



## Effect of Fatty Acid Composition on Thermal Stability of Extra Virgin Olive Oil

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### Article type

Original article

### Abstract

### Keywords

Olive Oil  
Fatty Acids  
Differential Thermal Analysis

Received: 29 Sep 2014

Revised: 8 Nov 2014

Accepted: 18 Dec 2014

**Background:** Fatty acids are the main compounds in edible oils. Oil thermal stability depends on the composition of fatty acids. So, this study was conducted to investigate the effect of fatty acid composition on the oxidative stability of extra virgin olive oil during heating process.

**Methods:** Totally, eight virgin olive oil samples, including five imported oils from market place of Mahabad and three local ones from Qazvin were analyzed to evaluate their thermal stability. Samples were heated at 120 °C for 4 h. Sampling was carried out in 2 h intervals, then fatty acid composition, peroxide, anisidine and totox values were assessed according to Iran national standards. All determinations were carried out in triplicates, and data were subjected to analysis of compare means (t test) and spearman correlation coefficients using SPSS 16.0.

**Results:** Results show that there is a significant correlation between palmitoleic acid and totox index in 2 h ( $r=0.786$ ) and 4 h ( $r=0.762$ ), and the same result is observed between linoleic and totox index in 2 h ( $r=0.643$ ) and 4 h ( $r=0.786$ ). But, there is an inverse relationship between oleic acid and totox index in 4 h ( $r=-0.833$ ).

**Conclusion:** Results indicated that linoleic acid and palmitoleic acid have a reducing effect on thermal stability of extra virgin olive oil in 2 h, but oleic acid increases it in 4 h.

### Introduction

The importance of oils and fats, apart from the health point of view, is their role in commerce and economy. The oil quality monitoring uses quality indicators based on quality attributes as hour of use, smoke evolution, foam height and changing of the original color or by measuring quality variables as percentage of free fatty acids, peroxide value, p-anisidine levels, total polar compounds, fatty acid composition and temperature (Formisano et al., 1971). Frying food is an old method of cooking (Hammond, 2002; Razali and Badri, 2003; Razali et al., 2003).

Extra virgin olive oil keeps all its vitamins, essential fatty acids and other nutrients including powerful antioxidants. So, it is recognized as one of the best food for its capacity to prevent some pathology such as cancer and cardiovascular diseases (Kontogianni et al., 2007). Olive oil is obtained from fruit of the olive tree and classified in the types of virgin, treated, and mix of treated and virgin olive oils. Virgin olive oil is prepared directly by mechanical methods without any changes (ISIRI, 2010). Phenolic compounds, polar and non-polar compounds and much more, mono unsaturated fatty acids in olive oil engender beneficial effects on health and dramatically reduce cardiovascular diseases (Covas et al., 2006). Ac-

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According to empirical studies, olive oil compounds have biological activities including anti-inflammatory, antioxidant, anti-arrhythmias and vasodilation effects (Covas et al., 2006). Olive oil contains vitamins E and A (Harwood and Aparicio, 2000), as well as the consequent squalene and beta-carotene (Huang et al., 2009). According to Iran national standards, sensory properties of extra virgin olive oils include organoleptic characteristics, smell, taste and color (ISIRI, 2010).

During the thermal processing of oil, oxygen, moisture and high temperature increase oxidation and reduce the nutritional quality (Razali and Badri, 2003; Razali et al., 2003). Studies have shown that the thermal stability of oil during heating is related to fatty acid compounds, the presence of antioxidants, blush and nutrient characteristics conditions (Huang et al., 2000; Pakash Kochhar, 2000). Studies have shown that high heat resistance of olive oil is due to the presence of fatty acids and natural antioxidants (Boskou and Elmadfa, 1999). The content of fatty acids is an important parameter for evaluating the quality of the oil (Kwon and Rhee, 1986).

Given the growing importance of extra virgin olive oil in cooking and its nutritional beneficial effects rather than other oils (Kwon and Rhee, 1986), this research was conducted with the aim to investigate the effect of fatty acids composition during heating process on oxidative stability of extra virgin olive oil.

## Materials and methods

### Chemicals

Solvents and chemicals used in this study were analytical grade and were purchased from Merck® (Germany).

### Sampling and thermal test

In this study, eight extra virgin olive oil samples, including five imported oils from market place of Mahabad and three local ones from Qazvin were purchased. Samples were heated by 120 °C adjusted oven for 4 h in a continuous manner. Sampling was done at intervals of 2 h and then, cooled samples were tested.

### Analysis parameters

Fatty acids were evaluated as their methyl esters after alkaline trans-esterification with cold methanolic solution of sodium methoxide 0.5 N. The gas chromatograph (Varian CP-3800) equipped with a flame ionization detector (FID) and a capillary column 50 m×0.25 mm×0.20 µm film thickness (BPX70), filled with diethylene glycol succinate each fatty acid including palmitic acid (C16:0), palmitoleic acid (C16:1), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2) and linolenic acid (C18:3). They were also quantified in terms of the area under the

reported peak. Helium gas was used as the carrier. The column temperature at first was 156 °C and immediately increased in the ratio of 0.4 °C/min up to 160 °C (holding for 2 min), then the increase ratio was 5 °C/min up to 210 °C (holding for 10 min). One µl of the sample was injected, when detector temperature was 230 °C and injection temperature was 250 °C. The automatic sampler was used for sample injection. In this study, fatty acid profile was measured based on Iran national standards No. 4091 and 4090 (ISIRI, 1992a; ISIRI, 1992b). Peroxide index was measured by iodometry method in accordance with the national standard No. 4179, by oil titration using sodium thiosulfate 0.1 N in the presence of starch and potassium iodide (ISIRI, 1998). To accomplish the anisidine index test, samples were prepared firstly by isooctane and reacted with the solution of paraanisidin acetic acid. Then, the increase in absorption area at 350 nm was measured and the anisidine number was calculated according to national standard No. 4093 (ISIRI, 1997). In this study, fatty acids profile, indexes of peroxide and anisidine were repeated 3 times.

### Definitions

Peroxide index illustrates the amount of peroxide in terms of mEq of active oxygen for 1 kg of oil. This index shows the amount of primary oxidation products (hydroperoxides) that are heat unstable (ISIRI, 1998). Anisidine index is empirically defined as 100 times the absorbance of a solution resulting from 1 g of fat or oil mixed with 100 ml of isooctane/acetic acid/p-anisidine reagent, measured at 350 nm in a 10 mm cell under the conditions of the test. This index represents the amount of secondary oxidation metabolite (aldehydes and ketones) which are more stable compared to the primary products of oxidation peroxides (ISIRI, 1997). Totox index is a criterion of the total oxidation that is calculated from sum of twice peroxide index and the anisidine index (ISIRI, 1997).

### Statistical analysis

All determinations were carried out in triplicates, and data were subjected to analysis of compare means (t test) and spearman correlation coefficients using SPSS 16.0 software.

## Results

At first, the fatty acid compositions of olive oil samples were investigated. Based on the results, we can confirm that all the oils selected according to Iran national standard were in the interval of the determined standards for extra virgin olive oils. In other words, it is obvious that all the oil samples were extra virgin olive oil (Table 1).

Next the mean, coefficient of variation, maximum and minimum of fatty acids compositions of extra virgin olive oil were calculated (Table 2). Then, the changes

in totox index and correlation coefficient between fatty acids and totox index were determined (Table 3 and 4).

**Table 1:** The fatty acids compounds of extra virgin olive oil

	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8
Palmitic acid	10.99±0.04 <sup>c</sup>	10.12±0.02 <sup>d</sup>	10.04±0.2 <sup>d</sup>	11.13±0.28 <sup>b</sup>	13.88±0.01 <sup>a</sup>	13.50±0.38 <sup>a</sup>	9.93±0.01 <sup>e</sup>	11.52±0.12 <sup>b</sup>
Palmitoleic acid	0.71±0.03 <sup>d</sup>	0.66±0.07 <sup>e</sup>	0.60±0.03 <sup>f</sup>	0.73±0.06 <sup>c</sup>	0.76±0.03 <sup>b</sup>	0.87±0.09 <sup>a</sup>	0.72±0.1 <sup>d</sup>	0.73±0.07 <sup>c</sup>
Stearic acid	2.65±0.04 <sup>c</sup>	3.27±0.03 <sup>b</sup>	3.16±0.04 <sup>b</sup>	1.79±0.01 <sup>f</sup>	3.88±0.09 <sup>a</sup>	2.04±0.03 <sup>e</sup>	2.39±0.19 <sup>d</sup>	2.23±0.05 <sup>d</sup>
Oleic acid	77.55±0.09 <sup>c</sup>	79.48±0.14 <sup>a</sup>	76.56±0.1 <sup>d</sup>	74.29±0.05 <sup>e</sup>	69.13±0.2 <sup>g</sup>	69.35±0.3 <sup>g</sup>	78.40±0.2 <sup>b</sup>	73.35±0.02 <sup>f</sup>
Linoleic acid	5.51±0.01 <sup>d</sup>	3.95±0.03 <sup>f</sup>	6.61±0.02 <sup>c</sup>	8.32±0.12 <sup>b</sup>	9.29±0.07 <sup>a</sup>	9.38±0.12 <sup>a</sup>	5.01±0.12 <sup>e</sup>	8.22±0.04 <sup>b</sup>
Linolenic acid	0.63±0.02 <sup>b</sup>	0.40±0.02 <sup>f</sup>	0.71±0.06 <sup>a</sup>	0.46±0.07 <sup>cd</sup>	0.57±0.09 <sup>c</sup>	0.52±0.03 <sup>c</sup>	0.43±0.03 <sup>e</sup>	0.49±0.02 <sup>d</sup>

Numbers within a row with the same lowercase letters are not significantly different ( $p < 0.05$ )

**Table 2:** Mean, coefficient of variation, minimum and maximum fatty acid structure (%) of extra virgin olive oil

Fatty acid	Coefficient of variation (%)	Mean	Maximum	Minimum
Palmitic acid	13.48	11.42±1.54	13.88	9.93
Palmitoleic acid	11.11	0.72±0.07	0.87	0.60
Stearic acid	26.21	2.67±0.7	3.88	1.79
Oleic acid	5.21	74.8±3.9	79.48	69.13
Linoleic acid	29.30	7.03±2.06	9.38	3.95
Linolenic acid	18.86	0.53±0.1	0.71	0.40

**Table 3:** Totox index of extra virgin olive oil samples

Oil samples	Heating time (h)		
	0	2	4
1	11.67	55.99	18.19
2	7.84	56.85	17.53
3	6.63	49.3	24.98
4	7.75	80.9	48.3
5	11.2	66.26	63.66
6	14.68	29.13	26.9
7	10.67	63.36	25.26
8	6.46	59.19	28.73

**Table 4:** The correlation coefficient between fatty acids and totox index

Fatty acid	h 0	h 2	h 4
Palmitic acid	0.286	0.548	0.690*
Palmitoleic acid	0.381	0.786*	0.762*
Stearic acid	0.71	-0.548	-0.286
Oleic acid	-0.143	-0.524	-0.833**
Linoleic acid	0.283	0.643*	0.786*
Linolenic acid	0.143	0.381	0.071

Mark \* in each column indicates statistically significant different ( $p < 0.05$ )

Mark \*\* in each column indicates statistically significant different ( $p < 0.01$ )

## Discussion

Oleic acid, as a monounsaturated fatty acid, is the most abundant fatty acids in oils and palmitic acid, linoleic acid, stearic acid, palmitoleic acid and linolenic acid are in the next rating, respectively. Based on this coefficient we can obtain extra virgin olive oil fatty acids dispersion. The results showed that fatty acids content of oils varied widely. Based on the obtained results, the average of oleic acid with the amount of 74.8% and palmitoleic acid with the amount of 72%, are the lowest and highest fatty acids found in extra virgin olive oils, respectively. The obtained results in this study were comparable with the results of the Boskou (1996) study. Since peroxide compounds were broken during thermal process, the peroxide

index is not a reliable index for oil oxidation determination, so totox index is used (Billek, 1978; Fatemi, 2005). The peroxide index was broken in 2 h, which is not considering in an oxidation reduction category. By regard to decomposition of the hydroperoxide to secondary compounds such as aldehyde and ketone, oxidation process will continue (Pokorny and Sakurai, 2002); therefore, totox index decreases after the second hour. Totox index is a criterion of the total oxidation that calculated from sum of two times of the peroxide index and the anisidine index. Totox index calculation showed that heating process changes the totox index at different stages, changes peroxide and anisidine index and subsequent changes totox index. This is similar to results of previous studies

(Abdulkarim et al., 2007; Ayadi et al., 2009; Billek, 1978; Casal et al., 2010).

There is an indirect correlation between totox index and thermal stability, which means increasing in the amount of totox index decreases thermal stability due to an increasing the rate of oxidation. As observed in the results, there is a significant relationship between palmitoleic acid and totox index in second and fourth hours at 5% level. This means that increasing the amount of palmitoleic acid in the olive oil increases totox index, which can reduce the thermal stability of the oil during heating. Also, the coefficient between linoleic and totox index in second and fourth hours showed a significant correlation ( $p < 0.05$ ). Linoleic acid is polyunsaturated fatty acids that contain two double bonds. It seems that increasing linoleic acid in olive oil increases the oxidation. These results are similar to results of study done by Mariod et al. (2005). The study by Xu et al. (1999) showed that linoleic acid amounts decrease will enhance the oxidative stability of oils and so reduce the bitter taste in food. Abdulkarim et al. (2005) compared also the thermal stability of vegetable oils containing high levels of polyunsaturated fatty acids such as linoleic and linolenic acid and concluded that they have higher totox value, which means the oxidation of the oil. Results showed direct relationship between linoleic acid and totox index in the second and fourth hours. There was a reverse relationship between oleic acid and totox index and the reverse relationship in the fourth hour of the heating in 1% level was highly significant. These results indicated that oleic acid is one of the most important fatty acids in olive oil and that can increase the thermal stability of oil during heating process. Also, these results correspond with the results of Mariod et al. (2005).

Based on the results, we can conclude that in the early hours of the oxidation, fatty acids have no significant effect on the totox index. This premise is probably due to the phenolic compounds and tocopherols presence in the olive oil that are destroyed quickly in the first hours of heating, and those compounds are responsible for the thermal resistance of the oil in the early hour (Mirrezaie and Sahari, 2013). The explanation is that, phenolic compounds affect the thermal stability in the early hours which are destroyed during the time and fatty acids, especially oleic acid, are then going to be more effective in increasing of the thermal stability.

## Conclusion

In thermal stability determination, fatty acids compounds are not the only factors which must be considered, but on the basis of the results obtained in this research, it can be noted that the composition of fatty acids can affect the stability of oil during the heating process. It

can be finally concluded that oleic acid from the outset has a reverse relationship with totox index and intense significant differences were observed in 4 h which means that the influence of this fatty acid on the thermal stability is expanded by increasing the time of heating. Whereas palmitoleic acid and linoleic acid in 2 h are effective and decreasing the thermal stability of oil.

## Conflicts of interest

The authors of this article declare that they have no conflict of interest.

## Acknowledgement

This work was financially supported by Qazvin University of Medical Sciences, Qazvin, Iran.

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