Effects of Phenolic Propyl Esters on the Oxidative Stability of Refined Sunflower Oil

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The oxidative stability of refined sunflower oil in the presence and in the absence of propyl caffeate (PC), propyl hydrocaffeate (PHC), propyl ferulate (PF), and propyl isoferulate (PI) has been evaluated according to the Rancimat method. The antioxidant activity of the phenolic derivatives was compared with that obtained with native [α-tocopherol (α-TOH)] and synthetic [propyl gallate (PG)] antioxidants. The results allow the establishment of a decreasing order of antioxidant power: PG > PHC > PC ≫ α-TOH > PI > PF. The oxidative stability was improved neither by the addition of PF nor by a supplement of α-TOH. Moreover, a positive antioxidant effect was obtained for PC that was placed between those of α-TOH and PG. The antioxidant activity of PHC was higher than that of its analogue (PC). A dose-dependent effect was observed for PG, PHC, and PC. A chain-breaking mechanism was proposed for the antioxidant activity of propyl phenolic esters because the same ranking order of efficacy was obtained for their antiradical activities evaluated by using the 2,2-diphenyl-1-picrylhydrazyl radical method.

Keywords: *Sunflower oil; antioxidant activity; propyl gallate; propyl caffeate; propyl hydrocaffeate; propyl ferulate; propyl isoferulate; α-tocopherol; Rancimat method; DPPH method*

INTRODUCTION

Refined sunflower oil is widely used for cooking and frying, as salad oil, and in margarine production. It represents an important source of polyunsaturated fatty acids, namely, linoleic acid (*cis*-9,*cis*-12-octadecadienoic acid), arousing, thus, particular interest from the nutritional point of view (*1*, *2*).

Linoleic acid represents ∼65−75% of the total fatty acids in sunflower oil (*1*, *3*). The high reactivity of the double-bond system present in this fatty acid is related to its susceptibility to oxidation, especially when submitted to high temperatures.

The oxidation of edible oils and lipids in general is one of the more important reactions that can cause deterioration in the quality of food products, which in turn promotes the shortening of their shelf life and reduces their nutritional quality (*4*).

Natural phenomena of oxidation are slow processes, extending frequently through several months. The evaluation of lipid oxidation under normal conditions for storage or distribution (stability in real time) becomes at times incompatible with quality control at the industrial level (*5*). For this reason, a set of tests is normally run using standard conditions of accelerated oxidation (intensive oxygenation, heat treatment, and/or forced initiation), which promotes the forced aging of fatty matter.

Resistance to oxidation (RO) is generally defined as the period of time necessary to attain the critical point of oxidation, whether it is a sensorial change or a sudden acceleration of the oxidative process (induction period or stability period).

The addition of antioxidants is one of the processes currently used to increase the oxidative resistance of unsaturated oils at the industrial level. Nevertheless, the natural or synthetic compounds actually permitted for oils and fats have limited use not only for economic and technological reasons but also for other reasons related to their physicochemical characteristics (e.g., solubility) and toxicological profiles.

The evaluation of antioxidant effectiveness frequently corresponds to an extension of the induction period (resistance to oxidation) as a result of the addition of the antioxidant compound. This delay is usually expressed as an antioxidant index or a factor of protection (*6*, *7*).

Among the assays currently used for the evaluation of the antioxidant efficiency of natural or synthetic compounds alone or in association, the Rancimat method is one of the most cited in the literature (*7*−*11*). Simple and easy to execute, it is a routine test in many quality control laboratories. Recent data pointed out a good correlation between the oxidative stabilities measured by using the Rancimat method and those obtained from other assays (*4*, *8*, *9*, *12*).

Previous research has shown that it is difficult to improve, to great extent, the oxidative stability of sunflower oil by adding antioxidant compounds either of natural or synthetic origin (*13*), although it seems to be important to promote the development of new antioxidants that could prevent or minimize the oxidative deleterious process in lipids and in lipid-containing products.

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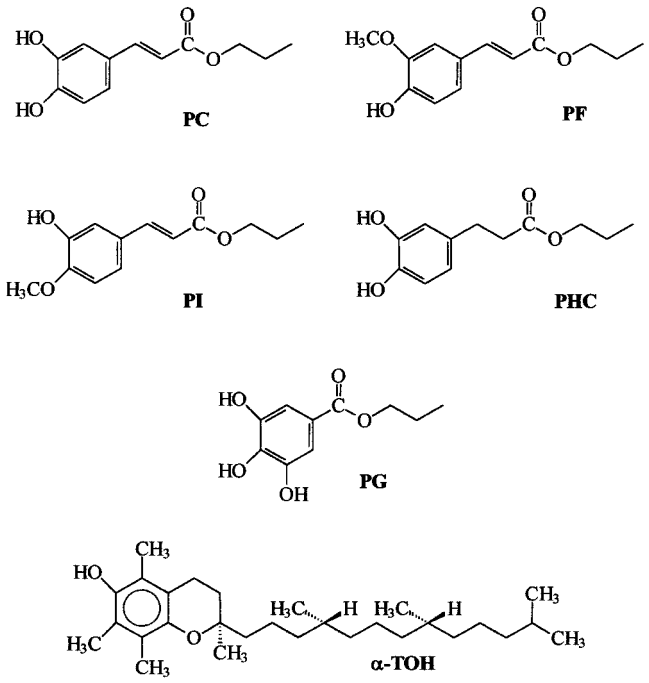

Figure 1. Chemical structures of the phenolic compounds under study.

Concerning the lack of safety of synthetic antioxidants as food additives, considerable interest has arisen in finding suitable compounds based on natural models that are widely distributed in the plant kingdom (e.g., phenolic acids).

The aim of this study was to develop additives for preventing oxidative deterioration of edible oils. With this purpose in mind, the antioxidant efficacy of several phenolic acid derivatives—propyl caffeate (PC), propyl hydrocaffeate (PHC), propyl ferulate (FP), and propyl isoferulate (PI)—in refined sunflower oil was evaluated. The results were compared with those obtained with α-tocopherol (α-TOH) and propyl gallate (PG) under the same experimental conditions. It is worthwhile to note that caffeic acid is the most predominant phenolic acid in sunflower seeds (*14*). The chemical structures of the compounds under study are shown in Figure 1.

As free radical scavenging is a generally accepted mechanism for phenolic antioxidants to inhibit lipid oxidation, the antiradical efficacy of the compounds under study toward a stable free radical—2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$)—was evaluated. The DPPH$^{\bullet}$ test is a nonenzymatic method currently used to provide basic information on the reactivity of compounds to scavenge free radicals (*15*).

MATERIALS AND METHODS

Reagents. The oil samples, belonging to the same lot, were provided from a local industrial manufacturer. The refined sunflower oil was stored in amber packages of 1 L capacity in a cool dark place. The analytical data are given in Table 1.

Deionized water was used as absorption solution for the conductivity measurements. *dl*-α-Tocopherol, propyl gallate, and ethanol absolute were acquired from Sigma/Aldrich (Sintra, Portugal) and Merck (Porto, Portugal), respectively. *trans*-Caffeic acid (3,4-dihydroxycinnamic acid), hydrocaffeic acid (3,4-dihydroxyhydrocinnamic acid), *trans*-ferulic acid (3-methoxy-4-hydroxycinnamic acid), *trans*-isoferulic acid (3-hydroxy-4-methoxycinnamic acid), *n*-propanol, and DPPH radical were purchased from Aldrich. All chemicals were of reagent grade.

Table 1. Analytical Data of Sunflower Oil[a]

acidity (%)	0.04
peroxide value (mequiv of O_2/kg)	0.00
oxidative stability (h)	5.6
fatty acid composition (%)	C14:0, 0.08; C16:0, 6.58; C16:1, 0.19; C17:0, 0.13; C18:0, 4.13; C18:1, 20.69; C18:2, 65.83; C18:3, 0.12; C20:0, 0.32; C20:1, 0.22; C22:0, 0.74; C22:1, 0; C24:0, 0.36

[a] Adapted from the original analysis sheet provided by the industry.

Apparatus. The synthesized compounds were identified by FTIR, UV, NMR, and EI-MS. Infrared spectra were recorded on an ATI Mattson Genesis Series FTIR spectrophotometer using potassium bromide disks; only the most significant absorption bands are reported (υ_{max}, cm^{-1}). Ultraviolet spectra were acquired on a UV−vis Varian Cary 1E spectrophotometer; absorption bands (λ_{max}) are reported in nanometers (ethanolic solutions). ^{1}H and ^{13}C NMR data were acquired, at room temperature, on a Brüker AMX 300 spectrometer operating at 300.13 and 75.47 MHz, respectively. Dimethyl-d_6 sulfoxide was used as solvent; chemical shifts are expressed in δ (parts per million) values relative to tetramethylsilane (TMS) as internal reference; coupling constants (J) are given in hertz. Electron impact mass spectra (EI-MS) were carried out on a VG AutoSpec instrument; the data are reported as m/z (percent of relative intensity of the most important fragments). Melting points were obtained on a Köfler microscope (Reichert Thermovar) and are uncorrected.

The evaluation of antioxidant activity was performed in a 617 Rancimat apparatus, from Metrohm-Herisau A.G.

Spectrophotometric data for the evaluation of radical scavenging activity were acquired at room temperature using a UV-160 Shimadzu dual-beam spectrophotometer and disposable cells from ATI Unicam (Porto, Portugal).

Synthetic Procedure. The propyl esters of ferulic and isoferulic acids were synthesized by Fischer esterification following a previously described procedure (*16*). After solvent evaporation, the residues were purified by column chromatography (silica gel; petroleum ether/ethyl ether 8:2) (*17*).

The structural data of propyl caffeate (PC) and propyl hydrocaffeate (PHC) were previously reported (*15*).

Propyl Ferulate (PF): yield 1.08 g, 79%; FTIR υ_{max} (cm^{-1}) 3437, 2961, 2929, 1696, 1631, 1595, 1514, 1459, 1264, 1165, 1125, 1031, 985; UV λ_{max} (nm) (log ϵ) 325 (4.3), 236 (4.0), 217 (4.1), 201 (4.1); ^{1}H NMR δ 0.92 (3H, *t*, J = 7.4, C$\underline{H}_3$), 1.64 (2H, *m*, C$\underline{H}_2$), 4.07 (2H, *t*, J = 6.6; OC$\underline{H}_2$), 3.81 (3H, *s*, OC$\underline{H}_3$), 6.48 [1H, *d*, J = 15.9, H(α)], 6.78 [1H, *d*, J = 8.2, H(5)], 7.11 [1H, *dd*, J = 8.2, 1.9, H(6)], 7.32 [1H, *d*, J = 1.8, H(2)], 7.54 [1H, *d*, J = 15.9, H(β)], 9.63 (1H, *s*, O$\underline{H}$); ^{13}C NMR δ 10.4 $\underline{C}$H$_3$, 21.7 $\underline{C}$H$_2$, 55.7 O$\underline{C}$H$_3$, 65.2 O$\underline{C}$H$_2$, 111.1 C(2), 114.5 C(α), 115.5 C(5), 123.2 C(6), 125.6 C(1), 145.0 C(4), 147.9 C(β), 149.3 C(3), 166.7 (C=O); EI-MS, m/z (%) 236 (M$^{\bullet+}$, 100), 194 (89), 177 (94), 150 (86), 149 (50), 134 (39), 133 (33), 117 (47), 105 (30), 89 (57), 77 (34); mp 34−37 °C.

Propyl Isoferulate (PI): yield 1.03 g, 84%; FTIR υ_{max} (cm^{-1}) 3417, 2968, 2888, 2844, 1711, 1630, 1587, 1515, 1446, 1388, 1350, 1311, 1262, 1132, 1059, 1027, 984; UV λ_{max} (nm) (log ϵ) 325 (4.2), 295 (4.1), 243 (4.0), 217 (4.1), 202 (4.1); ^{1}H NMR δ 0.91 (3H, *t*, J = 7.4, C$\underline{H}_3$), 1.63 (2H, *m*, C$\underline{H}_2$), 4.07 (2H, *t*, J = 6.7, OC$\underline{H}_2$), 3.80 (3H, *s*, OC$\underline{H}_3$), 6.34 [1H, *d*, J = 15.9, H(α)], 6.94 [1H, *d*, J = 8.1, H(5)], 7.10 [1H, *d*, J = 1.9, H(2)], 7.12 [1H, *dd*, J = 8.1, 1.9, H(6)], 7.51 [1H, *d*, J = 15.9, H(β)], 9.20 (1H, *s*, O$\underline{H}$); ^{13}C NMR δ 10.3 $\underline{C}$H$_3$, 21.7 $\underline{C}$H$_2$, 55.6 O$\underline{C}$H$_3$, 65.3 O$\underline{C}$H$_2$, 111.9 C(5), 114.2 C(2), 115.1 C(α), 121.2 C(6), 126.9 C(1), 144.7 C(3), 146.7 C(β), 150.1 C(4), 166.5 (C=O); EI-MS, m/z (%) 236 (M$^{\bullet+}$, 100), 194 (70), 177 (91), 150 (57), 149 (42), 148 (24), 147 (23), 145 (30), 135 (28), 134 (37), 133 (31), 117 (34), 105 (25), 89 (42); colorless oil.

Evaluation of the Antioxidant Activity (Rancimat Method). Oxidation was carried out by passing a dry air flow (~20 L/h) through an aliquot of sample (2.5 g) fitted in a reaction vessel heated to 110 ± 0.2 °C. The volatile compounds

Table 2. Results of the Solubility Study of Propyl Gallate in Refined Sunflower Oil[a]

	procedure 1		procedure 2		procedure 3		procedure 4	
	control	sample	control	sample	control	sample	control	sample
IP	5.7 ± 0.1	10.3 ± 0.1	5.3 ± 0.1	9.5 ± 0.2	5.4 ± 0.0	ND[b]	5.4 ± 0.1	ND
CV (%)	1.8	1.0	1.9	2.1	0.0		1.9	

[a] For experimental conditions see Materials and Methods. Mean ± SD. [b] ND, not determined.

Table 3. Resistance to Oxidation of Fortified Sunflower Oil Samples[a]

	IP (mean value ± SD)		AOP (%) (mean value)	
sample	160 ppm	200 ppm	160 ppm	200 ppm
control	5.7 ± 0.2[a]	5.7 ± 0.1[a]	0.00[a]	0.00[a]
oil + PF	5.7 ± 0.2[a]	ND[b]	0.00[a]	
oil + PI	5.8 ± 0.2[b]	ND	1.72[b]	
oil + α-TOH	5.9 ± 0.2[c]	ND	3.39[c]	
oil + PC	6.7 ± 0.2[d]	7.1 ± 0.1[b]	14.93[d]	19.72[b]
oil + PHC	7.7 ± 0.2[e]	8.5 ± 0.1[c]	25.97[e]	32.94[c]
oil + PG	10.4 ± 0.2[f]	12.0 ± 0.1[d]	45.19[f]	52.50[d]

[a] Values in the same column followed by the same letter are not significantly different within $P \leq 0.05$. [b] ND, not determined.

of oxidation formed during the experiment and swept along by the air flow were collected in a flask containing 50 mL of deionized water at room temperature. The increase in conductivity was registered automatically during the process with the aid of an electrode immersed in the solution. The determination was interrupted for each sample when the conductivity value attained the maximum (300 μS/cm).

In all tests, a control sample (refined nonfortified sunflower oil) was included and submitted to the same experimental conditions.

The antioxidant effectiveness was estimated on the basis of the induction period (IP), which was determined by the method of the tangents.

Study of the Solubility of Propyl Gallate in Refined Sunflower Oil. The resistance to oxidation of refined sunflower oil samples previously fortified with PG (160 ppm) was evaluated by using the Rancimat method according to the following procedures:

Procedure 1. PG was weighed and directly added to 50 mL of refined sunflower oil. After sonication (30 min), the sample was allowed to stand for 12 h in the dark, at room temperature, and then the volume was made up to 100.0 mL.

Procedure 2. PG was weighed and directly added to 50 mL of refined sunflower oil. After sonication (30 min, 40 °C), the sample was allowed to stand for 12 h in the dark, at room temperature, and then the volume was made up to 100.0 mL.

Procedure 3. PG was previously dissolved in 2 mL of ethanol absolute and then added to 100.0 mL of refined sunflower oil. After mixing, ethanol was removed by an extended flushing with N_2, at room temperature and with magnetic stirring.

Procedure 4. PG was previously dissolved in 2 mL of ethanol absolute and added to 100.0 mL of refined sunflower oil. After mixing, ethanol was evaporated with stirring under reduced pressure (40 °C/0 atm).

General Method for Sample Preparation. For the evaluation of antioxidant activity, the compounds were added individually to refined sunflower oil in concentrations of 160 ppm and, in some experiments, 200 ppm.

According to the desired final concentration, an amount of each compound was weighed directly in a volumetric flask and 50 mL of sunflower oil was added. After sonication (30 min), the samples were allowed to stand for 12 h, at room temperature and in the dark, and then the volume was made up to 100.0 mL.

The time elapsed between the preparation of the samples and the measurements did not exceed 24 h. The samples were stored away from light, at room temperature.

Evaluation of Radical Scavenging Activity. The free radical scavenging activities of test compounds were measured using DPPH radical method. The experimental procedure was adapted from that of Ohnishi et al. (*18*). Special care was taken to minimize the loss of free radical activity of the DPPH• solution, as recommended by Blois (*19*). For each compound and concentration tested (50, 100, 200, 400, and 800 μM), the reduction of DPPH• was followed by monitoring the decrease of absorbance at 517 nm until the reaction reached a plateau (steady state). The percentage of remaining DPPH• was then calculated, and the radical scavenging effects of the tested compounds were compared on the basis of $1/IC_{50}$ (IC_{50} represents the concentration needed to reduce 50% of the initial amount of DPPH•, and it was expressed as the molar ratio of each compound to radical). All tests were run in triplicate and averaged.

Description and Statistical Analysis of the Results. For each compound and concentration tested, the results are reported as mean values of the induction period ± standard deviations. The differences between mean values of the control and of the fortified samples were determined from variance analysis (ANOVA), followed by the Fisher test. All experiments were done in triplicate and averaged.

To establish a correlation between the analytical results, the data were ranked according to their antioxidant power (AOP), which was calculated by using the formula of Castera-Rosignol et al. (*7*)

$$AOP = 100 - [(IP_c/IP_s) \times 100]$$

where IP_c and IP_s are the induction periods of the control and of the sample, respectively.

AOP is expressed as a value between 0 and 100; the greater the AOP, the stronger the antioxidant capacity. A variance analysis (ANOVA), followed by the Fisher test, was performed.

In both cases the differences were considered to be significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

Study of the Solubility of Propyl Gallate in Refined Sunflower Oil. As some of the compounds were only partially soluble in sunflower oil at room temperature, it was found to be necessary to study the experimental conditions suitable for sample preparation. PG was used as a test compound because it presented major difficulties in dissolving when compared to the other compounds under study.

To achieve the objective, four protocols were selected for the addition of the compound to the oil, which were adapted from experimental procedures found in the literature (*20*−*24*).

From the results obtained (Table 2), procedure 1 was adopted as the general method for sample preparation due to its accuracy [translated by the lesser value of the coefficient of variation (CV%), which was greater than that obtained with procedure 2]. The IP values corresponding to the samples submitted to experimental procedures 3 and 4 were not evaluated due to the attainment of curves outside the usual pattern.

Study of the Oxidative Stability of Fortified Refined Sunflower Oil. The concentrations of putative and legal antioxidants used in the study of the oxidative stability (Rancimat method) of refined sunflower oil were in accordance with that permitted by European legislation. The maximum concentration established for

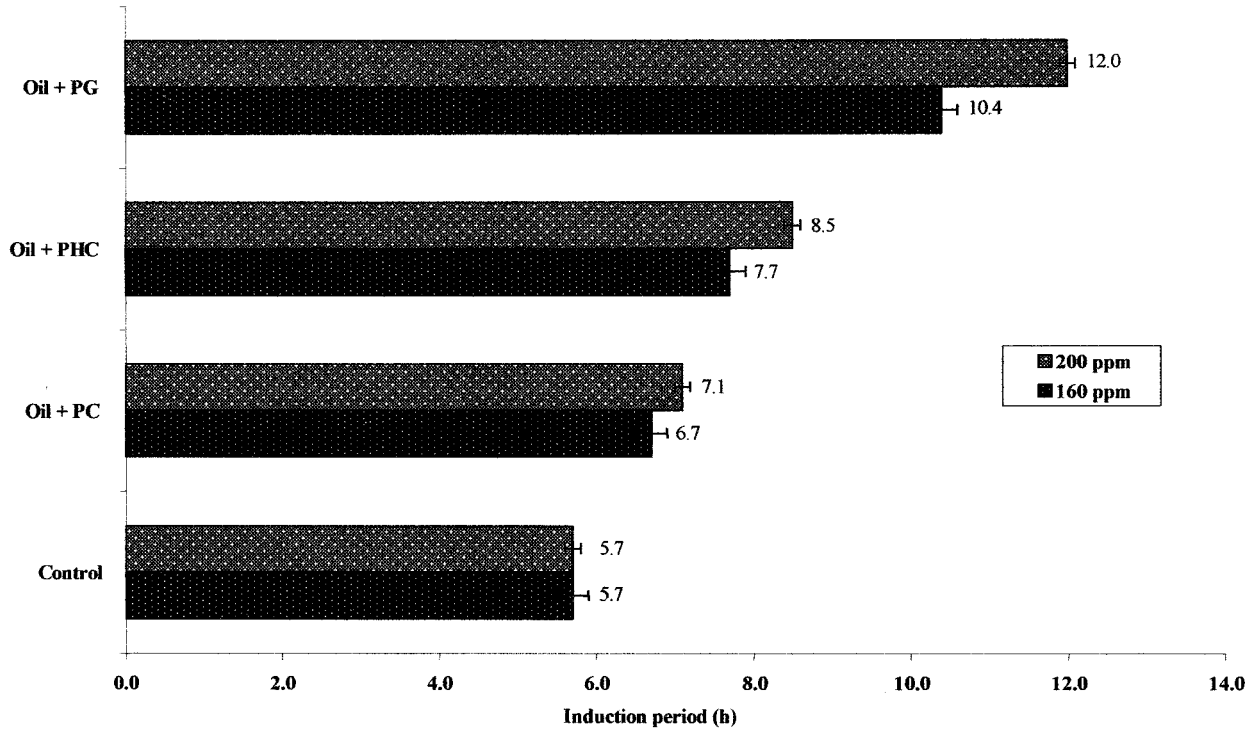

Figure 2. Comparison of the oxidative stability of fortified sunflower oil samples (160 versus 200 ppm).

the addition of synthetic antioxidants (e.g., PG) to the fats and oils used in the production or preparation of foodstuffs, submitted to heat treatments, is 200 ppm (*25*).

The results of the study of the oxidative stability of refined sunflower oil before and after the addition of each compound, at the concentration of 160 ppm, are presented in Table 3. Refined nonfortified sunflower oil (control) had an IP value of 5.7 h, which is in accordance with the data found in the literature (*1*, *13*). The addition of tested compounds causes, to different degrees, an improvement of the oxidative stability of the oil, with the exception of PF.

The resistance to oxidation of the fortified sunflower oil samples allows one to clearly distinguish two major groups:

Group 1: fortified samples containing PC, PHC, and PG, for which an increase in the stability of sunflower oil is observed. These compounds have higher AOP values than α-TOH, which means a better antioxidant efficiency.

Group 2: samples containing PF, PI, and α-TOH, for which the induction periods were not markedly different from that of the control (nonfortified sample). These compounds have AOP values of zero or near zero, indicating the absence of or weakened antioxidant activity.

The order of antioxidant effectiveness evident from the present data was as follows: PG > PHC > PC ≫ α-TOH > PI > PF.

The higher oxidative stability of sunflower oil, when compared with those of other oils, could be related to its content of tocopherols, which are naturally present in this type of vegetable oil. Some authors refer to the presence, even after processing, of tocopherols content of ∼600−700 ppm, of which 80−90% corresponds to the α form (*1*, *2*, *26*).

From the results obtained (Table 3) one can conclude that when α-TOH (160 ppm) was added to the oil, a significant increase in its oxidative stability was not observed (only 0.2 h, which corresponds to an increase of ∼3.5%). This fact is surprising if one considers, on the one hand, the high solubility of the compound in oils and fats and, on the other, its antioxidant properties (*7*, *27*, *28*). Nevertheless, the data are in agreement with previous studies from which it may be concluded that the content of α-TOH in sunflower oil could be very close to its optimum antioxidant concentration (*10*, *13*, *26*). However, as the Rancimat test was performed at 110 °C, other reasons must be pointed out, which are related to the thermal stability of the compound. Castera-Rosignol et al. (*7*) concluded that the α-TOH isomer is rapidly oxidized by temperatures >50 °C, a fact that also can explain the AOP value obtained for the oil fortified with α-TOH.

In an attempt to establish a relationship between the AOP and the concentration of antioxidant, another study was performed in which the refined sunflower oil was fortified (200 ppm) with the most active antioxidant compounds (group 1). The results are expressed in Table 3.

In all of the experiments an increase in the IP values was observed when compared to previous ones (concentation = 160 ppm); thus, a dose-dependent effect was found for PG, PHC, and PC. However, the relative order of antioxidant efficiency, expressed as values of AOP, remains the same (PG > PHC > PC) (Figure 2).

Evaluation of the DPPH Radical Scavenging Activity. The antioxidant activity of a phenolic compound is often associated with its scavenger activity toward several free radicals [e.g. alkyl radicals ($R^{\bullet}$), alkoxyl radicals ($RO^{\bullet}$), and peroxyl radicals ($ROO^{\bullet}$)], which were formed during the oxidation of fats and oils (*29*). That activity is related to the capacity of phenolic compounds to donate hydrogen and the stability of new radical species (phenoxyl radicals) formed during the process. Therefore, it was important to evaluate the radical scavenging activity of the phenolic propyl esters

toward a stable free radical—2,2-diphenyl-1-picrylhydrazyl (DPPH•). As the data corresponding to α-TOH, PC, and PHC were previously reported (*15*), the assays were performed only for PG, PF, and PI.

From these studies it was found that PG was the most potent compound ($1/IC_{50} = 10.01 \pm 0.02$) and that weak antiradical activity is associated with PF ($1/IC_{50} = 1.82 \pm 0.02$) and PI (negligible effect at the maximum concentration used in this study). The antiradical efficacy order of the phenolic compounds was as follows: PG > PHC > PC > α-TOH ≫ PF > PI.

As expected, the electron-donating ability was related to the degree of hydroxylation being higher for PG than for the other compounds. The results were in good agreement with the general statement that the antiradical efficiency is related to the type of chemical structures of the compounds (e.g., position and number of phenolic groups and the presence of other substituents) and that hydroxylation increased the activity (*30*).

The comparison of antiradical potential determined by using the DPPH• method and the AOP evaluated by the Rancimat method allows one to verify that compounds having greater antioxidant efficiencies (PG, PC, and PHC) show also greater antiradical activities. In the same way, compounds with weak AOP (PF and PI) also have reduced antiradical efficiencies. As in the Rancimat method the efficacy of the compounds is evaluated under drastic conditions of oxidation and in the apolar medium, and as the determination of the antiradical activity by the DPPH• method occurs in the polar medium (ethanol) at room temperature, it was not possible to compare quantitatively the values.

The present findings reinforce the hypothesis that the AOP of a phenolic-type compound is intrinsically related to its operating mechanism.

CONCLUSION

This research corresponds to a comparative study between the antioxidant efficiency of synthesized phenolic propyl esters and legal antioxidants of synthetic (PG) and natural origin (α-TOH), which are used as additives for edible oils and fats.

The measurement of accelerated stability (the Rancimat method) allowed the evaluation of the resistance to oxidation of samples of refined commercial sunflower oil in the presence or absence of compounds and established the following relative order of antioxidant activity: PG > PHC ≫ PC ≫ α-TOH > PI > PF.

The work developed confirms the effectiveness of PG and also points out the potential antioxidant strength of the tested ortho-diphenolic compounds (PC and PHC). In terms of oxidative stability, it seems that there is no advantage to the addition of either monophenolic compounds (PF and PI) or a supplement of α-TOH to sunflower oil.

The results obtained arouse particular interest in the propyl esters of caffeic and hydrocaffeic acids, with a view to the improvement of the oxidative stability of refined sunflower oil. Such results allow encouraging future prospects in the development of efficient new powerful antioxidants based on natural models. However, the extrapolation of the results requires some caution, and other tests should be carried out in real time and in normal conditions of storage and preservation of the products.

Be that as it may be, it is important to study the toxicity of the compounds. Their use as antioxidants in foods and pharmaceuticals will inevitably be dependent on their toxicological profiles.

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