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Influence of Simulated Deep Frying on the Antioxidant Fraction of Vegetable Oils after Enrichment with Extracts from Olive Oil Pomace

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ABSTRACT: The stability of the antioxidant fraction in edible vegetable oils has been evaluated during a simulated deep frying process at 180 °C. Four edible oils (i.e., extra-virgin olive oil with a 400 μg/mL overall content in naturally existing phenols; high-oleic sunflower oil without natural content of these compounds but enriched either with hydrophilic antioxidants isolated from olive pomace or with an oxidation inhibitor, dimethylsiloxane; and sunflower oil without enrichment) were subjected to deep heating consisting of 20 cycles at 180 °C for 5 min each. An oil aliquot was sampled after each heating cycle to study the influence of heating on the antioxidant fraction composed of hydrophilic and lipophilic antioxidants such as phenols and tocopherols, respectively. The decomposition curves for each group of compounds caused by the influence of deep heating were studied to compare their resistance to oxidation. Thus, the suitability of olive pomace as raw material to obtain these compounds offers an excellent alternative to the use of olive-tree materials different from leaves. The enrichment of refined edible oils with natural antioxidants from olive pomace is a sustainable strategy to take benefits from this residue.

KEYWORDS: edible oil enrichment, olive phenols, tocopherols, olive pomace, simulated deep frying, hydrophilic and lipophilic antioxidants

INTRODUCTION

Physicochemical changes occurring during the frying process dramatically deteriorate the quality of oils and fats. Physical changes mainly result in increased viscosity and foaming, color changes and decreased smoke-point. Simultaneously, the main chemical changes involve increased concentrations of free fatty acids and polar components as well as decreased unsaturation, flavor quality and nutritive value by degradation of minor compounds affecting the organoleptic and nutraceutical properties.[1]

It is widely known that oils and fats also deteriorate during storage in an oxidizing atmosphere by a process termed autoxidation. When fats or oils are heated in such an atmosphere up to relatively high temperature, autoxidation is not only accelerated but also followed by oxypolymerization and thermal-oxidative degradation. This overall process is referred to as thermal-oxidative decomposition, which involves a set of physicochemical reactions such as thermoxidation, hydrolysis, polymerization, isomerization or cyclization. As a result of thermal-oxidative decomposition, monomeric, polymeric, primary and secondary oxidative compounds are formed, thereby affecting the quality of oil and fried product.[2] The reactions occurring during deep-fat frying depend on factors such as temperature, heating time, type of fryer, initial quality of frying oil such as unsaturated fatty acid contents, food materials subjected to frying, oxygen concentration, humidity and, mainly, type and concentration of antioxidants. Antioxidants naturally present in oils encompass hydrophilic and lipophilic compounds. Thus, tocopherols are lipophilic compounds present in vegetable oils, including refined oils such as linseed and sunflower oils. On the contrary, hydrophilic antioxidants are exclusive of nonrefined oils such as olive oil, which is consumed as obtained. Among hydrophilic antioxidants, the most important are phenolic compounds, some of which (e.g., hydroxytyrosol, tyrosol, secoiridoids and their conjugated forms) are characteristic of olive oil. These compounds are primary antioxidants which either delay the oxidation step by reacting with free radicals or inhibit the propagation step by reacting with peroxy or alkoxy radicals thus avoiding degradation of vegetable oils.[3] The effectiveness of natural antioxidants on the resistance of oils to degradation has been well documented.[4−6]

Because of the relevant role of antioxidants in oil stability, prevention or minimization of oil degradation during frying processes can be achieved by enrichment with either natural or artificial antioxidants. However, the use of synthetic oxidation inhibitors such as butylated hydroxyl toluene (BHT) and butylated hydroxyl anisol (BHA) can cause harmful effects on humans. BHA has been shown to cause lesions in the rat forestomach.[7] Other studies have pointed out that BHT may cause internal and external hemorrhages at high doses, which are severe enough to cause death in some strains of mice and guinea pig.[8] According to the European Official Bulletin (Order of 24 August 2007), the maximum dose allowed of artificial additives such as BHT and BHA in frying oil and fats is 200 mg/kg of oil.[9]

Also comparative studies on the stabilization effect on oils of natural antioxidants versus oxidation inhibitors have promoted the wider use of the former.[5,10] With these premises, the use of these artificial additives has decreased because it is suspected that they may act as promoters of carcinogenesis. As a result, enrichment of edible oils with natural antioxidants becomes of great interest, especially after studying the fate of oxidation

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inhibitors[11] and their comparison with natural antioxidants when subjected to heating.[5,12]

Nowadays, vegetable plants are the main source of natural antioxidants. In this sense, *Olea europaeae* has been widely studied as a promising source of powerful hydrophilic antioxidants, mainly taking advantage of olive leaves after pruning,[6,13,14] while other very rich sources of these compounds (viz., olive pomace) have received scant attention.[15,16] In fact, because of their polar nature, phenolic compounds are found in olive pomace at concentrations up to 100 times higher than in olive oil, a fact explained by the polar nature of the semisolid residue versus the nonpolar character of olive oil.[16] The fact that oil pomace is a part of the oil fruit, used as food (table olives) for centuries, makes this a raw material for phenol extraction accepted by food regulations. Additionally, it is worth emphasizing the residual character of olive pomace generated after oil extraction, which increases the sustainability of antioxidant isolation from this raw material.

The aim of the present research was to evaluate natural antioxidants from olive pomace (both phenols and tocopherols as representatives of hydrophilic and lipophilic antioxidants) by studying their fate under simulated frying versus that of the same antioxidants naturally existing in the oil (use of extra-virgin olive oil, EVOO). Oils with quite similar lipid fraction have been used with this aim: EVOO and high-oleic sunflower oil (HOSO). Also, the influence of the lipid fraction on the fate of naturally existing antioxidants such as tocopherols and that of added natural antioxidants is considered in this study by using HOSO enriched with either natural antioxidants from oil pomace or an oxidation inhibitor (dimethylsiloxane, DMS) versus sunflower oil.

MATERIALS AND METHODS

Samples. Vegetable edible oils such as EVOO, HOSO and sunflower oil were provided by Koipesol (SOS Cuétara, S.A., Madrid).

Reagents. HPLC grade ethanol, methanol and acetonitrile from Panreac (Barcelona, Spain) were used as solvents for extraction and chromatographic separation. Deionized water (18 MΩ·cm) from a Millipore Milli-Q water purification system was used to prepare mobile chromatographic phases. Sodium carbonate, Folin−Ciocalteu (F−C) reagent and orthophosphoric acid were also from Panreac.

HPLC grade *n*-hexane and heptane to dissolve oils prior to the F−C test and analysis of fatty acid methyl esters (FAMEs) were provided by Panreac. Anhydrous sodium sulfate from Sigma-Aldrich (Steinheim, Germany) was used in the derivatization step as drying agent for the nonpolar phase. Methanolic solution of 0.4 M KOH, used in the derivatization of fatty acids, was from Panreac.

The phenolic compounds in olive oil and in pomace with enough abundance in them to be quantified (i.e., hydroxytyrosol, tyrosol, oleuropein, luteolin, apigenin, apigenin-7-glucoside, vanillic acid, *p*- and *o*-coumaric acids, ferulic acid and caffeic acid), and also syringic acid, used as internal standard, IS, were from Extrasynthese (Genay, France) and Sigma (St. Louis, USA). The stock standard solution of each phenol was prepared at 1000 μg/mL by dissolving 10 mg of each phenol in 10 mL of methanol. Tocopherols, both α and δ, were from Sigma, and their stock standard solutions were prepared at 1000 μg/mL by dissolving 10 mg of each compound in 10 mL of 2-propanol. Multistandard solutions were prepared by mixing the appropriate volume of each stock solution in methanol or 2-propanol. All solutions were stored in the dark at −20 °C in glass vials until use. All fatty acid analytical standards, supplied as FAMEs and used to prepare the multistandard, were from Sigma-Aldrich. Individual stock standard solutions were prepared by dilution of each compound in *n*-hexane (from C12 to C20) and heptane (C22:0). The stock standard solutions were stored at −20 °C.

Preparation of Edible Oils Enriched with Antioxidants. Four edible oils were prepared for this study. Different EVOOs with an intrinsic content of antioxidants were mixed up to a total concentration of phenolic antioxidants of 400 μg/mL, expressed as μg/mL of caffeic acid by the F−C test. HOSO was enriched with two types of antioxidants: a synthetic oxidation inhibitor (dimethylsiloxane, DMS, at 400 μg/mL) and a natural extract from olive pomace obtained by a protocol similar to that developed by Girón et al.[6] For enrichment with natural antioxidants, 20 g of olive pomace was extracted with 100 mL of ethanol and the ethanol partially evaporated to obtain a concentrated extract, which was put into contact with HOSO and vigorously shaken. In this way, the oil was enriched up to a total phenol concentration of 400 μg/mL, also expressed as caffeic acid. A mechanical electrical stirrer MS2 minishaker from Ika (Wilmington, NC, USA) was used to favor transfer of hydrophilic phenols from the extract to sunflower oil.

Simulated Frying Procedure. Two liters of the target oil was placed in a stainless-steel deep fryer and subjected to 20 cycles at 180 ± 5 °C for 5 min (total heating time: 100 min), with 30 min cooling intervals between heating cycles. 3 mL aliquots from the target oil were taken after each heating cycle and stored at −20 °C until analysis. A stainless deep fryer (Fagor F-206, Barcelona, Spain), without cover, was used for oil heating.

Extraction of the Hydrophilic and Lipophilic Antioxidants from Oil. Aliquots of ca. 2 g of enriched or pure oils were dissolved with 2 mL of hexane and shaken for 30 min with 20 mL of methanol. The methanolic phase containing the antioxidants was isolated by centrifugation, evaporated for preconcentration and stored at −20 °C for subsequent analysis. A Selecta Mixtasel centrifuge (Barcelona, Spain) was used for this step.

Determination of the Total Phenols Content. After extraction, the total concentration of phenols was estimated by the Folin−Ciocalteu method.[17] Briefly, 0.1 mL of methanolic extract was, in this order, mixed with 2 mL of water, 0.2 mL of F−C reagent and 0.6 mL of 20% (w/v) Na_2CO_3 aqueous solution. The resulting mixture was diluted with water to obtain a 5 mL final volume and then incubated for 30 min in a water bath at 50 °C. The reaction product was monitored at 725 nm using an Agilent 8453E UV−vis spectrophotometer. Caffeic acid was used as standard for calibration.

LC−DAD Separation−Quantification of Hydrophilic and Lipophilic Antioxidants. The applied method was that proposed by the International Oleic Council (IOC) for the individual determination of phenolic compounds in olive oil.[18] Briefly, the analytical column used was a 250 × 4 mm i.d., 5 μm particle size, reversed phase Inertisil ODS-2; the injection volume 10 μL; and the mobile phase a mixture of A (water acidified with 0.2% phosphoric acid) and B (acetonitrile−methanol, 1:1 v/v) at 1 mL/min. An initial linear gradient elution from 0 to 50% B in 40 min was followed by other linear elution gradient from 50 to 60% B in 5 min and a third gradient from 60 to 100% B in 10 min. Finally, the instrument was kept under isocratic conditions (100% B) for 2 min. A 5 min equilibration step enabled the initial conditions and mobile phase stabilization to be reached. The eluted phenols were monitored at 230, 280, 325, and 350 nm (elution time shorter than 57 min).

Additionally, tocopherols were eluted at the end of the chromatographic step with 100% B and monitored at 230 and 280.

An Agilent 1100 liquid chromatograph (Pittsburgh, PA, USA), consisting of a quaternary pump, a vacuum degasser, a diode array detector (DAD) and a Reodyne 7725 high pressure injection valve (20 μL injection loop), was used for the analysis of the target compounds. Calibration plots were run for the target analytes for which commercial standards are available using the peak area as a function of the standard concentration of each compound. Syringic acid, not present in any of the target oils, was used as IS. Compounds with no calibration standards were quantified by the calibration curve of the phenol with a more similar structure.

Calibration equations were set by using the ratio between the peak area of each compound and that of the IS of the given family as a function of concentration of each compound. The characterization of the method also

Table 1. Features of the Chromatographic Method for Determination of the Studied Phenols and Tocopherols: Limits of Detection (LODs), Limits of Quantitation (LOQs), Monitoring Wavelength and Experimental and Theoretical Response Factors[a]

compound	calibration curve	R^2	linear range	LOD (μg/mL)	LOQ (μg/mL)	wavelength (nm)	exptl factor	theor factor
hydroxytyrosol	$Y = 0.046\ (3.22) + 0.0087.03\ (0.43)$	0.996	LOQ−250	0.03	0.10	280	0.55	0.62
tyrosol	$Y = 0.0329\ (26.35) + 0.0359\ (0.55)$	0.996	LOQ−250	0.06	0.20	280	0.74	0.80
vanillic acid	$Y = 0.085\ (32.21) + 0.0902\ (0.86)$	0.995	LOQ−250	0.03	0.10	260	0.94	0.96
hydroxytyrosol acetate	as hydroxytyrosol							
p-coumaric acid	$Y = 0.2795\ (34.12) + 0.2991\ (0.62)$	0.995	LOQ−250	0.05	0.16	325	1.02	1.10
ferulic acid	$Y = 0.2971\ (48.23) + 0.322\ (0.32)$	0.995	LOQ−250	0.12	0.40	325	1.25	1.26
o-coumaric acid	$Y = 0.2526\ (43.55) + 0.0316\ (0.39)$	0.996	LOQ−250	0.09	0.30	325	1.42	1.31
oleuropein	$Y = 0.0603\ (28.42) + 0.0337\ (0.9953)$	0.998	LOQ−250	0.08	0.26	280	1.53	1.87
apigenin-7-glucoside	$Y = 0.0789\ (43.12) + 0.0587\ (0.64)$	0.998	LOQ−250	0.03	0.10	280	1.74	1.79
luteolin	$Y = 0.1322\ (32.55) + 0.0099\ (0.75)$	0.998	LOQ−250	0.11	0.35	325	1.83	1.88
apigenin	as apigenin-7-glucoside							
α-tocopherol	$Y = 0.0236\ (1.94) + 0.0188\ (0.71)$	0.998	LOQ−500	0.05	0.16	280	2.03	1.98
δ-tocopherol	$Y = 0.0721\ (4.10) + 0.0123\ (0.34)$	0.998	LOQ−500	0.05	0.16	280	2.06	2.01

[a] Concentration expressed as μg/mL. Experimental factor: retention time of each analyte/retention time of IS. Theoretical factor: retention time of each analyte/retention time of IS reported by the IOC.

involved calculation of the detection (LOD) and quantification (LOQ) limits. The LOD and LOQ for each analyte were expressed as the concentration of analyte which gives a signal 3σ and 10σ, respectively, above the mean blank signal (where σ is the standard deviation of the blank signal).

Determination of the Fatty Acid Profile. The fatty acids in the oil were derivatized to more volatile compounds (FAMEs) for proper individual separation by gas chromatography. The IOC method for FAMEs preparation was used.[19]

The individual separation of FAMEs was carried out by GC; then, they were detected and quantified by MS using the GC−MS method developed by Sánchez-Ávila et al.[20] modified in the injection step. Briefly, the injection volume was reduced to 1 μL, injected in splitless mode, and the injector temperature was 250 °C, maintained during the total run. A Varian CP-3900 gas chromatograph (Walnut Creek, CA, USA) equipped with a programmable-temperature injector and coupled to a Saturn 2100 ion-trap mass-spectrometer (Sunnyvalley, TX, USA) was used to obtain the fatty acid profiles. The chromatograph was furnished with a Varian CP 8400 autosampler and an SP-2380 fused silica capillary column (60 m × 0.25 mm i.d., 0.2 μm film thickness) from Supelco (Bellefonte, PA, USA).

Statistical Analysis. Statgraphics Centurion XV, Statpoint Technologies, Inc. (Warranton, VA, USA), was used as statistical software with this purpose. Additionally, statistical analysis to compare the levels of phenolic compounds for the different heating cycles was carried out by analysis of variance. In all cases, the confidence intervals were set at 95% and, thus, a *p*-value of 0.05.

RESULTS AND DISCUSSION

Characterization of the Method for the Determination of Antioxidants in Oils. The method proposed by the IOC for individual determination of phenols[18] is based on a chromatographic separation involving a solvent gradient from an acid aqueous phase to pure methanol for elution of the less polar organic compounds. This method does not include the determination of tocopherols that, despite their nonpolar character, can be eluted with pure methanol after apigenin and luteolin. For this reason, tocopherols can be determined at 280 nm with phenols in a single chromatographic run.

Identification of each phenol was based on comparison of the retention time and UV spectrum with those of the corresponding standards. Hydroxytyrosol acetate was identified by comparing its retention factor (retention time of each analyte/retention time of IS) and UV spectra with those reported by the IOC method.[18] Secoiridoid derivatives were similarly identified. Table 1 shows the maximum absorbance wavelength as well as the theoretical (IOC) and experimental retention factors for each analyte. Tocopherols were identified by individual standards of α- and δ-tocopherol. Figure 1, A and B, shows the chromatograms at the monitoring wavelength of the antioxidant fraction isolated from EVOO and HOSO enriched with extract from olive pomace prior to simulated frying. Quite similar profiles are obtained, the differences in

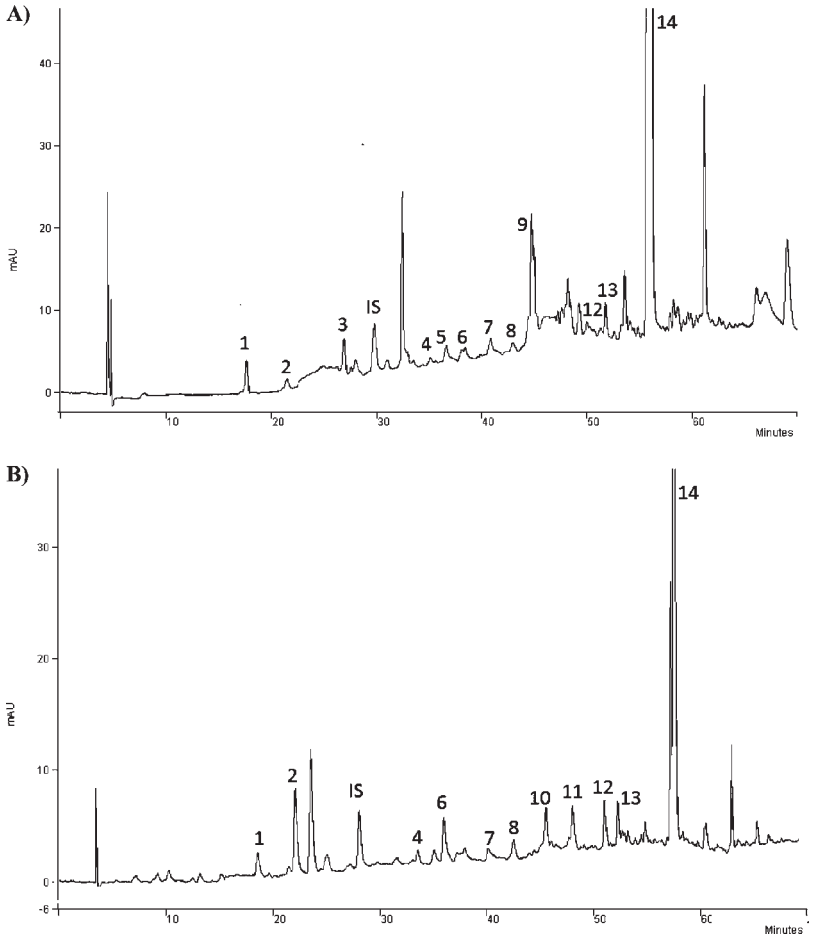

Figure 1. Chromatograms, at λ = 280 nm, provided by analysis of antioxidant extracts from extra-virgin olive oil (A), and high-oleic sunflower oil enriched with compounds from olive pomace (B). Peak identification: (A) 1, hydroxytyrosol; 2, tyrosol; IS, internal standard at 280 nm, syringic acid; 3, vanillic acid; 4, *p*-coumaric acid; 5, hydroxytyrosol acetate; 6, ferulic acid; 7, *o*-coumaric acid; 8, 9 and 10, secoiridoid derivatives; 11, apigenin-7-glucoside; 12, luteolin; 13, apigenin; 14, α-tocopherol.

concentration of the target antioxidants being a consequence of the different concentration of them in EVOO and olive pomace; obviously, the latter is richer in the most polar compounds as they have a more favorable partition factor to the pomace.

Calibration plots were run by using the peak area of each analyte/peak area of IS as a function of the concentration of each compound. The regression coefficients ranged between 0.995 and 0.998, as shown in Table 1. Compounds with no calibration standards were quantified by the calibration curve of the phenol with a more similar structure. Thus, hydroxytyrosol acetate was quantified by the hydroxytyrosol calibration curve, while secoiridoid derivatives were overally quantified by the oleuropein calibration curve. Syringic acid, not present in any of the target oils, was used as IS.

LODs and LOQs were then calculated as described in Materials and Methods. The LODs ranged between 0.03 and 0.12 μg/mL for all the analytes, and the LOQs between 0.10 and 0.40 μg/mL (Table 1).

Evaluation of the Antioxidant Fraction in Edible Oils Prior to Simulated Frying. The steps for extraction and individual separation−quantification of antioxidants described in Materials and Methods were applied to the target oils prior to heating and to the aliquots taken after each heating cycle. The first step was the characterization of pure and enriched oils before simulated deep frying to compare their content in hydrophilic and

Table 2. Concentrations (Expressed as μg/mL) of Phenols and Tocopherols in the Oils Prior to Heating

		high-oleic sunflower oil		
	sunflower oil	+ oxidation inhibitor	+ natural antioxidants	extra-virgin olive oil
hydroxytyrosol	<LOD[a]	<LOD	13.51	12.38
tyrosol	<LOD	<LOD	11.76	7.98
vanillic acid	<LOD	<LOD	4.61	<LOD
vanillin	<LOD	<LOD	<LOD	<LOD
p-coumaric acid	<LOD	<LOD	1.96	1.28
ferulic acid	<LOD	<LOD	1.69	1.13
hydroxytyrosol acetate	<LOD	<LOD	1.82	<LOD
o-coumaric acid	<LOD	<LOD	1.12	<LOD
secoridoids derivatives	<LOD	<LOD	2.98	22.85
apigenin 7-glucoside	<LOD	<LOD	<LOD	1.17
luteolin	<LOD	<LOD	2.43	3.01
apigenin	<LOD	<LOD	2.17	1.64
α-tocopherol	489.1	325.7	439.3	153.4
δ-tocopherol	15.22	<LOD	<LOD	<LOD

[a] LOD, limit of detection.

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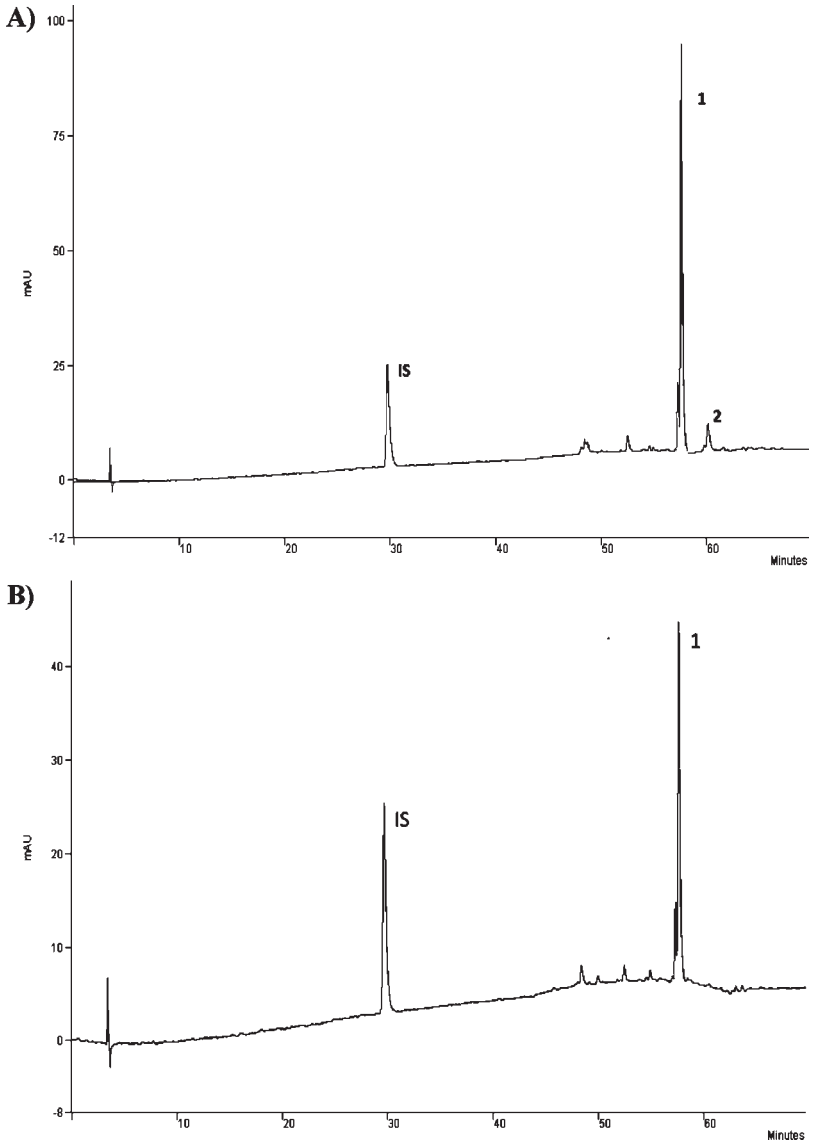

Figure 2. Chromatograms, at λ = 280 nm, provided by analysis of antioxidants extracts from high-oleic sunflower oil (A) and from high-oleic sunflower oil enriched with dimethylsiloxane, DMS (B). Peak identification: IS (internal standard, syringic acid); 1, α-tocopherol and 2, δ-tocopherol.

lipophilic antioxidants. Table 2 lists the concentrations of the monitored antioxidants in the oils under study. As can be seen in Figure 2, A and B, hydrophilic antioxidants were not present in HOSO enriched with DMS and in sunflower oil. This fact is ascribed to the refining process by which the oils are treated with no polar extractants and exposed to high temperature (above 180 °C) for short intervals with deodorization purposes. These results were confirmed by the F−C test that provided nil concentration of total phenols in both enriched HOSO and nonenriched sunflower oil. Obviously, DMS is not removed under the working conditions used for extraction of hydrophilic compounds because of its nonpolar nature.

Concerning hydrophilic antioxidants, EVOO and HOSO enriched with extract from olive pomace both obviously contained high concentrations of phenolic compounds (see Figure 1, A and B), which could be an indicator of good antioxidant capacity. Previous studies on transference of phenolic compounds from olive pomace extracts to vegetable edible oils with different composition of fatty acids showed that increased unsaturation in the oil (content of mono- and polyunsaturated fatty acids) favored its enrichment in phenolic antioxidants.[19,21,22] Attending to the fatty acid composition of HOSO, it seems to be suited for enrichment with olive phenols.[4,13] In this research, HOSO was selected for enrichment because of its fatty acid profile similar to that reported by EVOO. Table 3 shows the fatty acid composition of the target oils revealing this fact. As can be seen in Tables 2 and 3, EVOO and HOSO enriched with hydrophilic phenols presented a similar fatty acid profile, but also a similar composition in hydrophilic antioxidants as a result of the enrichment process. Compounds such as hydroxytyrosol, tyrosol, ferulic acid, vanillic acid, *o*- and *p*-coumaric acids, hydroxytyrosol acetate, luteolin and apigenin were found as simple phenols in both oils. Significant differences were found only in the concentration of secoiridoids in the HOSO oil as compared to EVOO. Secoiridoid derivatives are formed by conjugation of hydroxytyrosol or tyrosol with eleanolic acid,[23] and therefore, the high concentration of them in EVOO could explain its resistance against thermal oxidation with respect to other vegetable oils such as sunflower oil. Additionally,

Table 3. Fatty Acids Profile in the Target Edible Oils Determined before the Deep Heating Process[a]

		high-oleic sunflower oil		
fatty acid	sunflower oil	+ artificial antioxidant	+ natural antioxidant	extra-virgin olive oil
c12	0.00	0.00	0.00	0.00
c14	0.07	0.07	0.10	0.03
c16:0	8.47	9.39	9.63	14.50
c16:1 n7 n9	0.06	0.16	0.15	0.71
c17	0.45	1.06	0.37	0.52
c17:1 n10	0.00	0.00	0.00	0.10
c18	6.49	6.22	5.86	7.23
c18:1 n7	0.00	0.00	0.00	0.80
c18:1 n9 t	0.00	0.00	0.00	0.00
c18:1 n9	26.70	57.26	65.92	53.44
c18:2 t9, t12	0.00	0.00	0.00	0.00
c18:2 c, t	0.25	0.02	0.15	0.00
c18:2 t, c	0.00	0.02	0.00	0.00
c18:2 c, c	56.03	22.84	14.62	13.34
c19:1	0.00	1.61	1.72	0.46
c18:3 c, t, c	0.00	0.04	0.12	0.06
c18:3 c, c, c	0.13	0.20	0.06	0.38
c22:0	0.93	0.95	0.84	0.00
c20:4 n3	0.03	0.05	0.00	7.62
c22:2 n6	0.00	0.00	0.36	0.00
c20:5 n3	0.11	0.11	0.10	0.10
c22:5 n3	0.27	0.00	0.00	0.69

[a] Concentrations are expressed as percentage.

secoiridoid derivatives are directly responsible for the organoleptic properties of the oils.[24] As can be seen in Table 2, enriched HOSO presented a low concentration of secoiridoids as compared to EVOO (2.98 versus 22.85 μg/mL) before simulated frying. It is worth emphasizing the similar levels of hydroxytyrosol and tyrosol in EVOO and HOSO enriched with pomace extract. Taking into account these results, it would be foreseeable to predict the good stability of these two oils in the simulated frying process due to the presence of phenolic constituents with a reported high antioxidant activity. This behavior was predictable in the light of the results found by Chiou et al.[13] and Farag et al.[4] for similar enrichments by using olive leaves as a source of natural antioxidants.

The initial content of tocopherols was determined by the method exposed in Materials and Methods. Table 2 shows the concentration of α- and δ-tocopherol present in the four target edible oils before and during heating. The HOSO enriched with either natural antioxidants or with the artificial oxidation inhibitor presented higher concentration of α-tocopherol than EVOO (in the oils under study in the range 489.1−325.7 μg/mL vs 153.4 μg/mL of EVOO). δ-Tocopherol was only detected in sunflower oil but at 15.22 μg/mL, a much lower concentration than α-tocopherol. Despite the refining process, the concentration of lipophilic antioxidants in HOSO is still above the levels found in olive oil.

Evaluation of the Influence of the Simulated Frying Process on the Phenolic Fraction of Target Vegetable Oils. Before analysis of the results from this study, it is important to review the hypothesis in the literature that compares the efficiency of hydrophilic and lipophilic antioxidants to protect oils againts thermal degradation.[4,5] The presence of hydrophilic compounds in EVOO and their high antioxidant activity can be explained by the so-called "polar paradox",[25] which establishes that "polar antioxidants are more effective in non-polar lipids whereas non-polar antioxidants are more active in polar-lipid emulsions". This means that hydrophilic antioxidants protect more effectively against oxidation than lipophilic antioxidants, because the latter are dissolved in the oil, while phenolic compounds remain located at the air−oil interphase.[26] In order to study the evolution of degradation caused by the simulated frying process in the antioxidant fraction, the concentration of each compound was determined in the aliquots taken after each heating cycle. The thermal degradation of hydrophilic phenols in EVOO and HOSO enriched with olive pomace extract subjected to heating is plotted in Figure 3, A and B. As can be seen, the degradation trends observed for the monitored phenols made possible discrimination between two groups of compounds, the first of them composed of tyrosol, hydroxytyrosol and secoiridoid derivatives. Thus, tyrosol was quite stable during the complete heating process with a concentration decrease, after 20 cycles, close to 30% in both oils, results that agree with those reported by Carrasco-Pancorbo for EVOO.[27] This behavior was in contrast with that observed for hydroxytyrosol, the concentration of which experienced a drastic decrease of 80 and 100% for HOSO and EVOO, respectively, also in agreement with the results found by other authors.[28,29] Additionally, the degradation curve for hydroxytyrosol was clearly more pronounced in EVOO, in which this phenol practically disappeared after 10 heating cycles. A similar situation was observed for secoiridoid derivatives, which were rapidly decomposed in the early heating cycles, being almost completely degraded after 20 heating cycles. On the contrary, secoiridoids were not statistically affected (95% confidence level) in enriched HOSO during the whole heating process. Therefore, it is clear that these conjugates are indicators of the frying process for olive oil as well as other vegetable oils enriched with phenols from olive-tree materials. Thus, in this case, the resistance to oxidation during the heating process is superior for HOSO enriched with natural phenolic antioxidants than for EVOO. A similar behavior has not been reported so far. In EVOO, the effect of deep frying on the oil profile of hydrophilic antioxidants is particularly relevant with a direct influence on the organoleptic properties of the oil and self-protective antioxidant capacity.

The other group, formed by the rest of monitored phenols, shared a similar behavior to that of tyrosol. In HOSO enriched with pomace extract, a reduction between 3.5 and 40% was observed for vanillic acid, *p*-coumaric acid, hydroxytyrosol acetate, ferulic acid, luteolin and apigenin. In EVOO (see Figure 3, A and B), the final concentration of phenols such as *p*-coumaric acid, ferulic acid, apigenin-7-glucoside and apigenin as compared to their initial contents in oil was relatively constant, with estimated degradation between 9.4 and 20%.

Evaluation of the Influence of the Simulated Frying Process on Tocopherols in the Target Vegetable Oils. The lipophilic antioxidants that remain after the refining process of vegetable seed oils gain a special interest to compare the oxidative stability of oils and, particularly, taking into account their content in the different types of sunflower oil, which is above that in EVOO. The evolution of α-tocopherol in the four target oils during deep heating is shown in Figure 3C, which shows an identical behavior for sunflower oil and HOSO enriched with

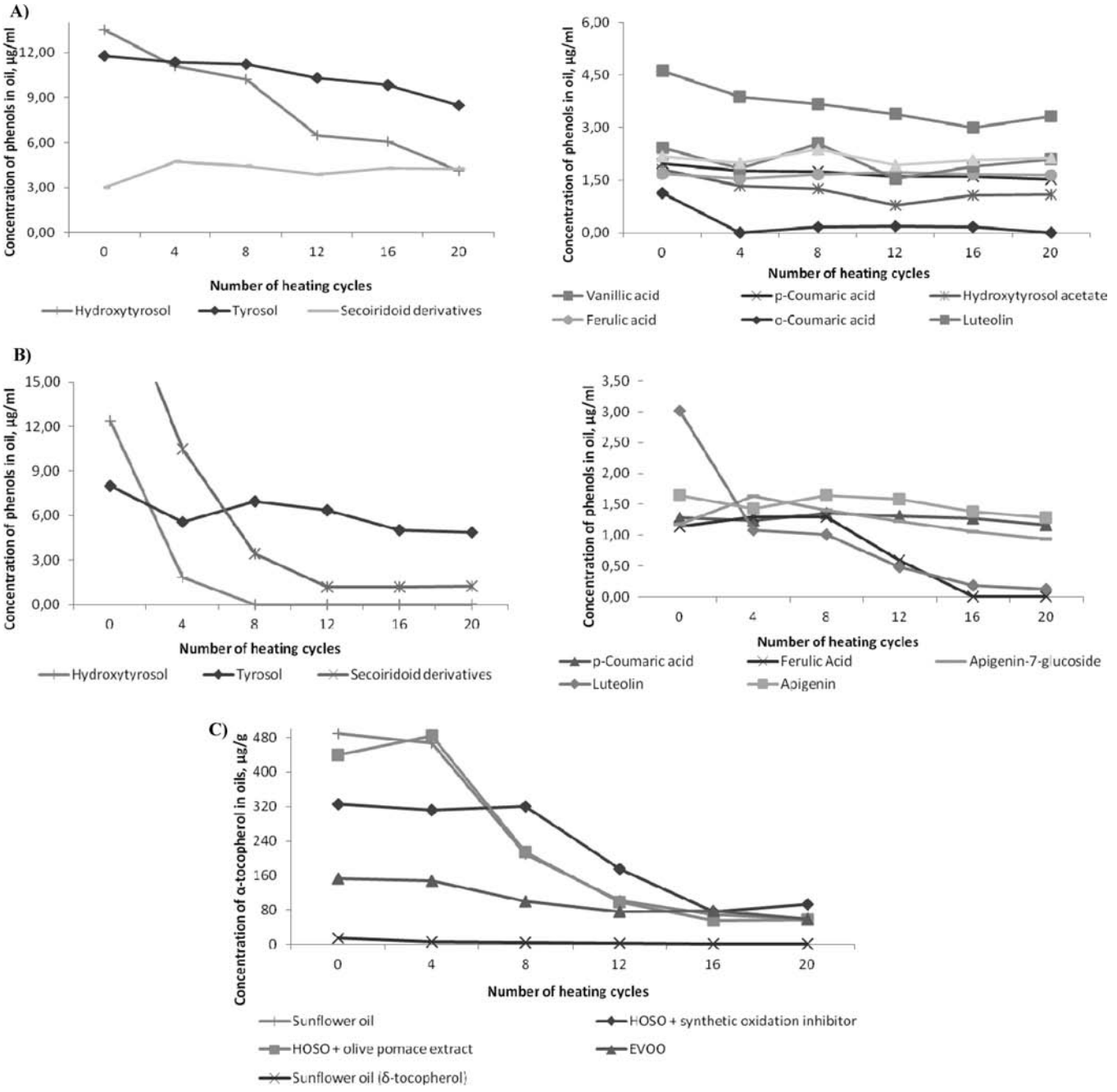

Figure 3. Behavior of the target antioxidants during the 20 cycles of the simulated frying process at 180 °C for (A) phenols in high-oleic sunflower oil (HOSO) enriched with olive pomace extract, (B) phenols in extra-virgin olive oil (EVOO) and, (C) α-tocopherol in the four target oils (δ-tocopherol is only present in sunflower oil).

natural antioxidants. A statistically significant degradation of α-tocopherol was observed just after 4 heating cycles. The level of α-tocopherol dropped around 50% after 8 heating cycles and 80% (concentrations below 70 μg/mL) after 16 heating cycles, when its concentration leveled off. This similar behavior of tocopherols in these oils is in disagreement with the literature, which points out a fate of these lipophilic antioxidants as a function of the insaturation degree of the given oil.[30,31]

In HOSO with added oxidation inhibitor the degradation of α-tocopherol was delayed up to the eighth cycle, when its concentration was significantly decreased to levels similar to those (viz., at the 16th cycle) for the other two sunflower oils: HOSO enriched with natural antioxidants and sunflower oil. HOSO concentration was also stabilized within heating cycles 16 to 20, which could be explained by the protective action of the oxidation inhibitor against decomposition of lipophilic antioxidants during deep heating. Furthermore, the comparison between the degradation curves of α-tocopherol for sunflower oil and HOSO enriched with oil pomace extract enables us to conclude that the enrichment with hydrophilic antioxidants does not exert any influence on the stability of lipophilic antioxidants. Also noticeable is the presence of δ-tocopherol only—and in low concentration—in sunflower oil. It is known that the relative stability of tocopherols decreases from δ- to α-tocopherol,[12]

which justifies the relatively fast degradation of the latter in the simulated frying process. δ-Tocopherol showed a behavior similar to that of α-tocopherol, but with a less pronounced degradation.

Degradation of α-tocopherol was more attenuated in the case of EVOO despite the fact that its concentration was considerably lower than in sunflower oil. Thus, after 16 heating cycles, the initial concentration of α-tocopherol was decreased to 50%, in contrast with the 75−85% decrease observed for sunflower oil and HOSO subjected to the same number of heating cycles. The less degradation of α-tocopherol in EVOO shown in Figure 3C is in agreement with Pellegrini et al.,[5] who suggested that hydrophilic antioxidants in EVOO are effective stabilizers of α-tocopherol during olive oil heating.

Concerning HOSO, this is not the case with dimethylsiloxane, the retardant effect of which on oxidation and polymerization of frying oils is well-known, the mechanism of action being based on the formation of a protective film at the oil−air interphase that limits access to oxygen.[32] A maximum effectiveness of DMS has been suggested by Márquez-Ruiz et al.[11] in discontinuous operations when the oil surface becomes unprotected against oxidation due to the absence of food, which is the situation in the present study.

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