

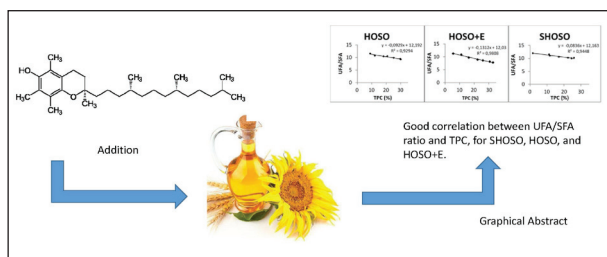
# Effects of $\alpha$ -tocopherol and oleic acid content in sunflower oil subjected to discontinuous and prolonged frying process

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**Summary.** Frying is a widespread cooking method that positively influences the sensory characteristics of food. Despite the advantages, physical and chemical changes leading to oil degradation can occur. The presence of antioxidants, such as  $\alpha$ -tocopherol, and a reduced content of polyunsaturated fatty acids are reported in the scientific literature as factors reducing oxidation processes. The aim of this study was to compare the effects of  $\alpha$ -tocopherol addition and the presence of a high concentration of oleic acid in sunflower oil subjected to a prolonged and discontinuous frying process. Every 8 hours of process, the following determinations were performed on oils: free fatty acids (FFA), peroxides value (PV), fatty acids composition (FA), total polar compounds (TPC). High oleic sunflower oil showed the best frying performance, with lower total polar compounds, lower octanoic acid formation and a lower unsaturated/saturated fatty acids (UFA)/(SFA) ratio decrease than oil to which  $\alpha$ -tocopherol was added. These results showed that the presence of monounsaturated fatty acids may largely influence the heat resistance of sunflower oil more than the presence of  $\alpha$ -tocopherol.

**Key words:** sunflower oil, lipid oxidation,  $\alpha$ -tocopherol acetate, oleic acid, deep frying, thermal stability



## Introduction

Deep fat frying is one of the most popular and oldest cooking techniques in the world. In the frying process, the food matrix is immersed in an oil bath at temperatures of 150–190 °C (1). During the thermal treatment, important changes occur. These changes can be physical, such as heat and mass transfer, or

chemical, such as hydrolysis, oxidation, isomerization, cyclization and polymerization (2); in addition, volatile compounds are formed (3). Several compounds are responsible for off-flavours, while others are toxic at high concentrations. To achieve high-quality frying, it is important to consider certain parameters, such as the oil/matrix ratio, filling up, oil degree of unsaturation and presence of antioxidant compounds (1). It is known that the higher the degree of oil unsaturation, the faster the degradation process occurs (4).

In a previous study, the better frying performance of high oleic sunflower oil than conventional sunflower oil was demonstrated (5). In recent years, oils with tocopherols or other vitamins added have appeared on the market because tocopherols are known for their antioxidant capacity (6,7). It was reported that the addition of 200 ppm of  $\alpha$ -tocopherol improves the sta-

bility of frying oil, prolonging its useful life (8,9). The antioxidant activity of tocopherols depends on several factors, such as oil type and concentration (10-12). In fact,  $\alpha$ -tocopherol at high levels may have a pro-oxidant effect (13).

The aim of the study was to compare the frying performance of a high oleic oil with an  $\alpha$ -tocopherol addition to that of a sunflower oil with a higher content of oleic acid and no added  $\alpha$ -tocopherol during discontinuous and prolonged heat treatment of French fries, a typical frying process of fast food establishments and restaurants.

## Materials and methods

Commercially refined and deodorized high oleic sunflower oils, differing in oleic acid content, and frozen French fries were obtained from a local market.  $\alpha$ -Tocopherol acetate was obtained from Azienda Chimica E Farmaceutica (A.C.E.F.) srl (Piacenza, Italy).

Normally, sunflower oil contains a maximum of 40% of oleic acid (14), but thanks to genetic improvement techniques it has been possible to select sunflower cultivars with seeds from which oil, richer in oleic acid, is obtained. In this study two different sunflower oils were used, with respectively about 75% and 85% of oleic acid. The oil containing the lower oleic acid content was reported as HOSO (high oleic sunflower oil), while the oil containing the higher content of oleic acid was reported as SHOSO ('super' high oleic sunflower oil).

To evaluate the performance of an oil with  $\alpha$ -tocopherol, 280 ppm  $\alpha$ -tocopherol was added to HOSO. This oil was reported as HOSO+E.

### *Frying Protocol*

The frying process was carried out according to the procedure described by Romano et al. in 2012 (15). Briefly, oil was heated for a total of 6 days, 8 h per day. Each day, the fryer was filled with fresh oil.

Aliquots of 100 g of frozen French fries were fried each hour to stress the oil, simulating fast food conditions. Sampling was carried out every 8 h.

Thermo-oxidized oils were heated under the same conditions, but no food matrix was added to the oil.

### *Analytical Methods*

Thermo-oxidized and frying oils were subjected to the following determinations: free fatty acids (FFA), peroxides value (PV), fatty acids composition (FA), total polar compounds (TPC) as described by Romano et al. in 2012 (15).

Tocopherol content has been determined by HPLC-UV according to the method proposed by Grilo et al. in 2014 (16). Briefly, after an appropriate dilution of the oil samples, 20  $\mu$ l were injected in an HPLC System (Shimadzu, mod.LC-10ADVP) equipped with a UV-detector (Shimadzu, mod.SPD-M10AVP) and a reversed phase column (Spherisorb S5 ODS3 250 x 4,6 mm i.d). An isocratic elution was used with 1.0 ml/min flow of methanol at 100%. The wavelength of the UV-Detector was set at 292 nm. The identification and quantification of  $\alpha$ -Tocopherol were performed by comparison with the time of retention and the area of the standard.

All determination and experiments were performed in triplicate and presented results are the average values of three determinations. Data were subjected to analysis of variance (ANOVA) (XLSTAT 2006). Differences at  $P \leq 0.05$  were considered significant.

## Results and Discussion

### *FFA, PV and TPC*

FFAs are degradation products formed by the hydrolysis of triacylglycerols (4). The FFA values obtained for the three oils are reported in Table 1. Free fatty acids increased during the heat treatment. In general, the fried oils showed higher FFA values than the corresponding thermo-oxidized samples. This could be due to the release of water in the oil bath from the food matrix (17,18). In fact, frying is a complex process in which the transition and evaporation of water can occur, depending on food characteristics (19).

HOSO+E and SHOSO showed lower FFA values during both the thermo-oxidation and frying processes than HOSO. Moreover, SHOSO showed the lowest FFA values at the end of frying (0.24 and 0.65% during thermo-oxidation and frying at 48 h, respectively).

The peroxides showed an irregular trend because they decompose rapidly in secondary products, such as

**Table 1.** FFA (% oleic acid) trend in HOSO, HOSO+E and SHOSO at different treatment times.

Time (h)	HOSO		HOSO+E		SHOSO	
	T	F	T	F	T	F
0	0.28 <sup>a</sup> ±0.02	0.28 <sup>a</sup> ±0.00	0.22 <sup>a</sup> ±0.01	0.24 <sup>a</sup> ±0.01	0.13±0.01	0.14 <sup>a</sup> ±0.01
16	0.49 <sup>a</sup> ±0.09	0.47 <sup>a</sup> ±0.11	0.36 <sup>ab</sup> ±0.01	0.39 <sup>ab</sup> ±0.01	0.20±0.04	0.28 <sup>a</sup> ±0.05
32	0.83 <sup>b</sup> ±0.07	0.99 <sup>b</sup> ±0.14	0.52 <sup>b</sup> ±0.00	0.59 <sup>b</sup> ±0.03	0.21±0.01	0.44 <sup>ab</sup> ±0.01
48	1.06 <sup>b</sup> ±0.08	1.08 <sup>b</sup> ±0.11	0.70 <sup>b</sup> ±0.00	0.81 <sup>b</sup> ±0.00	0.24±0.05	0.65 <sup>b</sup> ±0.02

FFA: free fatty acids; HOSO: high oleic sunflower oil; HOSO+E: high oleic sunflower oil with  $\alpha$ -tocopherol-acetate; SHOSO: super high oleic sunflower oil; T: thermo-oxidized oil; F: frying oil.

a-d: Different letters in the same column correspond to statistical significant differences ( $P \leq 0.05$ ).

**Table 2.** PV (mEqO<sub>2</sub>/Kg oil) trend in HOSO, HOSO+E and SHOSO at different treatment times.

Time (h)	HOSO		HOSO+E		SHOSO	
	T	F	T	F	T	F
0	2.20 <sup>c</sup> ±0.09	2.14 <sup>a</sup> ±0.08	2.25 <sup>a</sup> ±0.21	2.29 <sup>b</sup> ±0.21	2.20 <sup>ab</sup> ±0.12	2.23 <sup>ab</sup> ±0.09
8	2.45 <sup>c</sup> ±0.04	1.53 <sup>a</sup> ±0.10	2.26 <sup>a</sup> ±0.23	2.85 <sup>b</sup> ±0.18	1.10 <sup>a</sup> ±0.14	2.90 <sup>a</sup> ±0.14
16	4.85 <sup>d</sup> ±0.11	1.47 <sup>a</sup> ±0.11	1.65 <sup>b</sup> ±0.06	3.08 <sup>ab</sup> ±0.00	2.90 <sup>a</sup> ±0.14	0.95 <sup>c</sup> ±0.49
24	5.50 <sup>c</sup> ±0.10	1.90 <sup>b</sup> ±0.05	2.36 <sup>a</sup> ±0.33	3.40 <sup>b</sup> ±0.14	1.90 <sup>b</sup> ±0.14	1.60 <sup>b</sup> ±0.00
32	8.41 <sup>b</sup> ±0.12	1.99 <sup>b</sup> ±0.14	1.85 <sup>b</sup> ±0.02	2.18 <sup>b</sup> ±0.11	1.20 <sup>cd</sup> ±0.13	2.85 <sup>a</sup> ±0.64
40	8.50 <sup>b</sup> ±0.07	2.60 <sup>a</sup> ±0.16	1.46 <sup>bc</sup> ±0.11	1.82 <sup>c</sup> ±0.01	1.80 <sup>b</sup> ±0.07	3.17 <sup>a</sup> ±0.00
48	9.16 <sup>a</sup> ±0.09	2.08 <sup>b</sup> ±0.11	1.57 <sup>bc</sup> ±0.08	1.96 <sup>c</sup> ±0.03	1.40 <sup>a</sup> ±0.09	1.20 <sup>b</sup> ±0.29

PV: peroxides value; HOSO: high oleic sunflower oil; HOSO+E: high oleic sunflower oil with  $\alpha$ -tocopherol-acetate; SHOSO: super high oleic sunflower oil; T: thermo-oxidized oil; F: frying oil.

a-c: Different letters in the same column correspond to statistical significant differences ( $P \leq 0.05$ ).

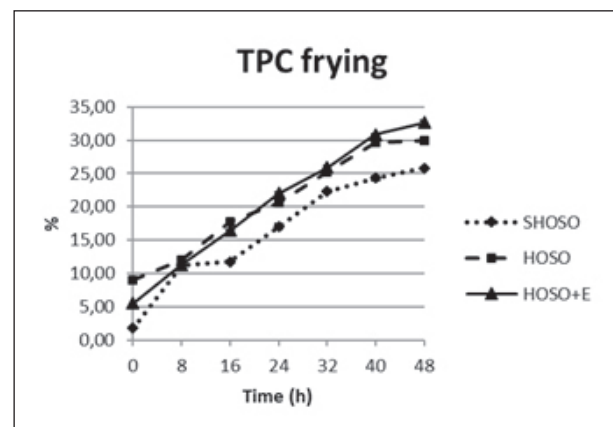
aldehydes, ketones and alcohols (17,18). The peroxides values are reported in Table 2. HOSO+E showed lower PV values than HOSO in the thermo-oxidized samples, with values of 1.57 and 9.16 mEqO<sub>2</sub>/Kg oil at 48 h, respectively. SHOSO showed lower PV values during the first hours of frying than the oil with added tocopherol.

The measurement of TPC is considered the most important test for assessing the degradation level of oils (4). In many countries, the maximum level of TPC allowed is 25%. As shown in Fig. 1, HOSO+E exceeded the maximum TPC limit at 32 h of frying, as did HOSO. In contrast, SHOSO exceeded the limit after 48 h of frying, with a value of 25.8%.

#### FA

Fatty acid composition has been recently indicated as a novel tool to differentiate similar food matrices by using specific markers: monounsaturated fatty acid

(MUFA) concentration, linolenic acid (18:3n3) concentration, C18:0/C18:1n9c and  $\Sigma$ MUFA/ $\Sigma$ SFA ratios (20,21).

**Figure 1.**

**Table 3.** Fatty acid composition of frying oils. HOSOE (1) and SHOSO (2).

<b>(1)</b>							
<b>HOSOE</b>							
	<b>t0</b>	<b>t8</b>	<b>t16</b>	<b>t24</b>	<b>t32</b>	<b>t40</b>	<b>t48</b>
<b>C8:0</b>	0.00 <sup>a</sup> ±0.00	0.04 <sup>de</sup> ±0.00	0.09 <sup>de</sup> ±0.01	0.13 <sup>bcd</sup> ±0.02	0.15 <sup>bc</sup> ±0.00	0.21 <sup>ab</sup> ±0.01	0.27 <sup>a</sup> ±0.06
<b>C16:0</b>	3.66 <sup>c</sup> ±0.03	3.53 <sup>c</sup> ±0.20	3.93 <sup>bc</sup> ±0.03	4.20 <sup>ab</sup> ±0.11	4.32 <sup>ab</sup> ±0.01	4.45 <sup>a</sup> ±0.22	4.63 <sup>a</sup> ±0.03
<b>C16:1</b>	0.18 ±0.01	0.16 ±0.02	0.17 ±0.00	0.18 ±0.02	0.17 ±0.00	0.17 ±0.01	0.18 ±0.00
<b>C18:0</b>	3.16 <sup>c</sup> ±0.01	3.31 <sup>d</sup> ±0.04	3.48 <sup>c</sup> ±0.01	3.67 <sup>b</sup> ±0.04	3.77 <sup>b</sup> ±0.02	3.88 <sup>a</sup> ±0.04	3.90 <sup>a</sup> ±0.00
<b>C18:1n9t</b>	0.04 <sup>e</sup> ±0.00	0.46 <sup>cde</sup> ±0.26	0.34 <sup>de</sup> ±0.08	0.85 <sup>bcd</sup> ±0.00	1.05 <sup>abc</sup> ±0.03	1.27 <sup>ab</sup> ±0.02	1.59 <sup>a</sup> ±0.36
<b>C18:1n9c</b>	77.53 <sup>a</sup> ±0.04	77.15 <sup>ab</sup> ±0.10	76.69 <sup>ab</sup> ±0.08	75.37 <sup>ab</sup> ±0.39	74.64 <sup>ab</sup> ±0.05	73.72 <sup>a</sup> ±0.13	75.10 <sup>ab</sup> ±2.42
<b>C18:2n6t</b>	0.06 <sup>d</sup> ±0.02	0.07 <sup>cd</sup> ±0.00	0.08 <sup>bcd</sup> ±0.02	0.15 <sup>abc</sup> ±0.02	0.14 <sup>abc</sup> ±0.02	0.15 <sup>ab</sup> ±0.00	0.16 <sup>a</sup> ±0.03
<b>C18:2n6c</b>	13.17 ±0.01	12.78 ±0.02	12.65 ±0.04	12.76 ±0.14	13.00 ±0.03	13.38 ±0.01	11.32 ±2.97
<b>C20:0</b>	0.28 <sup>h</sup> ±0.01	0.30 <sup>ab</sup> ±0.02	0.31 <sup>ab</sup> ±0.00	0.33 <sup>a</sup> ±0.01	0.34 <sup>a</sup> ±0.00	0.35 <sup>a</sup> ±0.02	0.33 <sup>a</sup> ±0.00
<b>C18:3n3</b>	0.25 <sup>a</sup> ±0.00	0.25 <sup>ab</sup> ±0.00	0.24 <sup>bc</sup> ±0.00	0.24 <sup>c</sup> ±0.00	0.21 <sup>d</sup> ±0.00	0.21 <sup>d</sup> ±0.00	0.21 <sup>d</sup> ±0.00
<b>C22:0</b>	0.67 <sup>c</sup> ±0.01	0.70 <sup>bc</sup> ±0.06	0.75 <sup>abc</sup> ±0.00	0.80 <sup>ab</sup> ±0.00	0.82 <sup>a</sup> ±0.00	0.83 <sup>a</sup> ±0.03	0.82 <sup>a</sup> ±0.00
<b>SFA</b>	7.77	7.88	8.56	9.13	9.40	9.72	9.95
<b>MUFA</b>	77.75	77.75	77.20	76.40	75.86	75.16	76.87
<b>PUFA</b>	13.48	13.48	12.97	13.15	13.35	13.74	11.69
<b>ΣTRANS</b>	0.11	0.53	0.42	1.00	1.19	1.42	1.75
<b>UFA/SFA</b>	11.74	11.57	10.91	9.81	9.49	9.15	8.90

HOSOE: high oleic sunflower oil + tocopherol; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; UFA: unsaturated fatty acids; a-e: Different letters indicate significant differences ( $p \leq 0.05$ ) between treatment times

**(2)****SHOSO**

	<b>t0</b>	<b>t8</b>	<b>t16</b>	<b>t24</b>	<b>t32</b>	<b>t40</b>	<b>t48</b>
<b>C8:0</b>	-	-	0.03 <sup>bc</sup> ±0.00	0.06 <sup>ab</sup> ±0.01	0.10 <sup>a</sup> ±0.02	0.05 <sup>ab</sup> ±0.01	0.05 <sup>ab</sup> ±0.02
<b>C16:0</b>	4.08 <sup>d</sup> ±0.09	4.25 <sup>d</sup> ±0.09	4.48 <sup>cd</sup> ±0.01	4.76 <sup>bc</sup> ±0.11	5.20 <sup>a</sup> ±0.11	5.16 <sup>ab</sup> ±0.16	4.84 <sup>abc</sup> ±0.11
<b>C16:1</b>	0.15 ±0.03	0.12 ±0.03	0.15 ±0.00	0.16 ±0.01	0.17 ±0.00	0.17 ±0.01	0.13 ±0.01
<b>C18:0</b>	2.61 <sup>d</sup> ±0.03	2.70 <sup>cd</sup> ±0.02	2.79 <sup>bc</sup> ±0.01	2.83 <sup>bc</sup> ±0.05	2.80 <sup>bc</sup> ±0.03	2.90 <sup>ab</sup> ±0.07	2.99 <sup>a</sup> ±0.01
<b>C18:1n9t</b>	0.05 <sup>f</sup> ±0.00	0.22 <sup>c</sup> ±0.00	0.45 <sup>d</sup> ±0.03	0.76 <sup>e</sup> ±0.05	1.04 <sup>bc</sup> ±0.02	1.37 <sup>a</sup> ±0.02	1.46 <sup>a</sup> ±0.05
<b>C18:1n9c</b>	85.38 <sup>a</sup> ±0.55	85.09 <sup>ab</sup> ±0.21	84.34 <sup>bc</sup> ±0.00	83.52 <sup>cd</sup> ±0.03	82.88 <sup>bc</sup> ±0.05	82.34 <sup>c</sup> ±0.11	82.25 <sup>c</sup> ±0.24
<b>C18:2n6t</b>	0.03 ±0.02	0.03 ±0.00	0.04 ±0.00	0.06 ±0.00	0.05 ±0.01	0.06 ±0.00	0.07 ±0.02
<b>C18:2n6c</b>	6.35 <sup>cd</sup> ±0.09	6.08 <sup>e</sup> ±0.02	6.21 <sup>de</sup> ±0.02	6.50 <sup>bc</sup> ±0.02	6.56 <sup>bc</sup> ±0.01	6.59 <sup>ab</sup> ±0.03	6.72 <sup>a</sup> ±0.05
<b>C20:0</b>	0.22 ±0.05	0.21 ±0.00	0.23 ±0.00	0.22 ±0.00	0.18 ±0.01	0.21 ±0.02	0.19 ±0.02
<b>C18:3n3</b>	0.13 ±0.03	0.12 ±0.04	0.11 ±0.03	0.00 ±0.00	0.09 ±0.01	0.09 ±0.06	0.06 ±0.00
<b>C22:0</b>	0.69 ±0.03	0.71 ±0.01	0.68 ±0.04	0.63 ±0.03	0.51 ±0.03	0.64 ±0.07	0.71 ±0.03
<b>Σ SFA</b>	7.69	8.00	8.30	8.61	8.89	9.09	8.91
<b>Σ MUFA</b>	85.68	85.56	85.08	84.59	84.19	83.91	83.98
<b>Σ PUFA</b>	6.62	6.43	6.57	6.75	6.86	6.95	7.05
<b>Σ TRANS</b>	0.08	0.25	0.49	0.82	1.09	1.43	1.53
<b>UFA/SFA</b>	12.00	11.50	11.04	10.61	10.24	10.00	10.22

SHOSO: superhigh oleic sunflower oil; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; UFA: unsaturated fatty acids; a-f: Different letters indicate significant differences ( $p \leq 0.05$ ) between treatment times

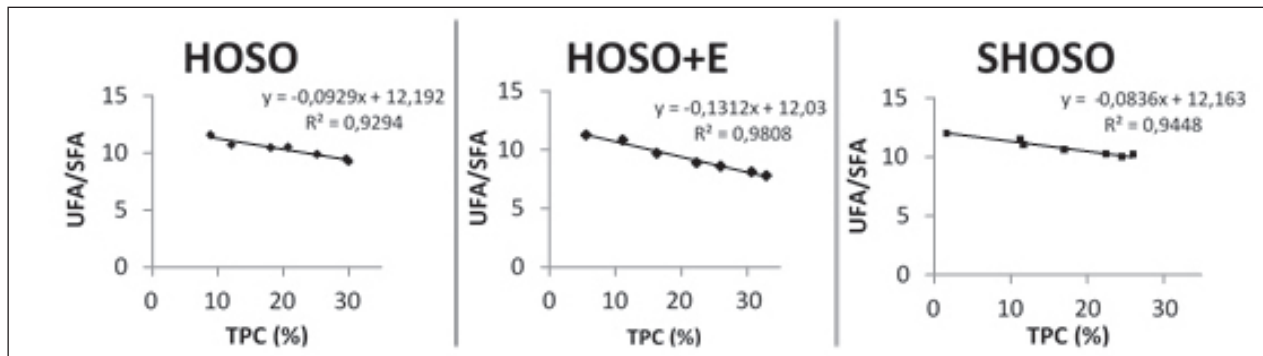


Figure 2.

The fatty acid compositions of HOSOE and SHOSO during the frying process are reported in Table 3. The fatty acid profile of fried HOSO was reported in a previous study (5). The tested oils had comparable unsaturated/saturated fatty acid ratios (UFA/SFA). Although the UFA/SFA ratio was nearly 12% for both oils, the oils presented a different distribution of mono- and polyunsaturated fatty acids. SHOSO had 85.4% oleic acid (OA), while 77.5% OA was detected in HOSO. SHOSO showed a lower concentration of linoleic acid (6.4%), while in HOSO+E, linoleic acid was 13.2%.

UFAs are the main target of lipid oxidation, so the UFA/SFA ratio could be considered a good indicator of thermal treatment. In general, the UFA/SFA ratio decreased during the heating processes. After 48 h of frying, SHOSO showed a smaller decrease in the UFA/SFA ratio (nearly 15%) than HOSO and HOSO+E (20% and 24%, respectively). In Fig. 2, a

correlation between the UFA/SFA ratio and TPC, which is the parameter used to decide when an oil must be discarded, was presented. The SHOSO, HOSO and HOSO+E samples showed a good correlation index (R<sup>2</sup> was 0.94, 0.92 and 0.98, respectively).

The C18:2/C16:0 ratios could be considered a good indicator of heat treatment. In Fig. 3, the normalized trends of C18:2/C16:0 for the three thermo-oxidized oils are shown. This ratio decreased with different kinetics during the treatment. In HOSO, a reduction of 64% was detected in the ratio at 48 h. When tocopherol was added, a reduction of 45% was obtained, while in SHOSO, the reduction was the smallest at approximately 23%.

Octanoic acid (C8:0) can also be considered a good marker of heat treatment due to its stability (22). In the fresh oils, C8:0 was absent. During thermo-oxidation, HOSO+E showed a lower octanoic acid content than HOSO. In SHOSO during thermo-oxidation, C8:0 was not detected until 48 h of treatment. During frying, HOSO+E showed a lower C8:0

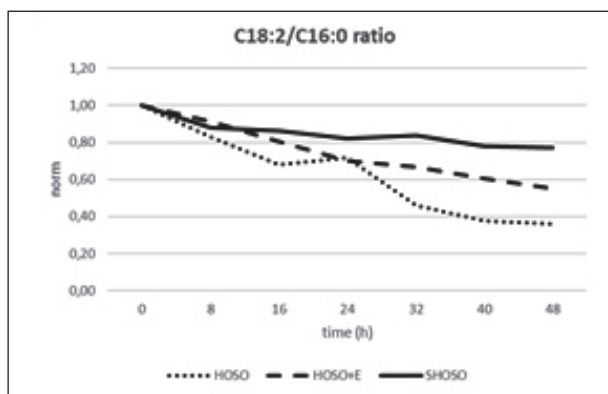


Figure 3.

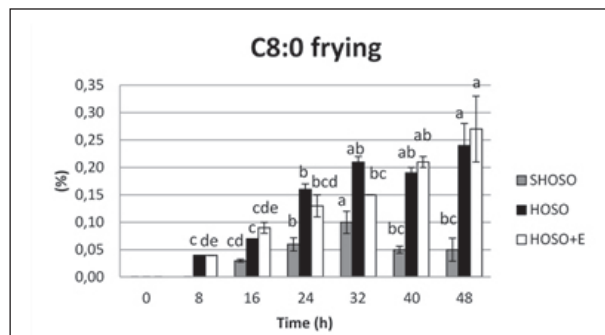


Figure 4.



content than HOSO until 32 h of treatment, while SHOSO showed the lowest content during all the treatments. Moreover, C8:0 was not detected up to 16 h of frying (Fig. 4). This showed that a higher oleic acid content in the oil could prevent C8:0 formation more than  $\alpha$ -tocopherol addition.

#### *$\alpha$ -tocopherol content*

The  $\alpha$ -tocopherol content decreased during the heat treatment. It has been reported that at high temperature,  $\alpha$ -tocopherol can be degraded into a wide range of oxidation products (23-26). The thermo-oxidized oils showed a smaller decrease in  $\alpha$ -tocopherol content (12.1%) than the fried samples (24.4%).

#### Conclusion

The results obtained in this study showed that the oil with the highest concentration of monounsaturated fatty acids (SHOSO) was significantly more resistant to degradation than the oil with added  $\alpha$ -tocopherol. In fact, lower FFA, TPC, and C8:0 contents and a smaller UFA/SFA decrease were detected in SHOSO than in HOSO and HOSO+E during frying. The addition of  $\alpha$ -tocopherol resulted in a reduction of FFA, even if the positive effects observed during thermo-oxidation, were erased by the presence of the food matrix during frying.

These results suggested that the presence of monounsaturated fatty acids, mainly C18:1, may largely influence the heat resistance of sunflower oil more than the presence of  $\alpha$ -tocopherol.

Moreover, the C8:0 content and UFA/SFA ratio were confirmed to be good indicators of thermal treatment, showing good correlation with TPC for all tested oils.

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