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ORIGINAL PAPER

Carob Fruit Polyphenols Reduce Tocopherol Loss, Triacylglycerol Polymerization and Oxidation in Heated Sunflower Oil

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Abstract Heated oils may contain potentially toxic altered compounds. A denatured carob fiber, very rich in non-extractable tannins (Exxenterol®), exhibits antioxidant activities in in vitro experiments. The present study was designed to evaluate in sunflower oil (SO) heated to frying temperature, the protective effect on oil thermal oxidation and polymerization of adding 10 mg Exxenterol/kg oil (SO-10) and 50 mg Exxenterol/kg oil (SO-50). After 2, 8 and 16 h at 180 °C, SO displayed a relevant increase in triacylglycerol-derived polar material (PM) and polymer contents and a decrease in α-tocopherol concentrations. Thermal oxidation changes were significantly checked in SO-50 throughout the 16-h heating, while SO-10 only displayed protection from thermal oxidation during the first 2 h of heating. Oil frying-life was doubled because formation of PM and polymers was inhibited by more than 50%. Results clearly show that this non-extractable tannin-rich fiber can be successfully employed as an additive to significantly prolong sunflower oil frying-life, and thus decrease the potential toxicity of the heated oil.

Keywords Condensed tannins · Frying · Sunflower oil · Tocopherols · Polar material · Thermal oxidation

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Introduction

The thermal oxidation and polymerization compounds formed in frying, show potential toxicity [1–3]. It has been reported that ingestion of oxidized lipids rich in linoleic acid causes profound alterations to membrane composition, fluidity and function [4]. Quiles et al. [5] confirmed in rats, that the loss of antioxidants and the increase in toxic compounds in fried oils affected microsomal membranes, suggesting that feeding fried foods may represent a direct source of oxidative stress for the organism. Any reduction in the amount of alteration compounds implies safer foods, with consequent health benefits for consumers.

Olive oil has traditionally been the frying oil of choice in Spain and other Mediterranean countries [6]. In recent years, however, other oils, including sunflower oil (SO), have partially replaced olive oil due to their lower cost. However, the higher linoleic acid content of these other oils makes them less suitable than monounsaturated oils for repeated frying use [7], as they produce a greater number of thermal oxidation products, polymers, cyclic fatty acid monomers and geometrical fatty acid monomers [8–10]. No replacement of used oil by fresh oil during frying accelerates these alterations [7]. The degradation and, therefore, the frying-life, of these oils are, thus, highly dependent on their type and quality [11–13]. Moreover, the amount of vitamin E lost in frying has been used to assess the progressive deterioration of the oil that occurs during repeated or continuous use [14–16].

Phenolic compounds are important natural antioxidants present in vegetable oils that may prevent deterioration by

quenching the radical reactions responsible for lipid rancidity [17]. Exxenterol, a denatured fiber obtained from carob fruit, is very rich in non-extractable condensed tannins and oligomeric proanthocyanidin complexes (OPCs). The OPCs belong to the condensed tannins category, primarily known for their antioxidant activity [18]. In vitro experimental results have demonstrated that OPCs have specificity for the hydroxyl radical [19]; thus, it can be hypothesized that carob OPCs would block, at least partially, the free radical formation and propagation at frying temperatures.

Previous studies in animal models [20–22] and humans [23] have shown that consumption of different carob fruit extracts has no deleterious effects on health. On the contrary, carob fruit extracts significantly decrease lipemia in rats [20, 24], while consumption of 8 g/day of Exxenterol significantly reduces LDL-cholesterol (26.1%) in hypercholesterolemic subjects [25].

Taking all these facts into account, the present study aimed to study under simulated frying conditions—a 1.5-l fryer, 180 °C, no replacement of used oil by fresh oil and free oxygen access to the oil—the effect of adding 10 and 50 mg of Exxenterol/kg sunflower oil (SO) on (a) the time-course on the increase of thermal oxidation and polymerization of the oil; (b) on tocopherol loss from the oil; and (c) to ascertain the possible relationships between the chemical changes undergone in the oil.

Materials and Methods

Materials

Refined SO (Coosur S.A., Vilches, Jaén, Spain) was purchased at a local store. A carob fiber pulp extract, "Exxenterol®" (Exxentia, S.A. Madrid, Spain) was employed [26]. Exxenterol has a high percentage of non-extractable condensed tannins (85%). The average composition of other Exxenterol compounds is: ash, 1%; moisture, 5%; soluble tannins, 2%; and extractable condensed tannins, 7%.

Heating Procedure

SO was placed in three domestic, 1.5 l capacity, stainless steel fryers (Solac FM 6600 Ideal Mpn-07884.1. Vitoria, Spain). The surface/volume ratio was 0.24 cm^{-1} in all fryers. Zero, 10 and 50 mg Exxenterol/kg oil were added to the oil in the three fryers, giving rise to the three experimental oils, SO, SO-10, and SO-50, respectively. Simulated frying conditions included an oil temperature of 180 °C (thermostat set and controlled) and heating intervals of 2, 8 and 16 h. After each of these respective periods, 20 ml of SO, SO-10 and SO-50 were taken for analysis. SO, SO-10 and SO-50 were cooled to room temperature after the first 8 h and after 16 h and passed through paper filters. Oils were kept under a nitrogen atmosphere, at −18 °C, until analysis.

Polar Material

Total PM content was determined by silica column chromatography [27] (with a slight modification using a 90:10 *n*-hexane/diethyl ether mixture to elute the non-polar fraction). Duplicate samples of approximately 1 g each of raw oil and oil heated for 2, 8 and 16 h were analyzed. All eluents and samples were filtered through a 0.45-μm filter (Econofilter, Agilent Technologies, Stuttgart, Germany). Separation was checked by TLC under iodine vapor and HPSEC.

High-Performance Size-Exclusion Chromatography

To obtain further information about the thermal oxidative changes that occurred during heating, polar fractions of the oils, previously obtained by column chromatography, were analyzed by HPSEC [27]. Samples (about 15 mg/ml in tetrahydrofuran) were applied to a 20-μl sample loop in a Water 501 chromatograph (Milford, Massachusetts). A Waters 410 refractive index detector and two 300 mm × 7.5 mm ID (5-μm particle size), 0.01 and 0.05 μm PLgel (polystyrene–divinylbenzene) columns (Hewlett-Packard, Palo Alto, CA), connected in series, were operated at 40 °C. High-performance liquid chromatography (HPLC)-grade tetrahydrofuran was used as the mobile phase with a flow of 1 ml/min. All eluents and samples were precleaned by passing them through a 0.45-μm filter (Econofilter, Agilent Technologies, Stuttgart, Germany).

Tocopherol Determination

Tocopherol concentrations were determined by HPLC, according to the IUPAC Standard Method 2.432 [28]. HPLC was performed using a Waters 600 HPLC pump with a 20-μl sample loop (Waters Associates, Milford, MA, USA) connected to a Hewlett-Packard 1046A programmable fluorescence detector (excitation at 290 nm and emission at 330 nm. A LiChrosorb (250 × 4 mm) column packed with 5-μm silica (Si 60) particles was used (Merck, Darmstadt, Germany). Precisely weighed oil samples (50 ± 0.001 mg) were dissolved in 1 ml *n*-hexane containing 22.4 μg *δ*-tocopherol that was used as an internal standard for quantification purposes. The mobile phase was *n*-hexane:isopropanol (99:1, vol/vol), with a flow rate of 1 ml/min.

Statistical Analysis

Data were analyzed using the SPSS 15.0 and SAS 9.01 statistical packages. Linear relationships between oil alteration compound and the heating time were studied in SO, SO-10 and SO-50. Comparisons between linear equation adjustments of three SO oils were performed by two-way analysis of covariance (ANCOVA). Significance levels were set at $P < 0.05$.

Results and Discussion

Figure 1 shows the PM content in SO, SO-10 and SO-50 unheated and after being heated for 2, 8 and 16 h. PM content determination is one of the most specific methods used to analyze the alteration and thermal oxidation that occurs during frying or at frying temperatures [9, 29]. Lumley [30], Romero et al. [31] found that the PM content of most unheated quality oils ranges between 2 and 6%. As expected, unheated SO displays an initial PM level within this range and similar levels were found in other studies [3, 31]. The PM content increased considerably—4.8-fold in SO, 4.6-fold in SO-10, and 2.4 in SO-50 after heating for 16 h (Fig. 1). Thus, the addition of 50 mg Exxenterol/kg oil greatly increased the stability of sunflower oil. According to the cut-off points of 20 and 25% for PM content established by several countries [29, 32, 33], the three oils were still considered edible after 8 h of heating. After 16 h of heating, however, SO and SO-10 became non-edible oils, while the PM content values of SO-50 were much lower, and thus, this oil should be considered still acceptable [7].

The polymer content also increased considerably in oils after heating for 16 h (Fig. 2). Current legislation in several countries dictates that oil whose polymer content exceeds 10–12 g/100 g oil must be discarded [29, 33]. In terms of polymer content, SO became non-edible after 16 h of heating. However, the addition of 50 mg Exxenterol/kg oil considerably delays polymerization in SO after 16 h of heating. Figure 2 shows that the addition of 50 mg Exxenterol/kg SO increased the oil life (in terms of polymer content) by more than 100%. Moreover, the addition of this carob fruit extract to oils used for frying decreases their content in polymer compounds, considered to be some of the most toxic components in heated oils [2, 10], resulting in safer fried foods. Moreover, the inclusion of 50 mg Exxenterol/kg oil decreased the TAG trimer versus TAG dimer formation after 16 h 180 °C heating. In fact, the trimer/dimer ratio was 0.26 in SO-50 and 0.45 in SO.

The major polar compounds of unheated SO were oxidized triacylglycerols, followed by diacylglycerols and TAG dimers (Fig. 3). These data were similar to those

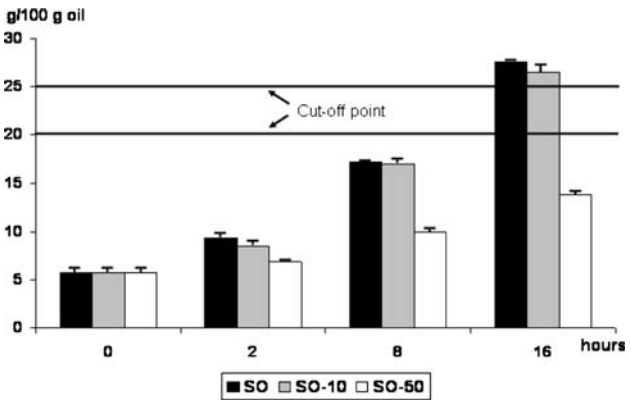

Fig. 1 Polar material content (g/100 g oil) after different heating times in sunflower oil (*SO*), sunflower oil plus 10 mg/kg of Exxenterol (*SO-10*) and sunflower oil plus 50 mg/kg of Exxenterol (*SO-50*)

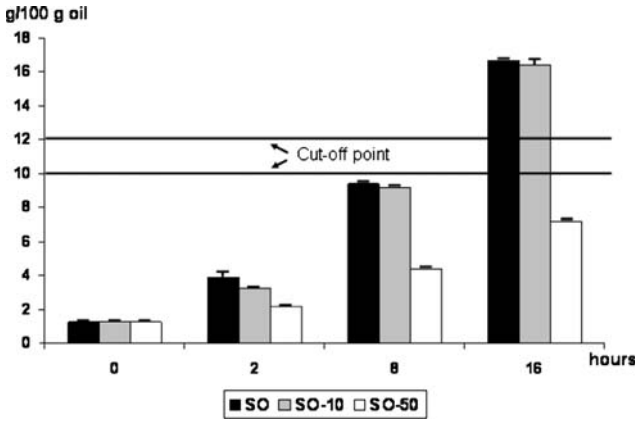

Fig. 2 Polymer content (g/100 g oil) after different heating times in sunflower oil (*SO*), sunflower oil plus 10 mg/kg of Exxenterol (*SO-10*) and sunflower oil plus 50 mg/kg of Exxenterol (*SO-50*)

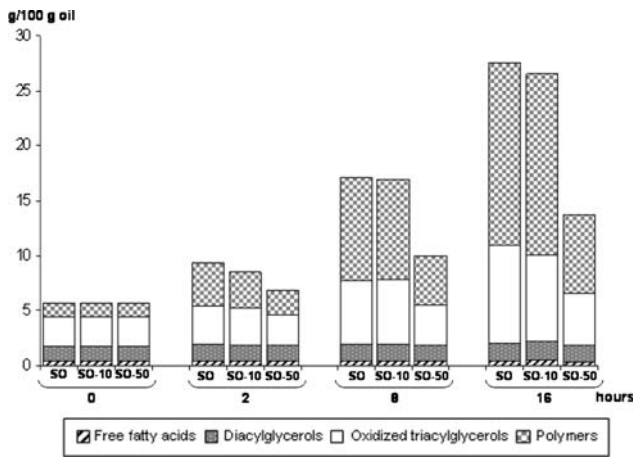

Fig. 3 Hydrolytic alteration (free fatty acids plus diacylglycerols) and thermal oxidation (oxidized triacylglycerols plus polymers) (g/100 g oil) after different heating times in sunflower oil (*SO*), sunflower oil plus 10 mg/kg of Exxenterol (*SO-10*) and sunflower oil plus 50 mg/kg of Exxenterol (*SO-50*)

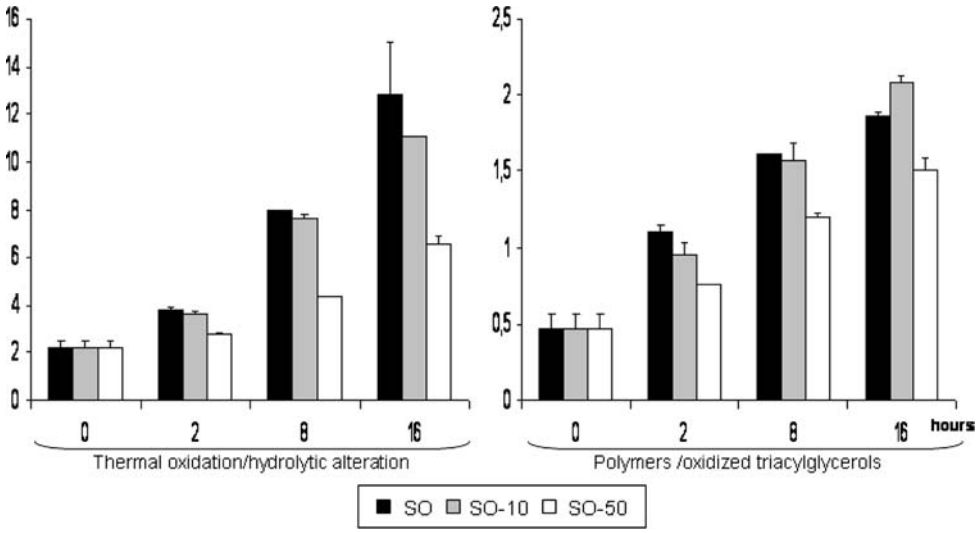

Fig. 4 Thermal oxidation/hydrolytic alteration ratio and polymers oxidized/triacylglycerols ratio after different heating times in sunflower oil (*SO*), sunflower oil plus 10 mg/kg of Exxenterol (*SO-10*) and sunflower oil plus 50 mg/kg of Exxenterol (*SO-50*)

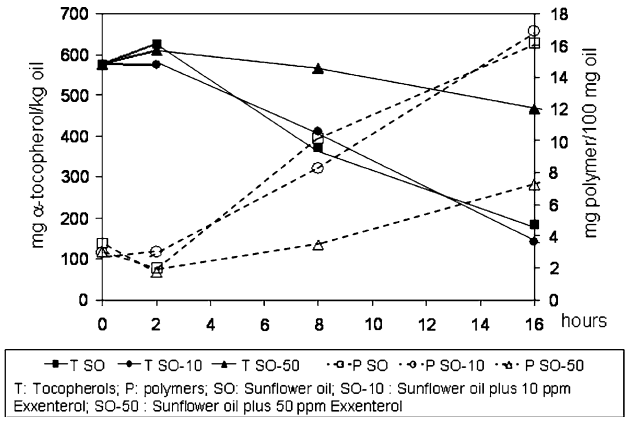

Fig. 5 Time-course evolution of polymer (mg/100 mg oil) and α-tocopherol (mg/kg oil) concentrations throughout 16 h of heating at 180 °C

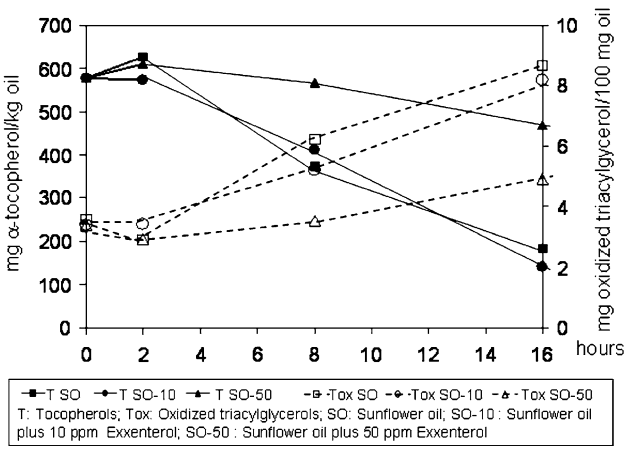

Fig. 6 Time-course evolution of oxidized triacylglycerol (mg/100 mg oil) and α-tocopherol (mg/kg oil) concentrations throughout 16 h of heating at 180 °C

found in previous studies in which different frozen pre-fried foods were fried [3, 8].

Figure 3 shows that concentration of thermal oxidation, hydrolytic alteration (mg/100 mg oil) while Fig. 4 the thermal oxidation/hydrolytic alteration and polymers/oxidized triacylglycerols ratios. All these parameters were markedly affected by heating time. Both SO and SO-10 displayed greater amounts of all altered compounds than SO-50, indicating the strong protective effect of 50 mg Exxenterol/kg sunflower oil against thermal oxidation. A detailed observation of the data suggests that the addition of 10 mg Exxenterol/kg oil reduced the formation of thermal oxidation compounds and the thermal oxidation/hydrolytic alteration and polymers/oxidized triacylglycerols ratios during the first 2 h of heating, but that SO and SO-10 displayed similar values for these parameters after further heating. The polymers/oxidized triacylglycerols ratio was about 20% lower in SO-50 than in SO, suggesting that although the addition of 50 mg Exxenterol/kg oil reduced both polymer and oxidized triacylglycerol levels, polymerization decreased more than oxidation. In turn, this finding indicates that this carob fiber extract displays both antioxidant and antipolymerizing activities in SO at frying temperatures.

Tocopherol content (Figs. 5, 6) in unheated SO coincided with that mentioned in various other publications [15, 16, 34, 35] and indicates the adequate quality of the unheated SO. Tocopherol levels have been shown to decrease at frying temperatures [15, 34]. Tocopherols act as scavengers, inhibiting the initial and propagation phases of thermal oxidation [36]. Exxenterol addition affected tocopherol content. Thus, 50 mg/kg oil of this Carob fruit extract maintained basal levels of tocopherol in SO during the first 8 h of heating (Figs. 5, 6). In SO and SO-10, these antioxidants decreased to one-third of their original levels after the oils were heated for 16 h. However, after 16 h, SO-50 presented a greater α-tocopherol content than that of

Table 1 Linear regression comparisons between polar material content, polymers, thermal oxidation, hydrolytic compounds, ratios and α-tocopherol and heating times in sunflower oil (SO), sunflower oil plus 10 mg/kg of Exxenterol (SO-10) and sunflower oil plus 50 mg/kg of Exxenterol (SO-50)

	R	Slope	Intercept	*P*	ANCOVA
SO					
Polar material	0.999	1.345 ± 0.027	6.211 ± 0.244	0.000	SO vs. SO-10 < 0.082
Polymers	0.999	0.947 ± 0.020	1.650 ± 0.177	0.000	SO vs. SO-10 < 0.061
Oxidized triacylglycerols	0.998	0.386 ± 0.011	2.720 ± 0.098	0.000	SO vs. SO-10NS
Diacylglycerols	0.541	0.009 ± 0.005	1.487 ± 0.049	0.166	SO vs. SO-10ND*
Free fatty acids	0.273	0.003 ± 0.004	0.355 ± 0.037	0.513	SO vs. SO-10ND*
Thermal oxidation[a]	0.999	1.333 ± 0.024	4.369 ± 0.212	0.000	SO vs. SO-10 < 0.058
Hydrolytic alteration[b]	0.452	0.011 ± 0.009	1.841 ± 0.083	0.261	SO vs. SO-10ND*
Thermal oxidation/hydrolytic alteration	0.981	0.658 ± 0.053	2.420 ± 0.481	0.000	SO vs. SO-10NS
Polymers/oxidized triacylglycerols	0.911	0.075 ± 0.015	0.758 ± 0.131	0.002	SO vs. SO-10NS
α-Tocopherol	0.973	−27.432 ± 2.651	618.310 ± 23.856	0.000	SO vs. SO-10NS
SO-10					
Polar material	0.998	1.301 ± 0.034	5.982 ± 0.307	0.000	SO-10 vs. SO-50 < 0.002
Polymers	1.000	0.947 ± 0.012	1.367 ± 0.106	0.000	SO-10 vs. SO-50 < 0.002
Oxidized triacylglycerols	0.984	0.328 ± 0.024	2.833 ± 0.215	0.000	SO-10 vs. SO-50 < 0.002
Diacylglycerols	0.974	0.017 ± 0.002	1.443 ± 0.015	0.000	SO-10 vs. SO-50ND*
Free fatty acids	0.935	0.009 ± 0.001	0.339 ± 0.013	0.001	SO-10 vs. SO-50ND*
Thermal oxidation[a]	0.998	1.275 ± 0.033	4.200 ± 0.299	0.000	SO-10 vs. SO 50 < 0.002
Hydrolytic alteration[b]	0.970	0.026 ± 0.003	1.783 ± 0.024	0.000	SO-10 vs. SO 50ND*
Thermal oxidation/hydrolytic alteration	0.993	0.551 ± 0.027	2.534 ± 0.243	0.000	SO-10 vs. SO 50 < 0.001
Polymers/oxidized triacylglycerols	0.968	0.095 ± 0.010	0.653 ± 0.091	0.000	SO-10 vs. SO 50 < 0.009
α-Tocopherol	0.989	−28.176 ± 1.689	609.26 ± 1.19	0.000	SO-10 vs. SO 50 < 0.005
SO-50					
Polar material	0.997	0.499 ± 0.015	5.831 ± 0.131	0.000	SO vs. SO-50 < 0.002
Polymers	0.999	0.367 ± 0.007	1.377 ± 0.064	0.000	SO vs. SO-50 < 0.001
Oxidized triacylglycerols	0.974	0.131 ± 0.012	2.645 ± 0.112	0.000	SO vs. SO-50 < 0.002
Diacylglycerols	0.602	0.004 ± 0.002	1.451 ± 0.018	0.114	SO vs. SO-50ND*
Free fatty acids	0.578	−0.002 ± 0.001	0.358 ± 0.011	0.134	SO vs. SO-50ND*
Thermal oxidation[a]	0.998	0.497 ± 0.014	4.022 ± 0.124	0.000	SO vs. SO-50 < 0.002
Hydrolytic alteration[b]	0.217	0.002 ± 0.003	1.809 ± 0.027	0.606	SO vs. SO-50ND*
Thermal oxidation/hydrolytic alteration	0.997	0.272 ± 0.009	2.212 ± 0.081	0.000	SO vs. SO-50 < 0.003
Polymers/oxidized triacylglycerols	0.967	0.062 ± 0.007	0.584 ± 0.060	0.000	SO vs. SO-50 < 0.015
α-Tocopherol	0.907	−7.745 ± 1.468	605.594 ± 13.214	0.002	SO vs. SO-50 < 0.010

Data ± SE

SO sunflower, *SO-10* sunflower oil plus 10 mg/kg of Exxenterol, *SO-50* sunflower oil plus 50 mg/kg of Exxenterol, *NS* not significantly different, *ND** not determined because one or two adjustments to be compared were not significantly adjusted to a linear equation

[a] Polymer plus oxidized triacylglycerols

[b] Diacylglycerols plus free fatty acids

the other two SO samples after only 8 h of heating, suggesting that Exxenterol protects sunflower oil from α-tocopherol losses.

Figures 5 and 6 show that the trend for the polymer and oxidized triacylglycerol formation, respectively, and the α-tocopherol content loss in the SO, SO-10 and SO-50 were inversely related. It has been proposed that an increase in the polymer content of oils is related to a decrease in tocopherols [34]. Nonetheless, it has also been reported that heated SO retains a considerable amount of α-tocopherol together with high levels of thermal oxidation compounds, while the amount of tocopherol decreases quickly in heated oils rich in monounsaturated fatty acids [34]. Figures 5 and 6 suggest that about 400–450 mg tocopherol coexist with a 10% polymer and 6% of oxidized triacylglycerol contents, respectively. Carob fiber contains

a large number of different polyphenols, including some that have shown strong antioxidant activity [37] that, in turn, should maintain relatively low the altered compound levels even after 16 h of heating at 180 °C. However, a minimum Exxenterol level (>10 mg/kg) seems essential to exert antioxidant and antipolymerizing activities at 180 °C.

Table 1 shows that the linear adjustments between heating time and the PM, polymer, oxidized triacylglycerol and tocopherol contents were significant (all $P < 0.001$) in SO, SO-10 and SO-50. Most linear adjustments between SO and SO-50 and between SO-10 and SO-50 (except for diacylglycerols, free fatty acids and hydrolytic alteration) were significantly different (at least $P < 0.05$). However, no significant differences were found between any linear adjustments in SO-10 and SO, although linear adjustments for PM, polymers and thermal oxidation contents tended to be different (all $P \leq 0.082$). Both PM content and polymer formation were highly affected by the increased presence of Exxenterol in the oils.

The decrease in thermal oxidation observed in the current study is also of nutritional interest because polymers, which are potentially toxic [9, 10, 13], are actively digested and their products absorbed [38, 39]. Hence, the addition of Exxenterol to SO would reduce its potential toxicity by limiting the levels of these thermal oxidation products formed during frying.

In short, the addition of 50 mg Exxenterol/kg SO considerably extends the life of sunflower oil by reducing thermal oxidation at frying temperatures, suggesting that the use of this carob fiber extract rich in condensed tannins, may offer both health and economic benefits in frying oils.

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