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GAS CHROMATOGRAPHIC DETERMINATION OF SYNTHETIC ANTIOXIDANTS IN LIQUID FRYING OIL SAMPLES

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ABSTRACT

Natural and synthetic antioxidants play a significant role in retarding the lipid oxidation reactions in food products. Prevention of lipid peroxidation has long become an important issue, since oils and fats, essential to our diets, tend to encounter problems of oxidation and rancidity, which affect food quality and also may threat human health. A gas chromatography system equipped by CP sil 8 CB capillary column (50m×0.53mm i.d×1.5µm film thickness) was used to determine the antioxidants tert-butyl-hydroquinone, tert-butylhydroxyanisole, and 3,5-di-tert-butylhydroxytoluene simultaneously in Iranian commercial liquid frying oils. The recoveries of BHA, BHT and TBHQ were 95.44-105.04, 96.03-105.47 and 95.62-108.89%, respectively. 53 commercial liquid frying oils were analyzed using the chromatographic method. The contents of BHA, BHT and TBHQ were found to be 0.00, 0.00 and 40.18±0.21 mg.kg⁻¹ respectively. The levels of synthetic antioxidants in studied samples were all below the legal limits of Iran (200 mg.kg⁻¹).

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INTRODUCTION

Edible fats and oils provide indispensable nutrition for body and they also add special flavour, texture and taste to food (Biglar *et al.*, 2012 a). However in contact with air they undergo autoxidation. Oxidized fats are harmful to health and reduce the quality of food (Chung 1999; Hajimahmoodi *et al.*, 2013; Moghaddam *et al.*, 2012 and Tryphonas *et al.*, 1999). Therefore it's essential to prevent or retard the autoxidation process. Synthetic antioxidants are food additives that are added to food to extend their shelf life (Kubow, 1990 and Nollet, 2000). They commonly be used as preservative in fat, oils and fat containing foods (Jacobsen *et al.*, 1999). Synthetic antioxidants such as butylated hydroxy anisole (BHA), butylated hydroxyl toluene (BHT), tertiary butyl hydroquinone

(TBHQ) and propyl gallate (PG) are widely used as food additives to prevent rancidification, owing to their high performance, low cost and wide availability (Shahidi, 2000). In the last 50 years food manufactures added food grade commercial antioxidants such as BHA, BHT, TBHQ and PG to their product as preservative in order to extend their shelf life (Gordon and Kourimská, 1995). Reports on synthetic antioxidants effects on human health are controversial. There are reports of harmful effects in high and long-term use, such as carcinogenic effects of BHA and BHT (Ito *et al.*, 1985; Kahl and Kappus, 1993 and Witschi, 1986). However most of synthetic antioxidants in recommended concentration that was used in drugs, foods and cosmetics are effective and also has beneficial effect to health such as anticarcinogenic, antimutagenic and antiviral effects (Keith *et al.*, 1982, Naidu, 2000 and Wojcik *et al.*, 2010). The use of antioxidants is subject to regulations that establish permitted substance and their concentration. In European, the antioxidants mentioned above are strictly regulated to use in foodstuffs,

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BHA is permitted in bouillons, gravies, dehydrated meat and dehydrated soups individually or combined with PG up to a maximum limit of 200 mg/kg, expressed on the fat content of the product and BHT is not permitted in these foods but it may be used in fats and oils. In the United States, TBHQ is permitted and can be used alone or in combination with BHA and/or BHT up to 200 mg/kg of fat (Burdock, 1997). TBHQ is also permitted in Australia, Brazil, New Zealand and Philippines (Karovičová and Šimko, 2000b). Maximum permitted amount of synthetic antioxidants (particularly BHA, BHT, TBHQ and PG) in food in many countries usually set to 100-200 mg/kg of fats and oils (de la Fuente *et al.*, 1999; González *et al.*, 1998; Noguera-Ortí *et al.*, 1999 and Viplava Prasad *et al.*, 1999).

Therefore a lot of researches has been conducted to determine presence and quantitate antioxidants especially synthetic phenolic antioxidants (André *et al.* 2010). Colorimetry and spectrophotometry methods were first used for the determination of these additives in different food samples and today it is possible to find different variations (Capitán-Vallvey *et al.*, 2004; Cruces-Blanco *et al.*, 1999 and Viplava Prasad *et al.*, 1987), later chromatographic methods developed (Karovičová and Šimko, 2000a; Ragazzi and Veronese, 1973; Yang *et al.*, 2002; Yankah *et al.*, 1998 and Biglar *et al.*, 2012 b) and there are electrochemical methods (Agui *et al.*, 1995; de la Fuente, Acuña, Vázquez, Tascón and Sánchez Batanero, 1999; dos Santos Raymundo *et al.*, 2007 and Guan *et al.* 2006). Many analytical methods have been described for quantification of synthetic antioxidants, Gas chromatography analysis is one of rapid and simple methods of this purpose. Therefore, the present study was undertaken to quantification of synthetic antioxidants (BHA, BHT, and TBHQ) in some frying oil samples.

MATERIALS AND METHODS

Chemicals

Reference compounds BHA (Sigma-Aldrich, USA), BHT (Sigma-Aldrich, USA), TBHQ (YASHO, India), Hydroquinone (Merck, Germany), acetonitrile (DUKSAN, South Korea) were purchased. All solvents/chemicals used were analytical grade. Double-distilled deionized water was used for the preparation of aqueous solutions.

Chromatographic analysis

GC condition

GC analyses were performed on a model A7890 Gas Chromatography (Agilent, USA) equipped with flame ionization detector. Analytical separation was achieved on a CP sil 8 CB capillary column (50m×0.53mm i.d×1.5µm film thickness) (Varian, USA). Nitrogen was used as carrier gas. Temperature setting was as follows: injector, 250°C and detector, 290°C.

Oven temperature program

Column temperature was initially held at 150°C for 1 minute then was increased gradually to 200°C at 7°C/min rate, and

finally was raised to 300°C at 20°C/min rate and analysis continued in this temperature for 35 minutes.

Sample preparation

Internal standard

100mg.kg⁻¹ hydroquinone in acetonitrile was made and was used as internal standard.

Antioxidants standards

BHA, BHT and TBHQ solutions in 40, 60, 80, 100, 120, 150 mg.kg⁻¹ were made and after addition of internal standard were used as GC injectable samples. A calibration curve was prepared relating concentration of antioxidants with area under the curve (AUC) of related peaks.

Frying oil samples

53 samples of frying oils were purchased from local supermarkets in Tehran, Iran. Each brand was coded with a letter. Lot numbers were checked to ensure that each unit belonged to a different lot. All oil samples were stored, labelled and analyzed before expiry dates. Samples were selected to include the major manufacturers of the oils in Iran. The composition of studied liquid frying oils was mixture of palm oil, soybean oil, canola oil, and sunflower oil.

Extraction

The first step of analyse was isolation of antioxidants from oil samples for this purpose 0.5 g (accurately weighed to 0.0001 g) of each sample were weighted to centrifuge tube and 0.5 ml acetonitrile was added and was homogenized with A1101 Shaker (Tajhiz-fan, Iran) at room temperature for 1 minute. It was centrifuged (Heraeus, Germany) for 3 minutes in 3500 RPM, then upper phase (acetonitrile phase) was collected and oil phase subsequently extracted once by this way. All acetonitrile were combined in a 2mL vial, 1mL hydroquinone (100mg.kg⁻¹) was added as internal standard then analyzed by GC. 1µl of each sample was injected to GC using 10µL syringe (Agilent, Australia) twice. Retention time and AUC of peaks were collected and statistical analyses were performed.

Data Analysis

Data were analyzed using statistical package for social sciences, version 16 (SPSS Inc., Chicago, IL, USA). Three independent oil samples of each cultivar were analyzed three times. Data are expressed as mean±SD. According to current data which meet the assumption of variances homogeneity, the Tukey post hoc was used (*p*-Value<0.05).

RESULTS

The repeatability of the peak areas and retention times were studied by repeatedly injecting samples. The reproducibility over different days was checked by injecting the same standard solution (100 mg.kg⁻¹) over five days. It was observed that both retention times and peak areas were reproducible and all the RSD values obtained were satisfactory.

Table 1. Recoveries of the spiked antioxidants from the liquid frying oil samples*

NO	TBHQ (mg.kg ⁻¹)	Amount Added (mg.kg ⁻¹)		AUC of TBHQ	AUC of Internal Standard		Amount of TBHQ that was found	Recovery (%)
		50	25		138.29	128.88		
51	69.48	50	25	1110.63	138.29	8.03	238.99	100.03
		25	12.5	809.04	128.88	6.28	188.60	99.27
52	41.14	50	25	801.93	129.77	6.18	185.80	103.52
		25	12.5	584.01	130.59	4.47	136.73	108.89
53	52.33	50	25	846.19	126.60	6.68	200.28	95.62
		25	12.5	635.26	125.45	5.06	153.73	98.14
NO	BHA (mg.kg ⁻¹)	Amount Added (mg.kg ⁻¹)		AUC of BHA	AUC of Internal Standard		Amount of BHA that was found	Recovery (%)
		50	25		138.29	128.88		
51	0	50	25	190.13	138.29	1.37	47.72	95.44
		25	12.5	78.90	128.88	0.61	25.81	103.22
52	0	50	25	200.08	129.77	1.54	52.52	105.04
		25	12.5	77.58	130.59	0.59	25.29	101.14
53	0	50	25	181.53	126.60	1.43	49.42	98.83
		25	12.5	72.46	125.45	0.58	24.81	99.24
NO	BHT (mg.kg ⁻¹)	Amount Added (mg.kg ⁻¹)		AUC of BHT	AUC of Internal Standard		Amount of BHT that was found	Recovery (%)
		50	25		138.29	128.88		
51	0	50	25	191.55	138.29	1.39	48.02	96.03
		25	12.5	74.56	128.88	0.58	24.84	99.35
52	0	50	25	192.43	129.77	1.48	50.83	101.65
		25	12.5	80.26	130.59	0.61	25.88	103.5
53	0	50	25	196.15	126.60	1.55	52.74	105.47
		25	12.5	71.53	125.45	0.57	24.60	98.39

*The content of each antioxidant in 0.05 g of the liquid frying oil, shown as mean±S.D. Data were shown as mean±S.D (n=3), Recovery (%)=[(C_A)/B]_100%, Coefficient of variation.

Table 2. TBHQ contents of commercial liquid frying oil*

NO	Peak of Samples		Peak of Internal standard		TBHQ (mg.kg ⁻¹)
	AUC (Mean±SD)	CV%	AUC (Mean±SD)	CV%	
1	231.50±2.59	1.12	293.16±2.90	0.99	30.91±0.48 ^d
2	232.48±7.31	3.14	377.83±7.79	2.06	25.89±0.19 ^d
3	597.22±31.15	5.22	231.24±7.43	3.21	82.40±1.49 ^a
4	229.25±1.89	0.82	302.21±3.70	1.22	30.01±0.45 ^d
5	198.81±10.18	5.12	302.62±16.30	5.39	27.09±0.05 ^d
6	364.30±29.39	8.07	305.81±23.07	7.54	42.44±0.18 ^c
7	501.43±24.67	4.92	316.34±10.24	3.24	53.75±0.77 ^c
8	362.29±32.25	8.90	323.31±29.66	9.17	40.42±0.09 ^c
9	272.81±83.26	30.52	267.65±22.79	8.52	37.23±6.47 ^c
10	232.27±13.65	5.88	338.70±7.87	2.32	27.91±0.70 ^d
11	208.99±1.56	0.74	288.45±0.98	0.34	29.03±0.23 ^d
12	213.41±16.55	7.75	315.14±28.67	9.10	27.68±0.26 ^d
13	512.53±9.12	1.78	340.98±25.65	7.52	51.50±2.49 ^c
14	37.38±12.86	34.41	325.57±1.29	0.40	11.51±1.15 ^c
15	425.16±0.07	0.02	397.54±2.29	0.58	38.95±0.17 ^c
16	472.50±6.98	1.48	363.97±0.06	0.02	45.52±0.56 ^c
17	374.48±30.97	8.27	307.17±22.90	7.45	43.23±0.29 ^c
18	514.46±15.86	3.08	349.45±8.44	2.41	50.51±0.28 ^c
19	275.00±22.36	8.13	305.68±33.58	10.98	34.11±0.74 ^d
20	480.62±28.67	5.96	294.13±5.20	1.77	55.15±1.97 ^c
21	342.83±36.22	10.57	328.24±29.97	9.13	38.21±0.43 ^c
22	243.43±19.96	8.20	305.06±21.72	7.12	31.13±0.25 ^d
23	484.26±11.80	2.44	304.18±3.03	1.00	53.96±0.66 ^c
24	480.03±2.50	0.52	329.24±11.46	3.48	50.13±1.24 ^c
25	260.83±11.67	4.47	301.23±9.39	3.12	33.09±0.34 ^d
26	278.50±1.24	0.44	336.41±8.57	2.55	32.01±0.71 ^d
27	152.79±6.33	4.14	308.35±22.09	7.16	22.47±0.43 ^d
28	491.11±29.49	6.01	337.48±16.84	4.99	50.02±0.42 ^c
29	14.59±5.71	39.16	329.15±2.28	0.69	9.49±0.51 ^c
30	329.31±6.55	1.99	301.46±15.95	5.29	39.63±1.04 ^c
31	337.74±24.38	7.22	314.25±7.26	2.31	39.13±2.94 ^c
32	284.99±13.70	4.81	289.21±1.49	0.51	36.53±1.22 ^d
33	322.08±6.24	1.94	335.35±17.80	5.31	35.84±0.93 ^d
34	502.88±15.35	3.05	341.97±24.73	7.23	50.63±4.36 ^c
35	260.54±9.75	3.74	327.44±6.14	1.88	31.07±0.43 ^d
36	176.82±5.15	2.91	296.46±7.75	2.62	25.35±0.05 ^d
37	439.67±27.91	6.35	302.09±2.99	0.99	50.05±3.07 ^c
38	353.88±3.64	1.03	315.16±14.73	4.67	40.51±1.18 ^c
39	277.43±4.33	1.56	327.62±0.28	0.08	32.55±0.40 ^d
40	261.83±17.62	6.73	290.41±1.30	0.45	34.12±1.63 ^d

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41	563.19±12.40	2.20	349.08±2.26	0.65	1.61±0.03	54.57±0.72 ^c
42	573.67±4.73	0.82	332.51±21.13	6.35	1.73±0.12	57.90±3.57 ^c
43	258.66±5.53	2.14	313.03±13.82	4.41	0.83±0.02	31.97±0.54 ^d
44	611.54±11.73	1.92	328.66±3.92	1.19	1.86±0.01	61.68±0.39 ^b
45	660.39±26.77	4.05	325.28±20.68	6.36	2.03±0.05	66.59±1.35 ^b
46	280.41±0.92	0.33	333.54±19.19	5.75	0.84±0.05	32.41±1.47 ^d
47	376.08±0.07	0.02	287.98±6.09	2.12	1.31±0.03	45.75±0.79 ^c
48	478.68±28.27	5.91	308.41±16.05	5.21	1.55±0.01	52.81±0.31 ^c
49	209.32±10.44	4.99	313.27±19.91	6.36	0.67±0.01	27.42±0.26 ^d
50	212.39±3.98	1.87	301.61±27.38	9.08	0.71±0.08	28.55±2.23 ^d
51	635.86±38.72	6.09	288.69±31.65	10.96	2.21±0.11	71.67±3.10 ^b
52	375.93±3.45	0.92	293.79±6.96	2.37	1.28±0.04	45.00±1.21 ^c
53	398.52±13.74	3.45	257.99±6.68	2.59	1.54±0.01	52.59±0.38 ^c
Total	352.21±3.60	1.02	315.12±5.27	1.67	1.12±0.01	40.33±0.21

^aValues in the same column bearing different superscripts are significantly different (P-value<0.05).

Table 3. Approved synthetic antioxidant range of Iran-standard in fat and oil samples

Food Products	Allowed Maximum Value of BHA (mg.kg ⁻¹)	Allowed Maximum Value of BHT (mg.kg ⁻¹)	Allowed Maximum Value of TBHQ (mg.kg ⁻¹)
Vegetable Oils, Animal Fats, Spreads, Oil in Watter Emulsions	200	200	200
Soups and Extracts	-	100	200
Butter Fat And Milk Fat (Without Watter)	175	75	-

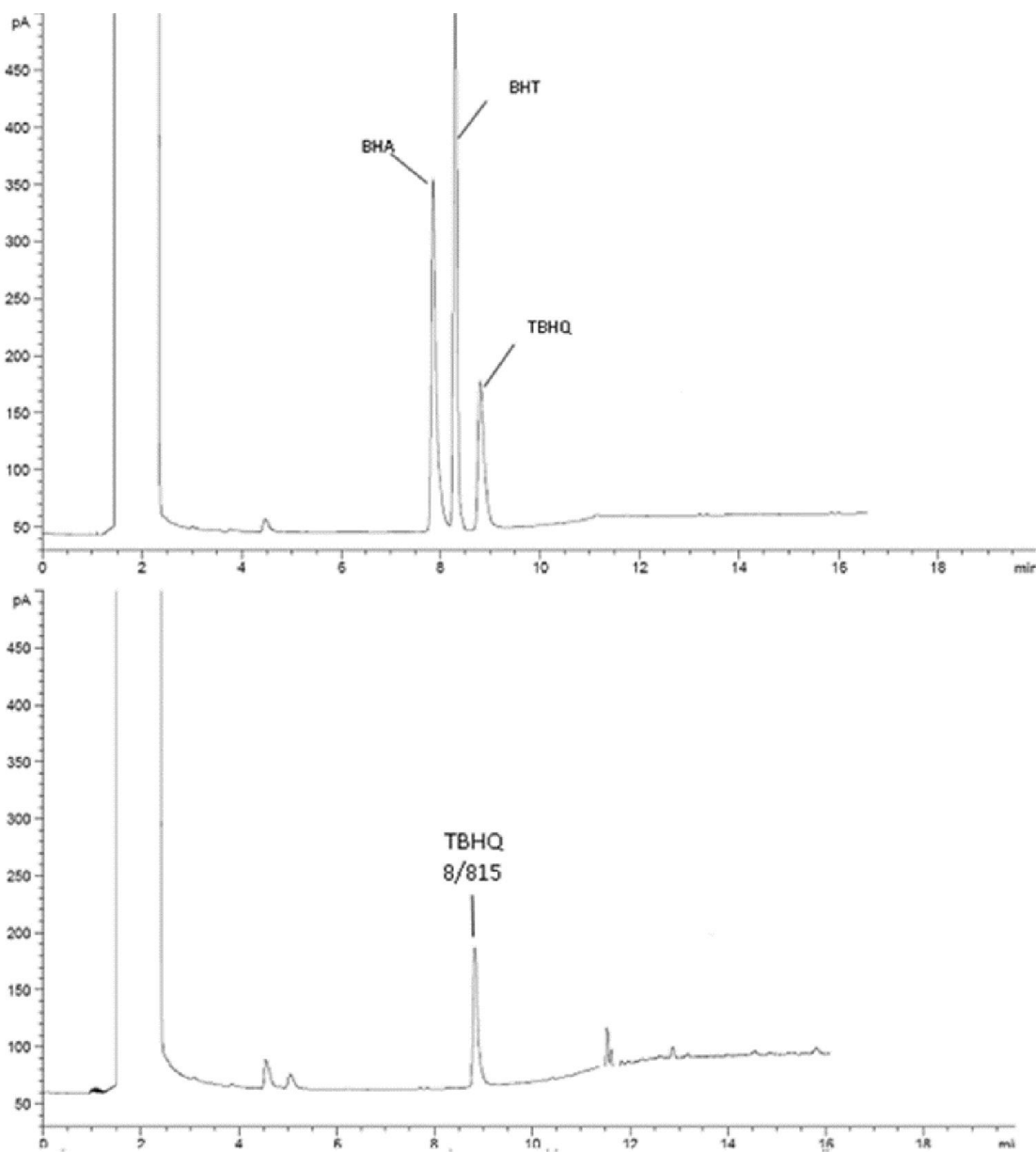


Figure 1. Chromatogram of synthetic antioxidant found in mixing standard by gas chromatography and one randomly selected liquid solid oil sample by gas chromatography

To understand the lowest detectable levels of BHA, BHT and TBHQ, standard solutions of six concentrations were prepared and analyzed. The recoveries of BHA, BHT and TBHQ were 95.44-105.04, 96.03-105.47 and 95.62-108.89%, respectively (Table 1). These results indicated the high accuracy of the present method. Details of the 53 liquid frying oil samples are shown in Table 2. All the samples were analysed according to the described procedure. Identification of the studied synthetic phenolic antioxidants was based on the comparison between the retention times of standard compounds and was confirmed by spiking known standards to the sample. Figure 1 shows the chromatogram of synthetic antioxidant found in one randomly selected liquid frying oil sample and also standard solution by gas chromatography. Quantification was based on the external standard method using calibration curves fitted by linear regression analysis. Although, BHA and BHT were not found in the samples, the range of TBHQ was from 9.85 ± 0.51 to 81.35 ± 1.49 mg.kg^{-1} (Table 2). On the other hand, the average amount of TBHQ was 40.33 ± 0.21 mg.kg^{-1} . For all the samples, the manufacturers declared the presence of TBHQ as synthetic antioxidants, but neither the BHA nor BHT was stated. Table 3 shows the synthetic antioxidants limitation of Iranian standard in the different samples. The analysis of the obtained results of all samples revealed that the mean TBHQ contents were lower than 200 mg.kg^{-1} therefore they could pass the Iranian standard.

DISCUSSION

Lipid oxidation is one of the major deteriorative reactions in cooking oils and often results in a significant loss of quality. Various natural and synthetic antioxidants are used in the prevention or retardation of lipid oxidation. Routine quality control of antioxidants in oil turns out to be more and more important due to the increased environmental concern on the use of large volumes of solvents for analysis. BHA is a synthetic antioxidant authorized as a food additive that was previously evaluated several times. Both committees established an ADI of $0.5 \text{ mg/kg bw.day}^{-1}$, with that of the SCF being classified as temporary. The ADI defined by both the SCF and JECFA was based on proliferative changes in the rat forestomach (BARLOW, 2013). Reports have shown that BHA has carcinogenic effects in non-rodents and causes lesion formation in the rat for stomach whereas BHT has carcinogenic effects in the liver of rats and mice (BARLOW, 2013).

In order to compare with other related study, the recoveries of antioxidants from cooking oils were 92.5-108.4% with coefficients of variation below 7.4% in study (Yang, Lin and Choong, 2002). Moreover in another research the synthetic antioxidants in food items including cooking oils, margarine, butter and cheese samples were evaluated (Saad *et al.*, 2007). The recoveries of the synthetic antioxidants when spiked to cooking oil, margarine, butter and cheese at 50 and 200 mg.L^{-1} were in the ranges 93.3-108.3% for PG, 85.3-108.3% for TBHQ, 96.7-101.2% for BHA and 73.9-94.6% for BHT. The levels of mentioned antioxidant in positive samples are all below the legal limits of Malaysia. In study the concentration ranges of BHA, BHT, and OMC in 12 commercial personal care samples were 0.13-4.85, 0.16-2.30, and 0.12-65.5 mg.g^{-1} ,

respectively (Akkbik *et al.*, 2011). In another Chinese study, 18 commercial edible fat and oil samples of olive, sunflower, peanut, soybean, vegetable and blend oils were analysed. The contents of BHA, BHT and TBHQ were found to be 0-50, 0-267 and $0-37 \mu\text{g.g}^{-1}$. 14 samples were detected to contain antioxidants. The total antioxidant contents in the three blend oils ranged 259-294 $\mu\text{g.g}^{-1}$, which exceeded the Chinese national standards control level of $200 \mu\text{g.g}^{-1}$ for edible oils (Choong and Lin, 2001). Since the toxicity of some synthetic antioxidants is not easily assessed, and as a result, a chemical may be considered safe by a country, tolerated in another country and forbidden in a third one. It was also found that TBHQ is authorized as an antioxidant in the US while it is forbidden in the European Union countries. For BHA, in United States, only 200 mg.kg^{-1} in fats, oils and chewing-gum and 50 mg.kg^{-1} in breakfast cereals or dehydrated soups are permitted (Miková, 2001). Normally, up to $100-200 \mu\text{g.g}^{-1}$ of several synthetic antioxidants especially PG, OG, TBHQ, BHA and BHT are used in oils and other fats, whether singly or in combinations, are allowed in many Countries (Yong, 2007).

Conclusion

The analytical method by GC-FID in this study has been developed for simultaneous determination of common synthetic antioxidants compounds in products. The satisfactory results of optimization and validation methods are quick, accurate, sensitive, excellent recoveries, convenient and effective. The evaluated amount of studied parameter clearly shows the status of antioxidants in liquid frying oil of Iran. Despite lack of BHA and BHT in studied samples, high content of TBHQ consumed daily by people since oils are the most commonly used in Iranian fast foods. Also substantial improvement of these products must be done. Likewise, people must be warned about the adverse health effects of synthetic antioxidants. Certainly, these assumptions warrant further control investigations in this field.

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REFERENCES

- Agui, M., Reviejo, A., Yanez-Sedeno, P. and Pingarrón, J. 1995. Analytical applications of cylindrical carbon fiber microelectrodes. Simultaneous voltammetric determination of phenolic antioxidants in food. *Analytical Chemistry*. 67:2195-2200.
- Akkbik, M., Assim, ZB. and Ahmad, FB. 2011. Optimization and validation of RP-HPLC-UV/Vis method for determination phenolic compounds in several personal care products. *International journal of analytical chemistry*. 2011.
- André, C., Castanheira, I., Cruz, J., Paseiro, P. and Sanches-Silva, A. 2010. Analytical strategies to evaluate antioxidants in food: a review. *Trends in food science & technology*. 21:229-246.

- BARLOW, SM. 2013. Safety of Food Additives in Europe. *Essential Guide to Food Additives*. 14.
- Biglar, M., Moghaddam, G., Sadeghi, N., Oveisi, MR., Jannat, B., Kaboli, Z., Hassani, S. and Hajimahmoodi, M. 2012 a. Profiling of major fatty acids in different raw and roasted sesame seeds cultivars. *African journal of biotechnology*. 11: 6619-6623.
- Biglar, M., Khanavi, M., Hajimahmoodi, M., Hassani, S., Moghaddam, G., Sadeghi, N. and Oveisi, MR. 2012 b. Tocopherol content and fatty acid profile of different Iranian date seed oils. *Iranian journal of pharmaceutical research*. 11: 873-878.
- Burdock, GA. 1997. Encyclopedia of food and color additives: CRC Press.
- Capitán-Vallvey, L., Valencia, M. and Arana Nicolas, E. 2004. Solid-phase ultraviolet absorbance spectrophotometric multisensor for the simultaneous determination of butylated hydroxytoluene and co-existing antioxidants. *Analytica chimica acta*. 503:179-186.
- Choong, Y-M. and Lin, H-J. 2001. Gas Chromatographic Determination of Synthetic Antioxidants in Edible Fats and Oils-A Simple Methylation Method. *Journal of Food and Drug Analysis*. 9:20-26.
- Chung, JG. 1999. Effects of butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) on the acetylation of 2-aminofluorene and DNA-2-aminofluorene adducts in the rat. *Toxicological Sciences*. 51:202-210.
- Cruces-Blanco, C., Segura Carretero, A., Merino Boyle, E. and Fernández Gutiérrez, A. 1999. The use of dansyl chloride in the spectrofluorimetric determination of the synthetic antioxidant butylated hydroxyanisole in food stuffs. *Talanta*. 12;50:1099-1108.
- de la Fuente, C., Acuña, JA., Vázquez, MD., Tascón, ML. and Sánchez Batanero, P. 1999. Voltammetric determination of the phenolic antioxidants 3-tert-butyl-4-hydroxyanisole and tert-butylhydroquinone at a polypyrrole electrode modified with a nickel phthalocyanine complex. *Talanta*. 6;49:441-452.
- dos Santos Raymundo, M., Marques da Silva Paula, M., Franco, C. and Fett, R. 2007. Quantitative determination of the phenolic antioxidants using voltammetric techniques. *LWT - Food Science and Technology*. 9;40:1133-1139.
- González, M., Gallego, M. and Valcárcel, M. 1998. Simultaneous gas chromatographic determination of food preservatives following solid-phase extraction. *Journal of Chromatography A*. 10/9;823:321-329.
- Gordon, MH. and Kourimská, L. 1995. Effect of antioxidants on losses of tocopherols during deep-fat frying. *Food Chemistry*; 52:175-177.
- Guan, Y., Chu, Q., Fu, L., Wu, T. and Ye, J. 2006. Determination of phenolic antioxidants by micellar electrokinetic capillary chromatography with electrochemical detection. *Food Chemistry*. 1;94:157-162.
- Hajimahmoodi, M., Arami, S., Nosrati, M., Moghaddam, G., Sadeghi, N., Reza Oveisi, M., Jannat, B. and Mazdeh, FZ. 2013. Trans Fatty Acid Content of Iranian Edible Oils. *Food & Nutrition Sciences*. 4.
- Ito, N., Fukushima, S. and Tsuda, H. 1985. Carcinogenicity and modification of the carcinogenic response by BHA, BHT, and other antioxidants. *CRC Critical reviews in Toxicology*. 15:109-150.
- Jacobsen, C., Schwarz, K., Stöckmann, H., Meyer, AS. and Adler-Nissen, J. 1999. Partitioning of selected antioxidants in mayonnaise. *Journal of agricultural and food chemistry*. 47:3601-3610.
- Kahl, R. and Kappus, H. 1993. Toxicology of the synthetic antioxidants BHA and BHT in comparison with the natural antioxidant vitamin E. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung*. 04/;196:329-338.
- Karovičová, J. and Šimko, P. 2000a. Determination of synthetic phenolic antioxidants in food by high-performance liquid chromatography. *Journal of Chromatography A*. 6/16;882:271-281.
- Karovičová, J. and Šimko, P. 2000b. Preservatives and antioxidants. In: *Food Analysis by HPLC* 2nd ed. New York: Marcel Dekker. p. 575-620.
- Keith, AD., Arruda, D., Snipes, W. and Frost, P. 1982. The antiviral effectiveness of butylated hydroxytoluene on herpes cutaneous infections in hairless mice. *Experimental Biology and Medicine*. 170:237-244.
- Kubow, S. 1990. Toxicity of dietary lipid peroxidation products. *Trends in Food Science & Technology*. 7;1:67-71.
- Miková, K. 2001. The regulation of antioxidants in food. In: Woodhead Publishing Ltd.: Cambridge, UK. p. 267-284.
- Moghaddam, G., Heyden, YV., Rabiei, Z., Sadeghi, N., Oveisi, MR., Jannat, B., Araghi, V., Hassani, S., Behzad, M. and Hajimahmoodi, M. 2012. Characterization of different olive pulp and kernel oils. *Journal of Food Composition and Analysis*. 28:54-60.
- Naidu, AS. 2000. Natural Food Antimicrobial Systems: Taylor & Francis.
- Noguera-Ortí, JF., Villanueva-Camañas, RM. and Ramis-Ramos, G. 1999. Direct injection of edible oils as microemulsions in a micellar mobile phase applied to the liquid chromatographic determination of synthetic antioxidants. *Analytica Chimica Acta*. 4/22;387:127-134.
- Nollet, LM. 2000. Food Analysis by HPLC: CRC Press.
- Ragazzi E, Veronese G. 1973. Quantitative analysis of phenolic compounds after thin-layer chromatographic separation. *Journal of Chromatography A*. 3/28;77:369-375.
- Saad, B., Sing, YY., Nawi, MA., Hashim, N., Mohamed Ali, AS., Saleh, MI., Sulaiman, SF., Talib, KM. and Ahmad, K. 2007. Determination of synthetic phenolic antioxidants in food items using reversed-phase HPLC. *Food Chemistry*. 105:389-394.
- Shahidi, F. 2000. Antioxidants in food and food antioxidants. *Die Nahrung*. Jun;44:158-163. Epub 2000/07/25.
- Tryphonas, H., Lacroix, F., Lok, E., Jee, P., Clayson, D., Hayward, S., Miller, D. and Mehta, R. 1999. The effect of butylated hydroxytoluene on selected immune surveillance parameters in rats bearing enzyme-altered hepatic preneoplastic lesions. *Food and Chemical Toxicology*. 37:671-681.
- Viplava Prasad, U., Divakar, TE., Hariprasad, K. and Sastry, CSP. 1987. Spectrophotometric determination of some antioxidants in oils and fats. *Food Chemistry*; 25:159-164.
- Viplava Prasad, U., Srinivasulu, V., Naidu, P. and Venkateswara Rao, G. 1999. Spectrophotometric methods for the determination of di-tert-butylhydroquinone in oils. *Journal of food science and technology*. 36:444-445.

- Witschi, HP. 1986. Enhanced tumour development by butylated hydroxytoluene (BHT) in the liver, lung and gastro-intestinal tract. *Food and Chemical Toxicology*. 10;24:1127-1130.
- Wojcik, M., Burzynska-Pedziwiatr, I. and Wozniak, LA. 2010. A Review of Natural and Synthetic Antioxidants Important for Health and Longevity. *Current Medicinal Chemistry*; 17:3262-3288.
- Yang, M-H., Lin, H-J. and Choong, Y-M. 2002. A rapid gas chromatographic method for direct determination of BHA, BHT and TBHQ in edible oils and fats. *Food Research International*. 35:627-633.
- Yankah, VV., Ushio, H., Ohshima, T. and Koizumi, C. 1998. Quantitative determination of butylated hydroxyanisole, butylated hydroxytoluene, and tert-butyl hydroquinone in oils, foods, and biological fluids by high-performance liquid chromatography with fluorometric detection. *Lipids*. Nov; 33:1139-1145. Epub 1998/12/31.
- Yong, YS. 2007. Determination Of Synthetic Phenolic Antioxidants In Food Items Using HPLC And Total Antioxidants Using FIA Approaches [TX553. A73 Y55 2007 f rb] Universiti Sains Malaysia.
