Changes in Phenolic Composition and Antioxidant Activity of Virgin Olive Oil during Frying

ARTICLE *in* JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY · FEBRUARY 2003

Impact Factor: 2.91 · DOI: 10.1021/jf025932w · Source: PubMed

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

J. Agric. Food Chem. **2003,** *51,* 667−672 **667**

Changes in Phenolic Composition and Antioxidant Activity of Virgin Olive Oil during Frying

SERGIO GÓMEZ-ALONSO,† GIUSEPPE FREGAPANE,†
M. DESAMPARADOS SALVADOR,† AND MICHAEL H. GORDON*,‡

Departamento de Química Analítica y Tecnología de los Alimentos, Facultad de Químicas, Universidad de Castilla-La Mancha, Avenida Camilo José Cela, 10, 13071 Ciudad Real, Spain, and Hugh Sinclair Unit of Human Nutrition, School of Food Biosciences, The University of Reading, Whiteknights, P.O. Box 226, Reading RG6 6AP, U.K.

The concentration of hydroxytyrosol (3,4-DHPEA) and its secoiridoid derivatives (3,4-DHPEA-EDA and 3,4-DHPEA-EA) in virgin olive oil decreased rapidly when the oil was repeatedly used for preparing french fries in deep-fat frying operations. At the end of the first frying process (10 min at 180 °C), the concentration of the dihydroxyphenol components was reduced to 50−60% of the original value, and after six frying operations only about 10% of the initial components remained. However, tyrosol (*p*-HPEA) and its derivatives (*p*-HPEA-EDA and *p*-HPEA-EA) in the oil were much more stable during 12 frying operations. The reduction in their original concentration was much smaller than that for hydroxytyrosol and its derivatives and showed a roughly linear relationship with the number of frying operations. The antioxidant activity of the phenolic extract measured using the DPPH test rapidly diminished during the first six frying processes, from a total antioxidant activity higher than 740 μmol of Trolox/kg down to less than 250 μmol/kg. On the other hand, the concentration of polar compounds, oxidized triacylglycerol monomers (oxTGs), dimeric TGs, and polymerized TGs rapidly increased from the sixth frying operation onward, when the antioxidant activity of the phenolic extract was very low, and as a consequence the oil was much more susceptible to oxidation. The loss of antioxidant activity in the phenolic fraction due to deep-fat frying was confirmed by the storage oil and oil-in-water emulsions containing added extracts from olive oil used for 12 frying operations.

KEYWORDS: Phenols; antioxidant activity; DPPH; virgin olive oil; deep-fat frying

INTRODUCTION

Virgin olive oil is the most commonly used cooking fat in Mediterranean countries, and frying is an important cooking technique employed in domestic and industrial food preparation. During this operation, due to the high temperature and the absorption of oxygen and water, triacylglycerols in the oil suffer a series of reactions, namely hydrolysis, oxidation, isomerization, and polymerization (*1*). Changes in minor components, e.g., sterols or steryl esters, including the elimination of water or organic acid, also occur.

Recent studies suggest that the phenolic compounds naturally contained in virgin olive oil improve its resistance to oxidative deterioration (*2*). Phenolic antioxidants interrupt the initiation and propagation stages of the oxidative chain reaction since they react with lipid radicals to form more stable products (*1*). The main components of the phenolic fraction of virgin olive oil are hydroxytyrosol (3,4-DHPEA), tyrosol (HPEA), and their derivatives linked to the aldehydic and dialdehydic forms of elenolic acid (3,4-DHPEA-EDA, 3,4-DHPEA-EA, HPEA-EDA, and HPEA-EA), which are described as secoiridoids (*3*−*7*). Moreover, significant amounts of the lignans pinoresinol and 1-acetoxypinoresinol are also present (*6*−*9*). Some of these compounds possess antioxidant activity (*10*, *11*) and therefore improve the oxidative stability of virgin olive oil and extend its shelf life (*11*−*13*). Previous studies have used the Folin−Ciocalteu method to investigate the residual total polyphenol content after frying (*14*, *15*), but this classic method is less specific and informative than the quantification of individual phenols by HPLC.

The higher oxidative stability of virgin olive oil, compared to that of other vegetable oils, is due to both the high oleic acid (monounsaturated) and low polyunsaturated fatty acid content of the triacylglycerols, and also to the level of natural phenolic components with antioxidant activity. Within Spanish virgin olive oil varieties, Cornicabra and Picual are those with the highest content of both oleic acid and phenolic compounds (*16*, *17*).

The purpose of this work was to study the changes in the phenols of virgin olive oil and their antioxidant activity following several deep-fat frying operations. The antioxidant

* Corresponding author (E-mail m.h.gordon@reading.ac.uk; telephone +44 118 3786723; fax + 44 118 9310080).
† Universidad de Castilla-La Mancha.
‡ The University of Reading.

10.1021/jf025932w CCC: $25.00
Published on Web 12/19/2002

activity of the phenolic fraction of virgin olive oil was characterized by its activity in stabilizing oils and emulsions against oxidative deterioration, and by the DPPH test. Both oils and emulsions were used to determine whether possible changes in the polarity of the antioxidants during frying would affect the activity differently in the two media. The DPPH test has previously been successfully employed in assessing the antioxidant activity of the phenolic extracts from virgin olive oil (*2*, *18*, *19*). Frying oil deterioration was monitored by the formation of oxidized triacylglycerols (monomers) (oxTGs) and dimeric and polymerized TGs during frying and under accelerated oxidation conditions at 60 °C.

MATERIALS AND METHODS

Olive Oil Samples and Reagents. Extra virgin olive oil (EVOO) of the Cornicabra variety was purchased from a Spanish industrial oil mill (Toledo, Spain). Refined olive oil (OO) was purchased from a local retailer (Reading, England). Syringic acid, *p*-hydroxyphenylacetic acid, Folin−Ciocalteu reagent, caffeic acid, α-tocopherol (95%), monoolein, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), Trolox, hexanal, and Tween 20 were purchased from Sigma Chemical Co. (Poole, UK). All solvents used throughout the study were HPLC or analytical grade, obtained from Merck (Poole, UK) or Rathburn (Walkerburn, UK).

Purified Antioxidant-Free Olive Oil (POO) Preparation. Phenols and tocopherols were removed from OO by column chromatography using alumina (Merck), according to the method of Yoshida (*20*).

Deep Frying. Potato slices (200 g) were cut into pieces (approximately 40−50 × 10 × 10 mm) and fried in a DeLonghi domestic fryer (model F620), filled initially with EVOO (2 L). The oil was heated to 180 °C, and the potato slices were added and fried for about 10 min. After each frying operation, the oil was allowed to cool to less than 50 °C. Twelve batches of potatoes were fried with the same oil during a 6-day period (two operations per day). Thus, the total frying period by the end of the experiment was 2 h, with 12 cooling stages in total. Samples of oil (30 g) were removed after each frying operation and stored at −20 °C until analysis.

Extraction of Phenolic Compounds. A sample of virgin olive oil (2.5 g) was weighed, and when the extract was intended for HPLC analysis, 250 μL of a solution of the internal standard (15 mg·kg^{-1} of syringic acid in methanol) was added and the solvent was evaporated with a rotary evaporator at 35 °C under vacuum. The oil was then dissolved in 6 mL of hexane. A diol-bonded phase cartridge (Supelco Co., Bellefonte, PA) was used to extract the phenolic fraction. The cartridge was conditioned by eluting with methanol (6 mL) and hexane (6 mL), and the oil solution was then applied to the SPE column. The column was washed with hexane (2 × 3 mL) and with hexane/ethyl acetate (85:15, v/v; 4 mL), which were run through the cartridge and discarded. Finally, the phenols were eluted with methanol (15 mL), and the solvent was removed with a rotary evaporator at 35 °C under vacuum until dryness. The phenolic residue was dissolved in methanol/water (1:1 v/v; 250 μL) for HPLC analysis or in methanol for the DPPH test.

For the accelerated oxidation assay, where a higher amount of phenolic extract was required, phenolic compounds from fresh EVOO or EVOO after frying were extracted by liquid−liquid extraction with aqueous methanol (80:20), as described by Montedoro et al. (*21*).

RP-HPLC Determination of Phenols [modified from Mateos et al., (*6*)]. HPLC analysis was performed using an HP series 1050 system equipped with an automatic injector and a diode array detector. A reverse-phase C18 column (250 × 4.6 mm i.d., 5 μm particle size) (Kromasil, Hichrom Ltd., Reading, UK) was used, with an injection volume of 20 μL and a flow rate of 1.0 mL/min. The mobile phase was a mixture of water/acetic acid (95:5 v/v) (solvent A), methanol (B), and acetonitrile (C). The mobile phase gradient was from 95% (A)−2.5% (B)−2.5% (C) to 34% (A)−33% (B)−33% (C) in 50 min, followed by 100% (B) for 15 min to clean the column. Chromatograms were analyzed at 240, 280, and 335 nm, and phenolic compounds were quantified at 280 nm using syringic acid as internal standard and the response factors determined by Mateos et al. (*6*).

Analysis of Polar Compounds [adapted from Márquez-Ruiz et al. (*22*)]. Oxidized polar compounds were determined by high-performance size-exclusion chromatography (HPSEC) with an Agilent 1100 HPLC (Agilent Technologies, Boeblingen, Germany) equipped with a refractive index detector (working at 35 °C), using two PL gel columns (300 × 7.5 mm, 5 μm particle size and 100 and 500 Å pore size, respectively; Agilent, Bracknell, UK) at 30 °C. Tetrahydrofuran at 1 mL/min was used as the mobile phase. The injection volume was 20 μL, and monoolein was added as an internal standard.

DPPH Radical Scavenging Effect. DPPH was used as a stable radical (*23*). Several concentrations of SPE phenolic extracts dissolved in methanol (0.1 mL) were added to a DPPH methanolic solution (2.9 mL, 6×10^{-5} M). The decrease in absorbance of the resulting solution was then measured at 515 nm at 0, 5, 10, 15 min, and then every 15 min until 1 h, with a Perkin-Elmer Lambda-Bio spectrometer (Beaconsfield, UK). The absorbance was plotted against time, and the percentage of absorbance reduction at 15 min was used as a measure of the antioxidant activity of the extract. A calibration curve was determined using Trolox as an external standard with a range of concentrations from 0.19 to 0.93 mM. The equation was remaining DPPH (%) = −132.08[Trolox] + 102.17 ($r^2 = 0.99$). The results were expressed as micromoles of Trolox per kilogram of EVOO.

Emulsion Preparation. Oil-in-water emulsions (30:70, 50 g) were prepared by dissolving Tween 20 (1%) in acetate buffer (0.1 M, pH 5.4) which was cooled in an ice bath. The oil sample was added dropwise while the sample was sonicated with a Vibracell high-intensity ultrasonic processor (Sonics & Materials Inc., Danbury, CT) (*25*). The separation of the oil from emulsions for analysis was performed by freezing at −70 °C, thawing, and centrifugation.

Accelerated Oxidation Experiments. Antioxidants and extracts were added to purified OO (20 g) before oil storage or to purified OO (15 g) before emulsion preparation. A control sample and samples containing 0.31 and/or 0.62 mmol·kg^{-1} of α-tocopherol or phenolic extract from fresh or from EVOO used for 12 frying operations were studied. The mean molecular mass of the phenolic compounds was assumed to be 300 for calculation of the extract concentration. Purified (antioxidant-free) bulk OO (20 g) and OO-in-water emulsions (50 g) were oxidized at 60 °C in darkness in a 50-mL beaker covered with aluminum foil for the bulk oil or in a 100-mL capped glass bottle for the emulsions. The progress of oxidation was monitored by measuring the peroxide value (PV) (AOCS Official Method Cd 8-53) and hexanal content by solid-phase microextraction (SPME) and GC as described previously (*24*). Each experiment was carried out in triplicate.

RESULTS AND DISCUSSION

Changes in Phenols Composition. The natural phenolic compounds in virgin olive oil suffered important changes during frying, in agreement with previous literature (*14*, *15*). The initial concentration of these compounds and their levels after 6 and 12 frying operations, determined by SPE, are reported in **Table 1**. All components decreased in concentration with an increase in the number of frying operations, although the rate of loss depended on chemical structure and antioxidant activity.

The concentration of 3,4-DHPEA and its derivatives 3,4-DHPEA-EDA and 3,4-DHPEA-EA in virgin olive oil rapidly diminished with the number of frying operations, as shown in **Figure 1**. By the end of the first process (10 min at 180 °C), these components had decreased by 40−50% of their original concentration, and after six frying operations less than 10% of the original content of these components remained (**Table 1**). The level of these components was very low after 12 frying operations.

The observed trend was consistent with the high antioxidant activity of hydroxytyrosol and its secoiridoid derivatives in virgin olive oil (*11*, *26*), since antioxidants act by reacting rapidly with lipid radicals and are thereby consumed. The level

Table 1. Changes in Phenolic Composition of Virgin Olive Oil during Frying[a]

compound	no. of frying operations		
	0	6	12
hydroxytyrosol (3,4-DHPEA)	4.88 ± 0.45	0.45 ± 0.01	nd
3,4-DHPEA-EDA	52.49 ± 4.24	3.80 ± 0.40	0.79 ± 0.05
3,4-DHPEA-EA	95.51 ± 7.57	8.81 ± 0.58	5.68 ± 0.64
tyrosol (*p*-HPEA)	6.59 ± 0.15	5.68 ± 0.02	5.26 ± 0.14
p-HPEA-EDA	96.70 ± 8.52	103.0 ± 0.75	77.46 ± 2.44
p-HPEA-EA	53.09 ± 2.11	29.92 ± 1.38	12.94 ± 0.26
pinoresinol	2.40 ± 0.28	0.80 ± 0.03	0.57 ± 0.00
1-acetoxypinoresinol	8.50 ± 1.34	0.99 ± 0.17	0.73 ± 0.06
elenolic acid	12959 ± 70	1060 ± 45	292 ± 9

[a] Concentrations expressed as milligrams per kilogram, except for elenolic acid which is in arbitrary peak area units. nd, not detected.

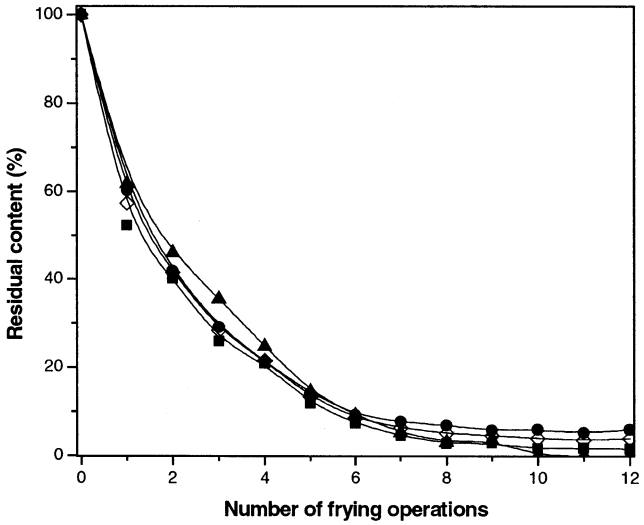

Figure 1. Changes in the content of hydroxytyrosol and its derivatives during frying. ▲, 3,4-DHPEA; ■, 3,4-DHPEA-EDA; ●, 3,4-DHPEA-EA; ◇, sum of the previous three compounds.

of hydroxytyrosol and its derivatives in virgin olive oil was reported to correlate well with the oxidative stability of the oil, as determined by the Rancimat method (*7*, *12*, *13*). Moreover, the similar rate of loss of these three compounds agrees with the results reported by Baldioli et al. (*11*), who found similar antioxidant activity for each of these compounds. The recent report that hydroxytyrosol and its derivatives were lost more rapidly during heating at 180 °C from an oil richer in linoleic acid (Arbequina variety) than from an oil richer in oleic acid (Picual variety) (*27*) supports the role of oxidative deterioration in the loss of the antioxidants. However, a contribution of non-oxidative thermal degradation to antioxidant loss cannot be discounted, and some of the phenols may have been lost by oxidative coupling, although losses by this route should be small due to the low concentration of phenols in the oil.

Another important family of compounds within the phenolic fraction of virgin olive oil is tyrosol (*p*-HPEA) and its secoiridoid derivatives (*p*-HPEA-EDA and *p*-HPEA-EA). This family showed a completely different behavior during the 12 frying processes studied. The reduction in the concentration of this group of compounds was much smaller than that observed for the hydroxytyrosol family and moreover showed an almost linear relationship with the number of frying operations (**Figure 2**). The observed trend is similar to that reported by Brenes et al. (*28*) in virgin olive oil of the Picual variety during storage at room temperature. The *p*-HPEA and *p*-HPEA-EDA content

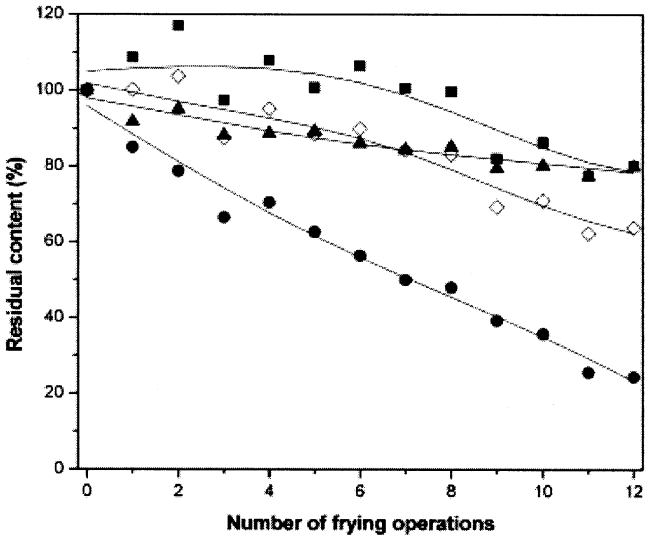

Figure 2. Changes in the content of tyrosol and its derivatives during frying. ▲, *p*-HPEA; ■, *p*-HPEA-EDA; ●, *p*-HPEA-EA; ◇, sum of the previous three compounds.

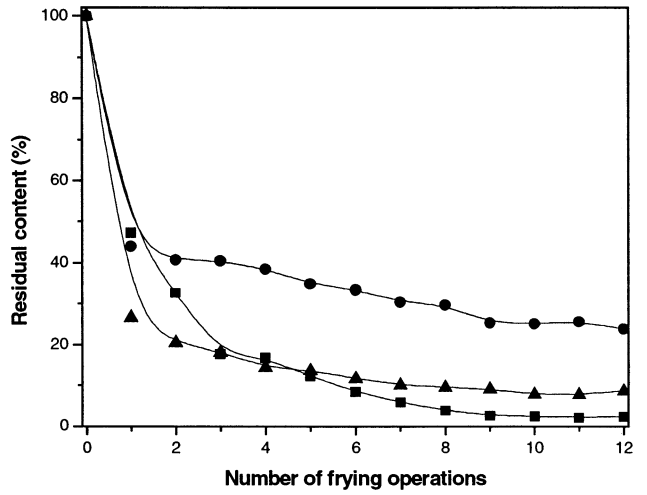

Figure 3. Changes in other polar compounds from the phenolic fraction during frying. ■, Elenolic acid; ●, pinoresinol; ▲, 1-acetoxypinoresinol.

after 12 frying operations decreased by only about 20% of the initial value, as compared to >95% for hydroxytyrosol and its secoiridoid derivatives. The loss of *p*-HPEA-EA was higher, although its concentration after 12 frying operations was still nearly 25% of the initial content. These results are consistent with the much higher antioxidant activity of hydroxytyrosol and its derivatives, as compared to that of the tyrosol family, in virgin olive oil, as reported by other authors (*11*, *26*). According to a recent report (*27*), most of the tyrosol derivatives were also lost by oxidation during prolonged heating at 180 °C, although the total heating period was much longer (up to 24 h compared with 2 h in the present frying experiments), and the change in *p*-HPEA-EDA was not clear due to coelution of an oxidized compound with this component.

Elenolic acid is not a phenol, but undoubtedly it is contained in the polar extract of virgin olive oil. The presence of this compound in a free form is due to the hydrolysis of oleuropein, ligstroside, and related compounds (*6*, *21*, *28*). The concentration of this acid rapidly diminished during frying, as shown in **Figure 3**, and the acid was present at only about 2% of its initial content after 12 frying operations. This does not mean that this compound possessed any antioxidant activity, but the observed

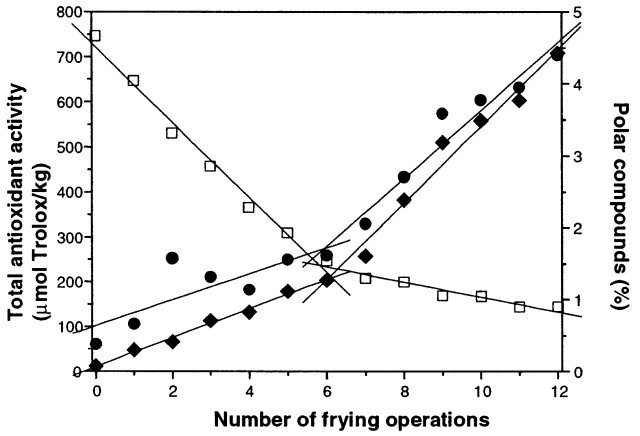

Figure 4. Changes in the antioxidant activity of the phenolic extract, assessed by the DPPH assay, and in the levels of oxidized polar compounds formed during frying. □, TAA; ●, OxTGs; ◆, PTGs + DTGs.

decrease was probably due to rapid oxidation. Briante et al. (*29*) showed that elenolic acid was a very poor antioxidant compared to hydroxytyrosol.

The lignans pinoresinol and 1-acetoxypinoresinol were described as major components of the phenolic fraction of virgin olive oil (*8*). According to the literature, these compounds possess in vitro antioxidant activity (*30*), and they may be able to inhibit lipid peroxidation in vivo if they are absorbed (*31*). As was also observed for elenolic acid, the content of these compounds fell drastically in the first frying operation, with a much lower reduction rate in subsequent frying operations (**Figure 3**). The increased stability of some lignans compared to that of hydroxytyrosol derivatives during the frying of potatoes is consistent with the recent report covering changes during dry heating at 180 °C (*27*).

Changes in the Antioxidant Activity. The antioxidant activity of the phenolic extract, determined by the DPPH test, rapidly decreased progressively during the first six frying processes, from a total antioxidant activity (TAA) higher than 740 μmol of Trolox/kg down to less than 250 μmol/kg, as shown in **Figure 4**. During subsequent frying operations, the total antioxidant activity of the phenolic extract continued to decrease progressively, but the rate of reduction was much lower. To confirm that the method of isolation with the SPE cartridge was able to extract the antioxidant components completely for both fresh and used virgin olive oil, liquid−liquid extraction of the phenolic fraction was performed with the original virgin olive oil and the oil after 12 frying batches, and the DPPH scavenging activity was nearly the same as for the samples isolated by SPE. The TAA for fresh oil was 693 and 746 μmol of Trolox/kg for liquid−liquid and solid-phase extraction, respectively, and 139 and 144 μmol/kg respectively for oil after 12 frying operations. Since large amounts of phenolic extract were required for accelerated oxidation experiments, liquid−liquid extraction was used to isolate the extract for this application, whereas SPE was used for the isolation of the extract for HPLC analysis due to its speed and convenience.

The concentration of total polar material (TPM), including oxidized triacylglycerol monomers (oxTGs), dimeric TGs (DTGs), and polymerized TGs (PTGs) (**Figure 4**), increased rapidly from the sixth frying operation onward, which is consistent with the low antioxidant activity of the phenolic extract after this point, with the consequence that the oil was more susceptible to oxidation. These findings are consistent with the low residual content of the main antioxidant compounds, hydroxytyrosol and its derivatives, in virgin olive oil after six

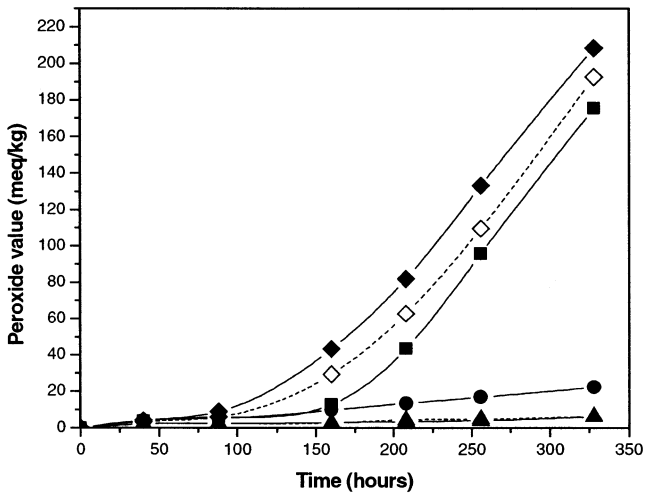

Figure 5. Oxidation at 60 °C of bulk oil containing phenolic extracts from fresh and used virgin olive oil. ■, Control; ●, α-tocopherol (0.62 mM); ▲, fresh oil extract (0.62 mM); △, extract from fresh virgin olive oil (0.31 mM); ◆, extract from virgin olive oil used for 12 frying operations (0.62 mM); ◇, extract from virgin olive oil used for 12 frying operations (0.31 mM).

frying operations. The loss of this family of antioxidants would allow the increase in the rate of formation of oxidized polar compounds in the oil.

The nutritional quality of the fried potatoes is likely to deteriorate after six frying operations as the level of oxidized polar compounds increases because oil absorbed by fried potatoes from poor-quality oil contains high levels of oxidized polar compounds (*32*), which showed negative physiological and biochemical effects in rats fed high doses of this polar material (*33*, *34*). Nevertheless, the levels of TPM (9%) and DTGs and PTGs (4.4%) in the olive oil after 12 frying operations (**Figure 4**) were much lower than the upper limits of 24% and 12% for TPM and DTGs + PTGs, respectively, recommended by the German Society for Fat Science (*35*).

To verify whether the residual phenolic compounds contained in the olive oil at the end of the 12 frying operations still maintained any antioxidant effects, the tocopherol-free phenolic extract from this oil was added to a purified antioxidant-free olive oil (POO). This olive oil was stored in the dark at 60 °C, and the peroxide value was periodically measured during the course of the oxidation process. The stability of this oil was compared with that of samples of the oil without additives (control) and with the addition of α-tocopherol (0.62 mM) and two levels of phenolic extract obtained from the fresh virgin olive oil (0.62 and 0.31 mM). These concentrations were chosen to reproduce the original concentration of phenolic extract and half the original concentration in the Cornicabra olive oil.

The stability of the olive oil samples containing the additives is shown in **Figure 5**. It is clear that the addition of fresh virgin olive oil phenolic extract gave a highly stable oil, and that after more than 300 h of storage at 60 °C, the peroxide value of the oil remained lower than 10 mequiv/kg. The oil containing phenolic extract from fresh virgin olive oil was even more stable than that obtained by the addition of an equimolar concentration of α-tocopherol when assessed by PV, but the sample containing α-tocopherol contained less hexanal than the sample containing phenolic extract from fresh virgin olive oil after 328 h of storage (**Table 2**). However, the phenolic extract from the oil used for 12 frying operations did not contribute any increased stability to the oil. Indeed, a small pro-oxidant effect was observed when changes in PV for this sample were compared to those of the

Table 2. Hexanal Content after Storage at 60 °C

prepared olive oil	hexanal content[a]	
	bulk oil[b]	emulsion[c]
fresh POO	164 ± 14	
control	26 260 ± 3104	16 119 ± 1803
α-tocopherol (0.62 mM)	286 ± 52	351 ± 53
fresh Cornicabra virgin olive oil extract (0.62 mM)	432 ± 96	3434 ± 666
12 frying extract (0.62 mM)	21 317 ± 2565	16 066 ± 1533

[a] Expressed as arbitrary peak area units. [b] Bulk oil after 328 h at 60 °C. [c] Emulsion after 208 h at 60 °C.

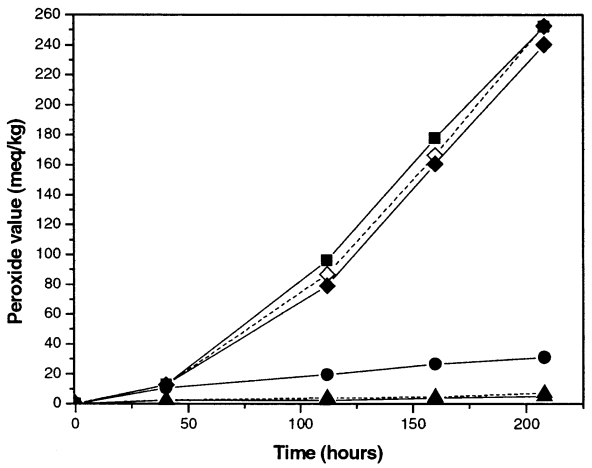

Figure 6. Oxidation at 60 °C of oil in water emulsions containing phenolic extracts from fresh and used virgin olive oil. ■, Control; ●, α-tocopherol (0.62 mM); ▲, fresh oil extract (0.62 mM); △, fresh oil extract (0.31 mM); ◆, extract from virgin olive oil used for 12 frying operations (0.62 mM); ◇, extract from virgin olive oil used for 12 frying operations (0.31 mM).

control oil, and the pro-oxidant effect was enhanced when the higher level of extract was present. The pro-oxidant effect was not evident from the hexanal determinations, but the lack of an antioxidant effect was confirmed. The pro-oxidant effect was probably caused by the presence of other compounds in the extract, like the oxidized polar compounds that favor the oxidation process. In fact, the level of oxidized triacylglycerols, and triacylglycerol dimers and polymers in the phenolic extract from oil used for 12 frying operations was increased by 11 and 20 times, respectively, compared to their content in the fresh virgin olive oil (**Figure 4**).

The fact that the phenolic extract from the frying oil used for 12 frying operations did not show any antioxidant activity in stored oil samples indicates that residual antioxidant activity detected by the DPPH method was due to compounds that react with DPPH radicals but do not interrupt the lipid peroxidation chain reaction. Tyrosol and its derivatives, which are known not to possess antioxidant activity in stored oil samples, may have been responsible for this misleading residual activity in the DPPH test.

The antioxidant behavior of the extracts in an oil-in-water emulsion was similar to that observed for the bulk oil. The main difference was that, in this case, the phenolic extract from oil used for 12 frying operations did not show any pro-oxidant effect when evaluated by PV measurements (**Figure 6**), possibly due to the oxidized compounds partitioning into the aqueous phase of the emulsion.

The analysis of hexanal in the volatile fraction of oil or emulsion samples stored for 328 and 208 h, respectively (**Table 2**), showed a large increase in this oxidation product in stored samples containing the phenolic extract after 12 frying operations as compared with the POO with added fresh phenolic extract (50 times higher) for both bulk oils and emulsions. This confirmed the absence of antioxidant activity in the extract after 12 frying operations, since the hexanal content was similar to that in the control sample.

Virgin olive oil shows a good behavior during frying in terms of resistance to formation of polar compounds and TPMs. This was found in the current frying experiment and is similar to the results found by other authors under similar conditions (*15*, *36*). The low linoleic acid content of the oil is significant in contributing to this effect, and the phenolic components may contribute to the oil stability in the early stages of frying.

LITERATURE CITED

(1) Frankel, N. *Lipid Oxidation*; The Oily Press Ltd.: Dundee, Scotland, 1998.

(2) Gordon, M. H.; Paiva-Martins, F.; Almeida, M. Antioxidant activity of hydroxytyrosol acetate compared with that of other olive oil polyphenols. *J. Agric. Food Chem.* **2001**, *49*, 2480−2485.

(3) Vázquez, A.; Janer, C.; Janer, M. L. Determinación de los polifenoles del aceite de oliva. *Grasas Aceites* **1973**, *24*, 350−357.

(4) Montedoro, G. F.; Servilli, M.; Baldioli, M.; Selvaggini, R.; Miniati, E.; Macchioni, A. Simple and hydrolyzable phenolic compounds in olive oil. Note 3. Spectroscopic characterization of the secoiridoid derivates. *J. Agric. Food Chem.* **1993**, *41*, 2228−2234.

(5) Brenes, M.; García, A.; García, P.; Rios, J. J.; Garrido, A. Phenolic compounds in Spanish olive oils. *J. Agric. Food Chem.* **1999**, *47*, 3535−3540.

(6) Mateos, R.; Espartero, J. L.; Trujillo, M.; Rios, J. J.; León-Camacho, M.; Alcudia, F.; Cert, A. Determination of phenols, flavones and lignans in virgin olive oils by solid-phase extraction and high-performance liquid chromatography with diode array ultraviolet detection. *J. Agric. Food Chem.* **2001**, *49*, 2185−2192.

(7) Gómez-Alonso, S.; Salvador, M. D.; Fregapane, G. Phenolic Compounds Profile of Cornicabra Virgin Olive Oil. *J. Agric. Food Chem.* **2002**, *50*, 6812−6817.

(8) Owen, R. W.; Mier, W.; Giacosa, A.; Hull, W. E.; Spiegelhalder, B.; Bartsch, H. Identification of lignans as major components in the phenolic fraction of olive oil. *Clin. Chem.* **2000**, *46*, 976−988.

(9) Brenes, M.; Hidalgo, F. J.; García, A.; Rios, J. J.; García, P.; Zamora, R.; Garrido, A. Pinoresinol, and 1-acetoxypinoresinol, two new phenolic compounds identified in olive oil. *J. Am. Oil Chem. Soc.* **2000**, *77*, 715−720.

(10) Gutfinger, T. Polyphenols in olive oils. *J. Am. Oil Chem. Soc.* **1981**, *58*, 966−968.

(11) Baldioli, M.; Servilli, M.; Perretti, G.; Montedoro, G. F. Antioxidant activity of tocopherols and phenolic compounds of virgin olive oil. *J. Am. Oil Chem. Soc.* **1996**, *73*, 1589−1593.

(12) Montedoro, G. F.; Servilli, M.; Baldioli, M.; Miniati, E. Simple and hydrolyzable phenolic compounds in olive oil. Note 2. Initial characterization of the hydrolyzable fraction. *J. Agric. Food Chem.* **1992**, *40*, 1577−1580.

(13) Caponio, F.; Alloggio, V.; Gomes, T. Phenolic compounds of virgin olive oil: influence of paste preparation techniques. *Food Chem.* **1999**, *64*, 203−209.

(14) Pellegrini, N.; Visioli, F.; Buratti, S.; Brighenti, F. Direct analysis of total antioxidant activitty of olive oil and studies on the influence of heating. *J. Agric. Food Chem.* **2001**, *49*, 2532−2538.

(15) Andrikopoulos, N. K.; Kalogeropoulos, N.; Falirea, A.; Barbagianni, M. N. Performance of virgin olive oil and vegetable shortening during domestic deep-frying and pan-frying of potatoes. *Int. J. Food Sci. Technol.* **2002**, *37*, 177−190.
(16) Aparicio, R.; Roda, L.; Albi, M. A.; Gutiérrez, F. Effects of various compounds on virgin olive oil stability measured by Rancimat. *J. Agric. Food Chem.* **1999**, *47*, 4150−4155.
(17) Salvador, M. D.; Aranda, F.; Gómez-Alonso, S.; Fregapane, G. Cornicabra virgin olive oil: a study of five crop seasons. Composition, quality and oxidative stability. *Food Chem.* **2001**, *74*, 267−274.
(18) Mosca, L.; De Marco, C.; Visioli, F.; Cannella, C. Enzymatic assay for the determination of olive oil polyphenol content: assay conditions and validation of the method. *J. Agric. Food Chem.* **2000**, *48*, 297−301.
(19) Frankel, E. N.; Meyer, A. S. Review. The problems of using one-dimensional methods to evaluate multifunctional food and biological antioxidants. *J. Sci. Food Agric.* **2000**, *80*, 1925−1941.
(20) Yoshida, H. Influence of fatty acids of different unsaturation in the oxidation of purified vegetable oils during microwave irradiation. *J. Sci. Food Agric.* **1993**, *62*, 41−47.
(21) Montedoro, G. F.; Servilli, M.; Baldioli, M.; Miniati, E. Simple and hydrolyzable phenolic compounds in olive oil. Note 1. Their extraction, separation, and quantitative and semiquantitative separation and evaluation by HPLC. *J. Agric. Food Chem.* **1992**, *40*, 1571−1576.
(22) Márquez-Ruiz, G.; Jorge, N.; Martín-Polvillo, M.; Dobarganes, M. C. Rapid, quantitative determination of polar compounds in fats and oils by solid-phase extraction and size-exclusion chromatography using monostearin as internal standard. *J. Chromatogr. A* **1996**, *749*, 55−60.
(23) Brand-Williams, W.; Cuvelier, M. E.; Berset, C. Use of a free radical method to evaluate antioxidant activity. *Lebensm.-Wiss. Technol.* **1995**, *28*, 25−30.
(24) Keceli, T.; Gordon, M. H. The antioxidant activity and stability of the phenolic fraction of green olives and extra virgin olive oil. *J. Sci. Food Agric.* **2001**, *81*, 1391−1396.
(25) Roedig-Penman, A.; Gordon, M. H. Antioxidant properties of myricetin and quercetin in oil and emulsions. *J. Am. Oil Chem. Soc.* **1998**, *75*, 169−180.
(26) Papadopoulos, G. K.; Boskou, D. Antioxidant effect of natural phenols on olive oil. *J. Am. Oil Chem. Soc.* **1991**, *68*, 669−671.
(27) Brenes, M.; García, A.; Dobarganes, M. C.; Velasco, J.; Romero, C. Influence of thermal treatments simulating cooking processes on the polyphenol content in virgin olive oil. *J. Agric. Food. Chem.* **2002**, *50*, 5962−5967.
(28) Brenes, M.; García, A.; García, P.; Garrido, A. Acid hydrolysis of secoiridoid aglycons during storage of virgin olive oil. *J. Agric. Food. Chem.* **2001**, *49*, 5609−5614.
(29) Briante, R.; La Cara, R.; Tonziello, M. P.; Febbraio, F.; Nicci, R. Antioxidant activity of the main bioactive derivatives from Oleuropein hydrolysis by hyperthermophilic β-glycosidase. *J. Agric. Food Chem.* **2001**, *49*, 3198−3203.
(30) Owen, R. W.; Mier, W.; Giacosa, A.; Hull, W. E.; Spiegelhalder, B.; Bartsch, H. Phenolic compounds and squalene in olive oils: the concentration and antioxidant potential of total phenols, simple phenols, secoiridoids, lignans and squalene. *Food Chem. Toxicol.* **2000**, *38*, 647−659.
(31) Kang M. H.; Mait, M.; Tsujihara, N.; Osawa, T. Sesamolin inhibits lipid peroxidation in rat liver and kidney. *J. Nutr.* **1998**, *128*, 1018−1022.
(32) Gordon, M. H.; Kourimská, L. The effects of antioxidants on changes in oils during heating and deep frying. *J. Sci. Food Agric.* **1995**, *68*, 347−353.
(33) Billek, G. Die Veränderungen von Nahrungsfetten bei höheren Temperaturen. *Fat Sci. Technol.* **1992**, *94*, 161−172.
(34) Lamboni, C.; Perkins, E. G. Effects of dietary heated fats on rat level enzyme activity. *Lipids* **1996**, *31*, 955−962.
(35) German Society for Fat (DGF): Recommendations of the 3rd International Symposium on deep-fat frying-optimal operation, 20−21 March 2000, Hagen (Germany). *Eur. J. Lipid Sci. Technol.* **2000**, *102*, 594.
(36) Andrikopoulos, N. K.; Dedoussis, G. V. Z.; Tzamtzis, V.; Chiou, A.; Boskou, G. Evaluation of medium polarity materials isolated from fried edible oils by RP-HPLC. *Eur. J. Lipid Sci. Technol.* **2002**, *104*, 110−115.

Received for review September 3, 2002. Revised manuscript received November 14, 2002. Accepted November 18, 2002. This research was partially supported by the CICYT (Comisión Interministerial de Ciencia y Tecnología; AGL 2001-0906).

JF025932W