

## Evaluation of *t*-Butylhydroquinone and Heating Effects on Variation of Fatty Acids of Three Edible Oils by Principle Component Analysis

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Principle component analysis, which is an important method of chemometrics, as a fast and efficient method for evaluating the effect of antioxidant on changing fatty acids in oils during heating, is proposed. Gas chromatography was used for quantitative determination of methyl esters of myristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid before and after the addition of *t*-butylhydroquinone during 8 h heating with 1 h periods. The fatty acid composition values and common indices were evaluated by Pearson correlation and principle component analysis. Present results indicates a significant high correlation ( $p < 0.01$ ) between the heating time and relative percentage of all fatty acids in the presence of antioxidant while the data has lower correlation coefficient in the absence of antioxidant. Principle component analysis results show and confirm that the changing of fatty acids in the presence of antioxidant is smoother. Comparison of the effect of the antioxidant on sunflower, frying oil (based on palmolein) and canola oil by principle component analysis shows that the effect of antioxidant on sunflower oil is stronger than frying oil and canola oil.

**Key Words:** *t*-Butylhydroquinone, Fatty acids, Edible oil.

### INTRODUCTION

Thermostability of vegetable oils is associated with fatty acid composition, especially with diunsaturated and polyunsaturated acyl groups<sup>1</sup>. Regarding, oils with a low degree of unsaturation such as hydrogenated oils and palmolein are preferred by the catering industry and snacks manufacturers due to high thermo stability<sup>2</sup>. Several studies<sup>3-5</sup> have indicated that oils with different fatty acid composition behave distinctive during heating performance. In this regards, reducing the content of C18:3 and C18:2 in vegetable oils increased oxidative stability of oils<sup>6</sup>.

The monitoring of fatty acid changing in oils during heating performance is an effective method to assess thermal oxidative changes in the oils. Cuesta *et al.*<sup>7</sup> used level of total altered fatty acids and the fatty acid pattern of a fryer oil to evaluate the alteration of sunflower oil during the frying various frozen foods, they suggested that total fatty acid alteration measure is a useful tool for frying assessments and that they must be related not only to the degradation of unsaturated fatty acids but also to migrations of some fatty acids from the frozen pre fried foods to the fryer oil. Xu *et al.*<sup>8</sup> found that the properties of C16:0 and poly unsaturated fatty acids (PUFA) in the oils were strongly correlated with hours of deep frying and

increased significantly. Takeoka *et al.*<sup>9</sup> reported that decreasing the unsaturated fatty acids level increased the oil stability during frying, significantly. Bastida and Sánchez-Muniz<sup>10</sup> reported that oils rich in linoleic acid became more polymerized in frying than those rich in oleic acid.

Antioxidants avoid or delay lipid oxidation during food processing. Vegetable oils contain many types of natural antioxidant like tocopherols and carotenoids. Meanwhile canola oils have the highest rate of  $\gamma$ -tocopherol and sunflower oil includes highest  $\alpha$ -tocopherol. But refining, bleaching and deodorization stages (RBD), especially deodorization eliminate substantial portion of these compounds<sup>11</sup>. Several studies<sup>12-15</sup> investigated enhancing processing oil with natural antioxidant of specific plants-such as rosemary, sage, sorghum, garlic, tea and coriander extract-inhibits oxidation. But using synthetic antioxidant still is the most practical way to prevent oxidation because of many reasons such as economy and difficulties in extracting antioxidant of noted plants. Therefore for increasing the stability of vegetable oils in industries, processing oils are enhancing with synthetic antioxidant such as BHA, BHT and TBHQ (*t*-butylhydroquinone) besides chelating agent like citric acid and ascorbic acid. Evidence shows that TBHQ has stronger protective effect<sup>16</sup>, hence certain concentration of TBHQ and citric acid are used in industries to inhibit oxidation.

Many researches lead to evaluate physico-chemical properties of edible oils with adding antioxidants during heating performance. Merrill *et al.*<sup>17</sup> studied the addition of TBHQ, alone and in combination with other antioxidants and reported the greatest increase in oxidation stability of safflower oil and other oleic acid. Wang *et al.*<sup>18</sup> indicated that the addition of (50-200 mg/kg) TBHQ alone and in combination with ascorbic palmitate resulted in a significant increase of oxidation stability of soybean oil.

Multivariate methods have widespread applications in analysis and interpretation of the experimental results. It is based on the statistical principle of multivariate statistics, which involves observation and analysis of more than one statistical variable at a time, in analysis, the technique is used to perform trade studies across multiple dimensions while taking into account the effects of all variables on the responses of interest. Factor analysis as an important multivariate technique, is used to uncover the latent structure (dimensions) of a set of variables. It reduces attribute space from a larger number of variables to a smaller number of factors. Principle component analysis (PCA) is probably the most widespread multivariate statistical technique and because of the importance of multivariate measurements in chemometrics, it is regarded by many as the technique that most significantly changed the scientist's view of data analysis.

In recent decade multivariate analysis method have applied for fast and efficient class determination of oils samples in routine analysis performed of food control laboratories<sup>19-21</sup>. The PCA was applied to gas chromatographic data for fatty acid composition of commercial edible oils. Khan *et al.*<sup>22</sup> used chemometric assessment to compare effect of microwave heating on some edible oils.

Fundamental studies describe performance mechanism of antioxidants as free radicals obstructer that blocks chain reaction in first stage of oxidation. Fatty acids oxidation is the most important compositional reason to make free radicals<sup>23</sup>. Regarding to above reports, authors interested in evaluating effect of TBHQ as a conventional antioxidant on main fatty acids by chemometric methods.

The aim of this study is to compare changing of individual main fatty acids during heating performance before and after adding TBHQ (AO) (100 ppm TBHQ as a conventional concentration in edible oils industry). In this context, three types of edible oils with different typical fatty acid are used: high-linolenic canola with 7 % linolenic acid (HLCO) of oleic group, medium-linolenic sunflower oil with 3 % linolenic (MLSO) of linoleic group and one type of frying oil that formulated based on palm olein (FPO) with indicated palmitic acid value.

## EXPERIMENTAL

Canola oil, sunflower oil and one type of frying oil formulated based on palmolen were obtained from NAZ oil manufacture (Isfahan, IRAN).

All Solvents were analytical grade, glacial acid acetic, toluene and methanol except *n*-hexane that was HPLC-grade. External standards that contained C14:0, C16:0, C16:1, C18:0, C18:1, C18:2, C18:3 were obtained from Sigma-Aldrich (Taufkirchen, Germany).

**Sampling:** The sample oils were taken in 3 series during oil processing in one shift work of manufacturing. In each series 2 L before adding antioxidant (after deodorization, before flash cooler) and 2 L after adding antioxidant were taken. Commercial oils of the most Iranian manufacturers contain TBHQ (100 ppm) as antioxidant. All samples were stored at 4 °C in dark glass until starting heating cycles (up to 3 days later).

**Heating cycle:** The oils heating operation carried out in two domestic electric deep fryer (Vidas, model number: VI: 220, capacity 2 L, China) that were used for heating sample before and after adding antioxidant and 8 h heating trials were conducted for each oil types (before and after adding antioxidant) simultaneously. The oils (2 L) were heated  $190 \pm 5$  °C and kept at this temperature for 8 h each day. The oil samples were taken every 1 h (20 mL) and stored at -22 °C for further chemical analysis. Oils were not topped during heating cycle (heating cycles were carried out in three replications).

**Fatty acid analysis:** Fatty acids were converted to their methyl esters prior to determine by gas-liquid chromatography (Agilent 6890N) and was reported in relative area percentages. Fatty acids were transesterified by vigorous shaking of solution contained oil (50  $\mu$ L) in 1 mL toluene +2 mL sodium methoxide 0.5 N at 50 °C for 0.5 h then provide two phase by addition of 0.1 mL glacial acid acetic +5 mL distilled water and eventually extracted fatty acid methyl esters with HPLC grade hexane<sup>24</sup>, kept in ice bath and analyzed by GC as fast as possible, using a fused silica capillary column (INOWax, 30 m  $\times$  0.25 mm id  $\times$  0.5  $\mu$ m), a flame-ionization detector and nitrogen as the carrier gas (2.5 mL min<sup>-1</sup>). Gas chromatography split ratio was 10:1. Initial column temperature was 180 °C and held 5 min, then ramped to 210 °C (3 °C/min) and held 5 min. Injector and FID temperature were 260 and 270 °C, respectively. Fatty acid methyl esters samples (1  $\mu$ L) were injected by auto sampler. Run time regulated in 20 min.

**Injection program:** Every sample injected in two repetitions at a same time. So there are 6 replicated injections for every oil sample. However outliers were deleted. To eliminate any biasness all samples of each oil type (before and after adding antioxidant) were injected in one day.

## RESULTS AND DISCUSSION

**Fatty acid composition of fresh oils:** The marked fatty acid compositions C14:0, C16:0, C18:0, C18:1, C18:2, C18:3 in MLSO, FPO and HLCO, before and after adding AO (TBHQ) are reported in Table-1. There is no significant difference between fresh oil composition before and after adding antioxidant ( $p < 0.01$ ). C16:1 in MLSO and FPO and C14:0 in MLSO were not considerable in the sensitivity domain of the chromatographic test.

**Evaluation of fatty acid changing by pearson correlation in oils with and without AO:** Trends of changing common fatty acids (C14:0, C16:0, C18:0, C18:1, C18:2, C18:3) in three types of oils, before and after addition of antioxidant were investigated simultaneously. Significant correlation ( $p < 0.01$  and  $p < 0.05$ ) has found between time of heating and relative percentage of fatty acids in both of oils with and without TBHQ. It is worthy to mention that the linear trend of these

TABLE-1  
FATTY ACID COMPOSITION OF VEGETABLE OILS DURING HEATING CYCLE WITH AND WITHOUT TBHQ

Heating time (h)		14:0	16:0	18:0	18:1	18:2	18:3	
Sunflower oil (MLSO)	0	----	6.3 ± 0.1 <sup>a</sup> 6.2 ± 0.0 <sup>b</sup>	2.7 ± 0.0 <sup>a</sup> 2.1 ± 0.4 <sup>b</sup>	21.8 ± 0.1 <sup>a</sup> 21.7 ± 0.0 <sup>b</sup>	60.7 ± 0.1 <sup>a</sup> 60.6 ± 0.0 <sup>b</sup>	3.1 ± 0.1 <sup>a</sup> 3.1 ± 0.0 <sup>b</sup>	
	1	----	6.9 ± 0.2 <sup>a</sup> 7.9 ± 0.1 <sup>b</sup>	2.3 ± 0.1 <sup>a</sup> 2.1 ± 0.1 <sup>b</sup>	21.7 ± 0.0 <sup>a</sup> 29.0 ± 0.2 <sup>b</sup>	61.2 ± 0.3 <sup>a</sup> 59.8 ± 0.4 <sup>b</sup>	3.2 ± 0.1 <sup>a</sup> 3.1 ± 0.1 <sup>b</sup>	
	2	----	8.1 ± 0.0 <sup>a</sup> 8.3 ± 0.3 <sup>b</sup>	3.2 ± 0.2 <sup>a</sup> 3.5 ± 0.3 <sup>b</sup>	22.1 ± 0.1 <sup>a</sup> 30.2 ± 0.2 <sup>b</sup>	57.1 ± 0.0 <sup>a</sup> 56.9 ± 0.1 <sup>b</sup>	3.1 ± 0.0 <sup>a</sup> 2.6 ± 0.0 <sup>b</sup>	
	3	----	8.4 ± 0.2 <sup>a</sup> 8.6 ± 0.4 <sup>b</sup>	3.2 ± 0.1 <sup>a</sup> 2.1 ± 0.0 <sup>b</sup>	22.9 ± 0.1 <sup>a</sup> 32.3 ± 0.1 <sup>b</sup>	56.2 ± 0.0 <sup>a</sup> 57.3 ± 0.1 <sup>b</sup>	2.9 ± 0.2 <sup>a</sup> 2.3 ± 0.3 <sup>b</sup>	
	4	----	9.2 ± 0.1 <sup>a</sup> 8.6 ± 0.2 <sup>b</sup>	3.3 ± 0.2 <sup>a</sup> 4.5 ± 0.1 <sup>b</sup>	23.2 ± 0.3 <sup>a</sup> 29.6 ± 0.1 <sup>b</sup>	55.9 ± 0.2 <sup>a</sup> 54.7 ± 0.1 <sup>b</sup>	3.0 ± 0.1 <sup>a</sup> 2.3 ± 0.0 <sup>b</sup>	
	5	----	9.3 ± 0.0 <sup>a</sup> 9.1 ± 0.1 <sup>b</sup>	3.4 ± 0.3 <sup>a</sup> 5.9 ± 0.0 <sup>b</sup>	25.5 ± 0.1 <sup>a</sup> 33.2 ± 0.1 <sup>b</sup>	45.1 ± 0.0 <sup>a</sup> 54.1 ± 0.1 <sup>b</sup>	2.8 ± 0.1 <sup>a</sup> 2.2 ± 0.2 <sup>b</sup>	
	6	----	9.2 ± 0.3 <sup>a</sup> 9.8 ± 0.1 <sup>b</sup>	3.5 ± 0.1 <sup>a</sup> 4.0 ± 0.1 <sup>b</sup>	24.3 ± 0.2 <sup>a</sup> 30.1 ± 0.1 <sup>b</sup>	45.8 ± 0.1 <sup>a</sup> 53.7 ± 0.2 <sup>b</sup>	2.8 ± 0.1 <sup>a</sup> 2.3 ± 0.2 <sup>b</sup>	
	7	----	9.3 ± 0.4 <sup>a</sup> 9.4 ± 0.2 <sup>b</sup>	3.4 ± 0.3 <sup>a</sup> 4.4 ± 0.2 <sup>b</sup>	24.5 ± 0.0 <sup>a</sup> 32.0 ± 0.3 <sup>b</sup>	50.2 ± 0.2 <sup>a</sup> 55.8 ± 0.1 <sup>b</sup>	2.7 ± 0.3 <sup>a</sup> 2.1 ± 0.1 <sup>b</sup>	
	8	----	9.3 ± 0.4 <sup>a</sup> 9.3 ± 0.1 <sup>b</sup>	3.7 ± 0.4 <sup>a</sup> 4.8 ± 0.1 <sup>b</sup>	27.7 ± 0.1 <sup>a</sup> 40.0 ± 0.2 <sup>b</sup>	37.3 ± 0.4 <sup>a</sup> 52.9 ± 0.1 <sup>b</sup>	2.3 ± 0.2 <sup>a</sup> 1.9 ± 0.0 <sup>b</sup>	
	Correlation			0.890 <sup>a</sup> 0.873 <sup>b</sup>	0.873 <sup>a</sup> 0.750 <sup>b*</sup>	0.873 <sup>a</sup> 0.793 <sup>b*</sup>	-0.904 <sup>a</sup> -0.888 <sup>b</sup>	-0.903 <sup>a</sup> -0.876 <sup>b</sup>
	Frying oil (FPO)	0	0.82 ± 0.01 <sup>a</sup> 0.81 ± 0.02 <sup>b</sup>	39.6 ± 0.1 <sup>a</sup> 39.6 ± 0.1 <sup>b</sup>	4.2 ± 0.2 <sup>a</sup> 4.2 ± 0.0 <sup>b</sup>	32.8 ± 0.0 <sup>a</sup> 32.9 ± 0.0 <sup>b</sup>	20.6 ± 0.2 <sup>a</sup> 21.2 ± 0.1 <sup>b</sup>	1.07 ± 0.00 <sup>a</sup> 1.04 ± 0.01 <sup>b</sup>
		1	0.81 ± 0.01 <sup>a</sup> 0.82 ± 0.02 <sup>b</sup>	39.7 ± 0.3 <sup>a</sup> 39.7 ± 0.2 <sup>b</sup>	4.2 ± 0.2 <sup>a</sup> 4.2 ± 0.3 <sup>b</sup>	32.8 ± 0.4 <sup>a</sup> 33.4 ± 0.3 <sup>b</sup>	20.1 ± 0.0 <sup>a</sup> 20.6 ± 0.1 <sup>b</sup>	1.05 ± 0.02 <sup>a</sup> 0.99 ± 0.05 <sup>b</sup>
		2	0.82 ± 0.02 <sup>a</sup> 0.81 ± 0.02 <sup>b</sup>	39.7 ± 0.4 <sup>a</sup> 39.8 ± 0.3 <sup>b</sup>	4.3 ± 0.1 <sup>a</sup> 4.2 ± 0.4 <sup>b</sup>	32.8 ± 0.3 <sup>a</sup> 33.1 ± 0.3 <sup>b</sup>	20.6 ± 0.3 <sup>a</sup> 20.4 ± 0.1 <sup>b</sup>	1.03 ± 0.02 <sup>a</sup> 0.96 ± 0.04 <sup>b</sup>
		3	0.82 ± 0.01 <sup>a</sup> 0.82 ± 0.03 <sup>b</sup>	39.7 ± 0.4 <sup>a</sup> 39.7 ± 0.2 <sup>b</sup>	4.3 ± 0.1 <sup>a</sup> 4.4 ± 0.3 <sup>b</sup>	32.9 ± 0.3 <sup>a</sup> 33.7 ± 0.3 <sup>b</sup>	20.2 ± 0.0 <sup>a</sup> 20.2 ± 0.2 <sup>b</sup>	0.89 ± 0.01 <sup>a</sup> 0.95 ± 0.03 <sup>b</sup>
		4	0.83 ± 0.03 <sup>a</sup> 0.82 ± 0.01 <sup>b</sup>	39.8 ± 0.2 <sup>a</sup> 39.8 ± 0.2 <sup>b</sup>	4.3 ± 0.3 <sup>a</sup> 4.6 ± 0.4 <sup>b</sup>	33.1 ± 0.3 <sup>a</sup> 33.5 ± 0.0 <sup>b</sup>	20.1 ± 0.0 <sup>a</sup> 18.8 ± 0.1 <sup>b</sup>	0.91 ± 0.01 <sup>a</sup> 0.90 ± 0.04 <sup>b</sup>
		5	0.83 ± 0.02 <sup>a</sup> 0.82 ± 0.03 <sup>b</sup>	39.8 ± 0.3 <sup>a</sup> 39.8 ± 0.3 <sup>b</sup>	4.3 ± 0.2 <sup>a</sup> 4.4 ± 0.2 <sup>b</sup>	33.1 ± 0.4 <sup>a</sup> 35.0 ± 0.0 <sup>b</sup>	19.7 ± 0.0 <sup>a</sup> 19.8 ± 0.2 <sup>b</sup>	0.91 ± 0.03 <sup>a</sup> 0.78 ± 0.03 <sup>b</sup>
		6	0.84 ± 0.01 <sup>a</sup> 0.84 ± 0.01 <sup>b</sup>	39.8 ± 0.2 <sup>a</sup> 39.9 ± 0.4 <sup>b</sup>	4.3 ± 0.4 <sup>a</sup> 4.2 ± 0.0 <sup>b</sup>	33.4 ± 0.2 <sup>a</sup> 33.4 ± 0.3 <sup>b</sup>	19.3 ± 0.2 <sup>a</sup> 19.1 ± 0.3 <sup>b</sup>	0.88 ± 0.04 <sup>a</sup> 0.85 ± 0.04 <sup>b</sup>
7		0.84 ± 0.02 <sup>a</sup> 0.84 ± 0.01 <sup>b</sup>	39.9 ± 0.2 <sup>a</sup> 39.9 ± 0.4 <sup>b</sup>	4.4 ± 0.0 <sup>a</sup> 4.5 ± 0.1 <sup>b</sup>	33.4 ± 0.1 <sup>a</sup> 35.1 ± 0.1 <sup>b</sup>	19.0 ± 0.0 <sup>a</sup> 18.9 ± 0.1 <sup>b</sup>	0.80 ± 0.03 <sup>a</sup> 0.80 ± 0.03 <sup>b</sup>	
8		0.85 ± 0.01 <sup>a</sup> 0.85 ± 0.02 <sup>b</sup>	40.1 ± 0.0 <sup>a</sup> 39.9 ± 0.4 <sup>b</sup>	4.5 ± 0.0 <sup>a</sup> 4.9 ± 0.3 <sup>b</sup>	33.5 ± 0.5 <sup>a</sup> 36.9 ± 0.2 <sup>b</sup>	18.9 ± 0.0 <sup>a</sup> 15.7 ± 0.0 <sup>b</sup>	0.78 ± 0.03 <sup>a</sup> 0.76 ± 0.03 <sup>b</sup>	
Correlation		0.935 <sup>a</sup> 0.897 <sup>b</sup>	0.911 <sup>a</sup> 0.909 <sup>b</sup>	0.885 <sup>a</sup> 0.704 <sup>b*</sup>	0.962 <sup>a</sup> 0.805 <sup>b</sup>	-0.935 <sup>a</sup> -0.851 <sup>b</sup>	-0.953 <sup>a</sup> -0.951 <sup>b</sup>	
Canola oil (HLCO)		0	0.06 ± 0.00 <sup>a</sup> 0.06 ± 0.01 <sup>b</sup>	4.3 ± 0.1 <sup>a</sup> 4.3 ± 0.0 <sup>b</sup>	1.8 ± 0.1 <sup>a</sup> 2.1 ± 0.0 <sup>b</sup>	56.5 ± 0.0 <sup>a</sup> 57.0 ± 0.0 <sup>b</sup>	24.9 ± 0.0 <sup>a</sup> 25.1 ± 0.1 <sup>b</sup>	7.2 ± 0.0 <sup>a</sup> 7.2 ± 0.0 <sup>b</sup>
		1	0.06 ± 0.05 <sup>a</sup> 0.07 ± 0.03 <sup>b</sup>	4.5 ± 0.1 <sup>a</sup> 3.5 ± 0.2 <sup>b</sup>	1.7 ± 0.1 <sup>a</sup> 2.7 ± 0.2 <sup>b</sup>	57.2 ± 0.2 <sup>a</sup> 62.0 ± 00.4 <sup>b</sup>	24.8 ± 0.0 <sup>a</sup> 21.6 ± 0.0 <sup>b</sup>	5.6 ± 0.1 <sup>a</sup> 6.1 ± 0.1 <sup>b</sup>
		2	0.07 ± 0.06 <sup>a</sup> 0.08 ± 0.05 <sup>b</sup>	4.6 ± 0.1 <sup>a</sup> 4.9 ± 0.1 <sup>b</sup>	1.8 ± 0.2 <sup>a</sup> 2.9 ± 0.2 <sup>b</sup>	58.1 ± 0.1 <sup>a</sup> 65.4 ± 0.1 <sup>b</sup>	24.6 ± 0.1 <sup>a</sup> 20.1 ± 0.2 <sup>b</sup>	5.5 ± 0.2 <sup>a</sup> 4.7 ± 0.1 <sup>b</sup>
		3	0.08 ± 0.03 <sup>a</sup> 0.08 ± 0.04 <sup>b</sup>	4.9 ± 0.0 <sup>a</sup> 4.0 ± 0.1 <sup>b</sup>	1.9 ± 0.1 <sup>a</sup> 2.9 ± 0.2 <sup>b</sup>	59.0 ± 0.2 <sup>a</sup> 65.4 ± 0.1 <sup>b</sup>	23.7 ± 0.2 <sup>a</sup> 20.1 ± 0.2 <sup>b</sup>	5.0 ± 0.0 <sup>a</sup> 4.8 ± 0.2 <sup>b</sup>
		4	0.09 ± 0.08 <sup>a</sup> 0.10 ± 0.06 <sup>b</sup>	5.0 ± 0.4 <sup>a</sup> 5.4 ± 0.1 <sup>b</sup>	1.9 ± 0.1 <sup>a</sup> 2.5 ± 0.0 <sup>b</sup>	59.7 ± 0.1 <sup>a</sup> 67.3 ± 0.1 <sup>b</sup>	22.8 ± 0.2 <sup>a</sup> 19.9 ± 0.0 <sup>b</sup>	4.5 ± 0.1 <sup>a</sup> 4.5 ± 0.1 <sup>b</sup>
		5	0.08 ± 0.05 <sup>a</sup> 0.09 ± 0.04 <sup>b</sup>	5.1 ± 0.1 <sup>a</sup> 5.5 ± 0.2 <sup>b</sup>	2.1 ± 0.0 <sup>a</sup> 2.8 ± 0.3 <sup>b</sup>	60.4 ± 0.3 <sup>a</sup> 67.0 ± 0.0 <sup>b</sup>	21.2 ± 0.1 <sup>a</sup> 18.9 ± 0.1 <sup>b</sup>	5.1 ± 0.1 <sup>a</sup> 3.2 ± 0.1 <sup>b</sup>
		6	0.10 ± 0.04 <sup>a</sup> 0.10 ± 0.01 <sup>b</sup>	5.1 ± 0.1 <sup>a</sup> 4.7 ± 0.1 <sup>b</sup>	2.1 ± 0.2 <sup>a</sup> 3.0 ± 0.3 <sup>b</sup>	62.7 ± 0.1 <sup>a</sup> 70.1 ± 0.4 <sup>b</sup>	19.5 ± 0.3 <sup>a</sup> 16.7 ± 0.4 <sup>b</sup>	3.7 ± 0.1 <sup>a</sup> 2.7 ± 0.2 <sup>b</sup>
	7	0.09 ± 0.04 <sup>a</sup> 0.10 ± 0.02 <sup>b</sup>	5.1 ± 0.2 <sup>a</sup> 4.9 ± 0.1 <sup>b</sup>	2.1 ± 0.1 <sup>a</sup> 3.1 ± 0.2 <sup>b</sup>	62.2 ± 0.4 <sup>a</sup> 78.8 ± 0.1 <sup>b</sup>	18.1 ± 0.2 <sup>a</sup> 15.4 ± 0.2 <sup>b</sup>	3.2 ± 0.2 <sup>a</sup> 3.5 ± 0.2 <sup>b</sup>	
	8	0.10 ± 0.07 <sup>a</sup> 0.10 ± 0.01 <sup>b</sup>	5.2 ± 0.2 <sup>a</sup> 6.3 ± 0.2 <sup>b</sup>	2.2 ± 0.1 <sup>a</sup> 3.5 ± 0.1 <sup>b</sup>	62.3 ± 0.4 <sup>a</sup> 76.3 ± 0.2 <sup>b</sup>	18.3 ± 0.3 <sup>a</sup> 15.3 ± 0.0 <sup>b</sup>	3.3 ± 0.2 <sup>a</sup> 2.9 ± 0.2 <sup>b</sup>	
	Correlation		0.921 <sup>a</sup> 0.913 <sup>b</sup>	0.941 <sup>a</sup> 0.719 <sup>b*</sup>	0.944 <sup>a</sup> 0.806 <sup>b</sup>	0.972 <sup>a</sup> 0.974 <sup>b</sup>	-0.968 <sup>a</sup> -0.958 <sup>b</sup>	-0.937 <sup>a</sup> -0.921 <sup>b</sup>

<sup>a</sup>Samples after adding TBHQ; <sup>b</sup>Samples before adding TBHQ; Mean value of samples <sup>a</sup> and <sup>b</sup> are not significantly different ( $p < 0.01$ ); Values are expressed as mean ± SD ( $n \leq 3$ )

patterns is more obvious for oils in the presence of TBHQ. According to the Table-1, Pearson correlation coefficients of C14:0, C16:0, C18:0, C18:1 is positive while correlation of C18:2 and C18:3 are negative. Nzikou *et al.*<sup>25</sup> reported high correlation coefficient (0.989) between time of frying and decreasing sunflower oil C18:3.

**Principle component analysis on the effect of antioxidant on the thermoxidation of edible oils:** To quantify the effect of antioxidant on the thermoxidation of edible oils we used PCA as a multivariate statistical analysis tool<sup>26</sup>. Principle component analysis reduces the effect of too many observable and original variables to the few very compact variables, while keeps all the information of the original one. In this manner, the inter correlation of the original variables are eliminated, which results in orthogonal variables without any interrelation. Here the data matrix X has 54 rows, which each row is an edible oil sample and 10 columns, which are 7 fatty acids and three common indices. The column variables are as follows: relative percentage of marked seven fatty acids C14:0, C16:0, C16:1, C18:0, C18:1, C18:2, C18:3 and three common indicators as peroxide, anisidine and free fatty acid index. The row object variables are edible samples before and after 1 h of heating step and with and without TBHQ.

The application of the PCA on the resulting data matrix gives two very important matrix, scores and loadings. The plot of the two first columns of the score matrix is known as score-score or score-plot. This plot shows the map or distribution of the object or samples in the column or variable space.

The effect of the TBHQ on the thermoxidation of the sunflower oil (MLSO) can be seen in Fig. 1. It can be seen that samples after adding TBHQ (a) vary along PC2 which can be related to the AO effect, where samples (a) can be classified in a separate class, while samples before adding AO (b) scatters along PC1 and PC2. It should be noted that unused MLSO samples (0) are included in the class of samples (a).

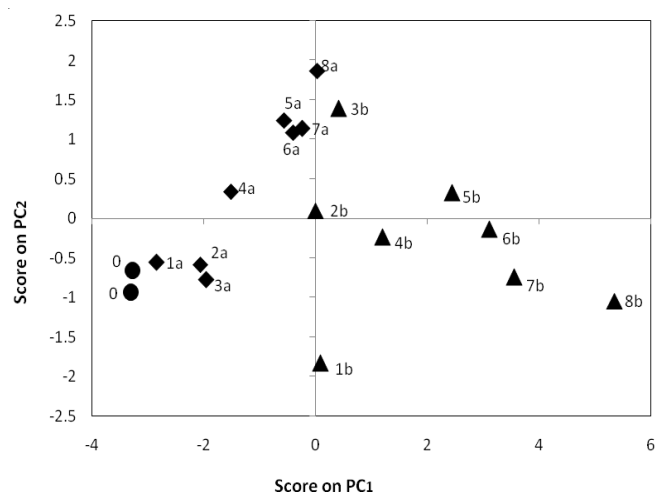


Fig. 1. Score plot of medium-linolenic sunflower oil (MLSO) with and without TBHQ; <sup>0</sup>Unused MLS; <sup>1b</sup>MLS before adding TBHQ, after 1 h heating; <sup>1a</sup>MLS after adding TBHQ, after 1 h heating

The same study has been examined for frying oil formulated based on palmolein (FPO) in Fig. 2. Samples (a) vary along PC1, thus PC1 can reflect the time of heating. The difference between samples (a) and (b) can be seen along PC2.

Hence PC2 can reflect the effect of TBHQ. On the other hand, the differences between samples (a) are negligible and they can be classified in a separate class. Again samples (0) are included in class (a), however, samples (b) scatter across the plane.

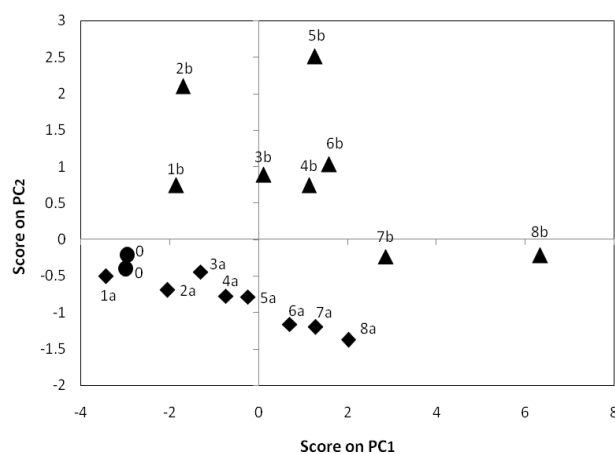


Fig. 2. Score plot of frying oil based on palm olein (FPO) with and without TBHQ; <sup>0</sup>Unused FPO; <sup>1b</sup>FPO before adding TBHQ, after 1 h heating; <sup>1a</sup>FPO after adding TBHQ, after 1 h heating

Fig. 3 shows the same study for canola oil (HLCO). Again we can recognize separate classes for samples (a). Moreover, samples (6a), (7a) and (8a) are classified in a different group, which shows that heating variations of this oil are similar in time 6-8.

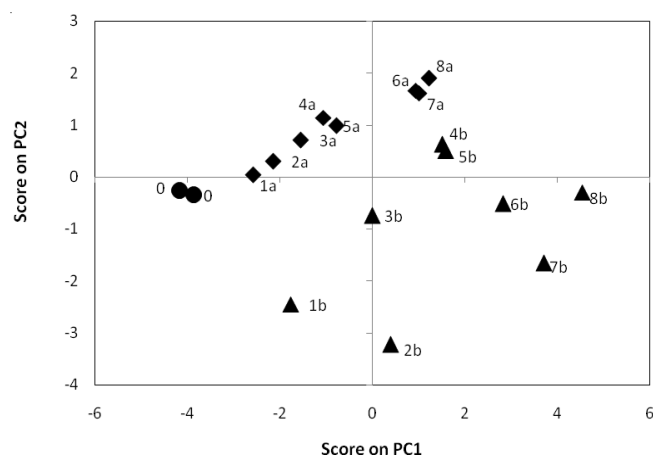


Fig. 3. Score plot of high-linolenic Canola oil (HLCO) with and without TBHQ; <sup>0</sup>Unused Canola oil; <sup>1b</sup>Canola oil before adding TBHQ, after 1 h heating; <sup>1a</sup>Canola oil after adding TBHQ, after 1 h heating

In this regard that in all examined oil types; the variations due to heating in the samples with TBHQ are small where we are able to classify them in specific groups.

Fig. 4a illustrates the plot of score matrix including 54 samples or objects. Three oil types can be classified in three different classes. Samples (a) and (b) in FPO and HLCO are not separable while PC1 component of samples (a) and (b) is different in MLSO which shows that PC1 reflects antioxidant effect. Also in this oil type, samples (a) are grouped in the same class with samples (0) with a slight difference. As a result, we conclude that the effect of antioxidant on the thermoxidation of the sunflower oil type is more than HLCO and FPO types.

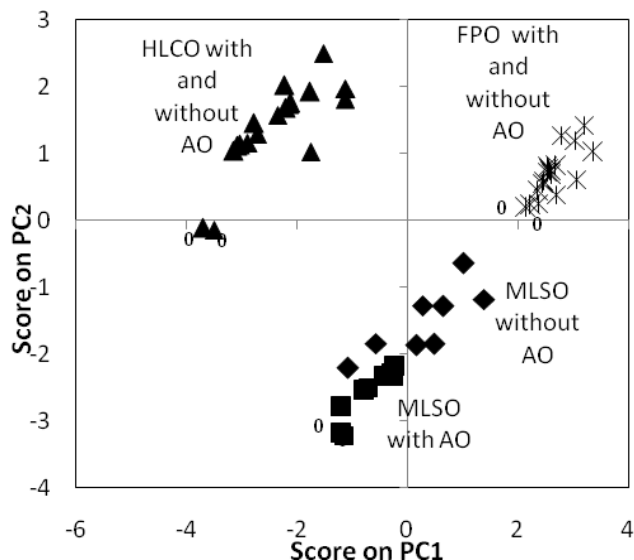


Fig. 4a. PCA plot of triplet comparison of antioxidant effect on MLSO, FPO and of HLCO

Fig. 4b shows PCA analysis of score matrix of MLSO and FPO samples. The PC2 components of samples (a) and (b) in MLSO are different, where PC2 components of samples (a) are very close, though it is not the case for samples (b). So in this comparison, PC2 axis reflects the effect of antioxidant. On the other hand samples (a) and (b) in FPO shows less variation and all of them can be classified in a unique class. Again, we can see the significant effect of antioxidant in MLSO.

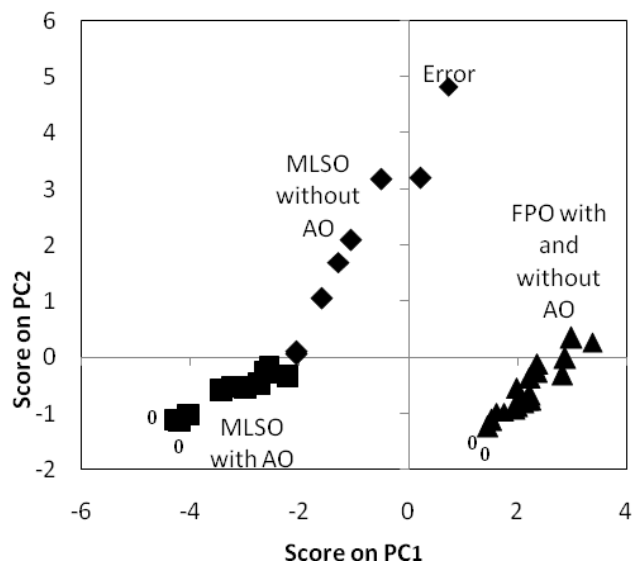


Fig. 4b. PCA plot of MLSO and FPO comparison

In Fig. 4c we can see the comparison of HLCO and FPO samples. Although samples (a) and (b) can not be completely took apart, these samples varieties along PC2. The variations of palm oil samples are less than HLCO ones. This shows that the effect of antioxidant on HLCO is more extensive than FPO. It should be noted that samples (0) in HLCO show a large amount of difference to first time of heating.

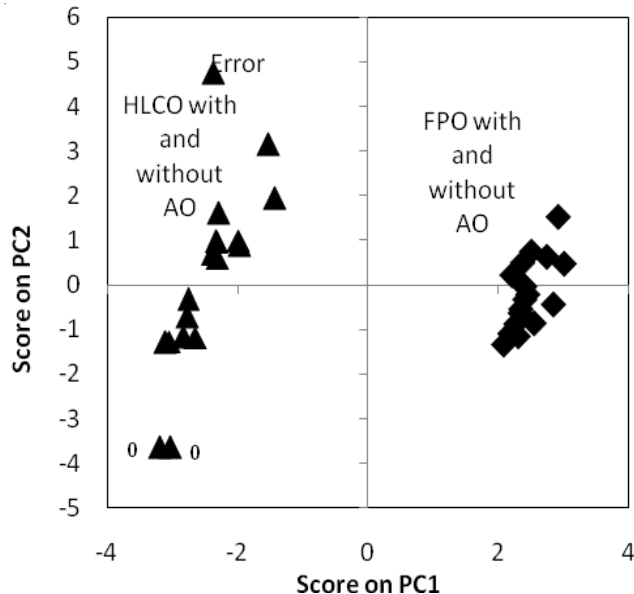


Fig. 4c. PCA plot of HLCO and FPO comparison

Fig. 4d illustrates the comparison analysis of HLCO and MLSO samples. In MLSO samples, we can see a large amount of variation along PC2 and samples (a) to some extent can be separated from samples (b). These variations determine that PC2 reflects the effect of antioxidant. On the other hand, although PC2 component of HLCO samples varies during heating, we can not separate samples (a) from (b); it determines the more effect of antioxidant on the thermoxidation of MLSO.

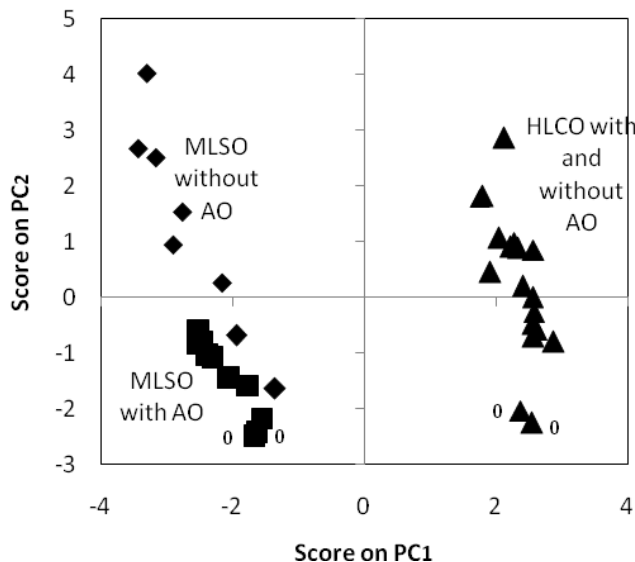


Fig. 4d. PCA plot of HLCO and MLSO comparison

**Conclusion**

Fatty acid profile and antioxidant are two important parameters to inhibit thermoxidation of edible oils. Oils with high proportion of saturated fatty acids in compare with unsaturated ones are naturally more resistant to thermo processing. Principle component analysis comparison of the effect of antioxidant on the thermoxidation process of frying oil formulated based on palm olein, sunflower and canola oil indicates that TBHQ affects weakly on heating variations of



palm and affects strongly on sunflower oil. It seems that there is a correlation between fatty acid profile and antioxidant such that the effect of antioxidant on the thermoxidation process of oils with different fatty acid compositions are not the same and in oils with high proportion of unsaturated fatty acids the effect of antioxidant are more significant. Consequently, profile of fatty acids is a more determinant parameter in thermoxidation than antioxidant.

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