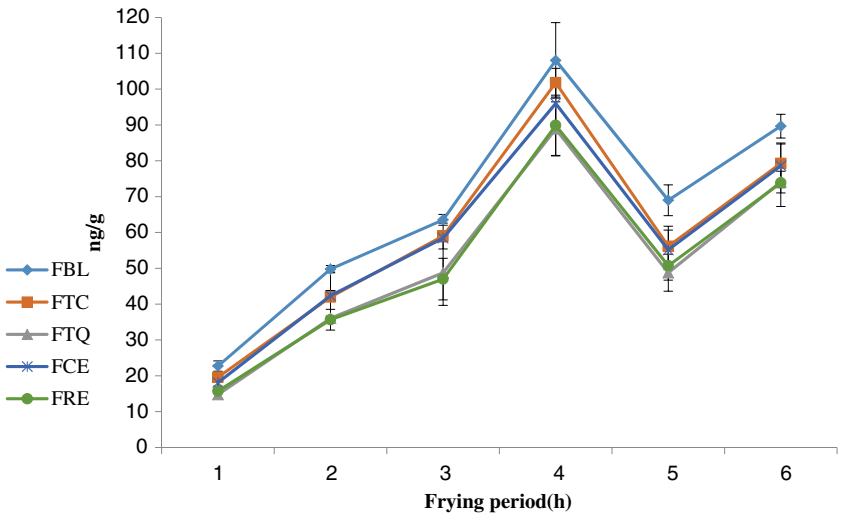

Fig. 4 Changes in Concentration of Heptanal (ng/g) of French fries prepared by antioxidant treatments during six frying periods

Table 5 Mean values for overall acceptability of fried potatoes prepared by antioxidant treatments during six frying periods

Treatment	1	2	3	4	5	6
FTQ	5.00 ± 0.00^{Bd}	5.00 ± 0.00^{Cd}	4.67 ± 0.09^{Ccd}	4.33 ± 0.21^{Cc}	3.50 ± 0.22^{Bb}	2.50 ± 0.12^{Ca}
FRE	5.00 ± 0.00^{Bd}	5.00 ± 0.00^{Cd}	4.72 ± 0.11^{Ccd}	4.33 ± 0.21^{Cc}	3.50 ± 0.22^{Bb}	2.33 ± 0.21^{BCa}
FCE	1.00 ± 0.00^{Aa}	2.22 ± 0.15^{Ac}	3.17 ± 0.17^{Ad}	3.38 ± 0.12^{ABd}	2.33 ± 0.21^{Ac}	1.33 ± 0.00^{Ab}
FTC	4.83 ± 0.17^{Bd}	4.72 ± 0.11^{BCd}	4.00 ± 0.00^{Bc}	3.50 ± 0.22^{Bb}	2.33 ± 0.21^{Aa}	2.17 ± 0.31^{Ba}
FBL	4.94 ± 0.05^{Be}	4.50 ± 0.12^{Be}	4.00 ± 0.00^{Bd}	3.17 ± 0.17^{Ac}	2.22 ± 0.15^{Ab}	1.17 ± 0.17^{Aa}

*Means followed by the same lower case letters are not significantly ($P > 0.05$) different over time (rows). Means followed by the same upper case letter are not significantly ($P > 0.05$) different among treatments (columns)

*Data are given as means ± SD, $n = 3$

after the fourth frying period. Sensory quality scores corresponded to p-anisidine value, headspace volatile analysis and polymer analysis. The lowest score for overall acceptability of (1.17) was obtained by control sample after the six frying periods. It was 2.14-fold, 2-fold, 1.85-fold and 1.14-fold lower than that of samples fried in oil treated with TBHQ, rosemary extract, tocopherol and ferulago extract, respectively. The samples fried in the oils treated with TBHQ and rosemary extract were the most preferred samples with the final scores of (2.50) and (2.33), respectively. Additionally there was no significant difference ($p > 0.05$) between the overall acceptability scores of these two samples throughout the frying time. This meant that the natural flavor of rosemary extract was compatible with the fried potatoes flavor profile, so it did not have flavor interruption with other components. The discrimination between tocopherol and TBHQ treatments was pronounced from the third frying period. As mentioned in the former part, tocopherol acts as a primary antioxidant and it is less effective in retarding formation of secondary oxidation products, responsible for the development of rancid odors and off-flavor of the oil. The overall acceptability of samples fried in the oil treated with ferulago extract followed a different trend and the scores were 1.00, 2.22, 3.17, 3.38, 2.33 and 1.33 for 1, 2, 3, 4, 5 and 6th frying periods, respectively. At the beginning, the sensory scores of these samples was low, but then followed the increasing trend until the end of the fourth frying period and finally started to decrease. Considering the slow rate of secondary oxidation process in the earlier stages of frying, the less overall acceptability of samples is attributed to the negative effect of extract ingredients. The observed decrease in the sensory score of samples following the fourth frying period is due to progression of oxidation.

Conclusion

The present study compared the antioxidative activities of tocopherol, rosemary extracts and ferulago, with synthetic antioxidant TBHQ, on oxidation in a mixture (1:1, *w*/*w*) of sunflower seed oil and palm olein, during deep frying of potato slices. The rosemary extract exhibited inhibitory effects towards both primary and secondary oxidation changes, as effectively as TBHQ. On the other hand, tocopherol was more effective in controlling the primary stage of oxidation while the ferulago extract showed strong protective effect toward the secondary oxidation products. Sensory evaluation illustrated that the organoleptic results of the rosemary extract treatment were same as TBHQ. Based on the overall results of this study, rosemary extract could be recommended as a good alternative to TBHQ, but further studies on evaluation of ferulago extract regarding its organoleptic behavior and antioxidative performance for deep frying are also needed.

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