Direct Analysis of Total Antioxidant Activity of Olive Oil and Studies on the Influence of Heating

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Aim of this study was to evaluate the total antioxidant activity (TAA) of extra virgin olive oil (EVOO) and the effect of heating on the α-tocopherol content and TAA in relation to the presence of polyphenols, heating time, and temperature. Experiments included the measurement by ABTS decolorization assay of antioxidant capacity of α-tocopherol and 14 simple phenolic compounds present in EVOO, either dissolved in ethanol or added to refined olive oil, and the evaluation of TAA, total phenols, and α-tocopherol of six commercial EVOO and three olive oils. Finally, four experimental oils were prepared from refined olive oil containing a fixed amount (300 ppm) of α-tocopherol and increasing amounts of polyphenols (25, 125, 225, and 326 ppm) extracted from EVOO. The thermal stability of experimental oils under domestic heating conditions (heating time from 30 to 120 min, heating temperature from 160 to 190 °C) was studied by evaluating the loss of α-tocopherol and TAA according to a Latin square design. Results indicate that TAA of commercial oils is mainly due to their phenol and α-tocopherol content. Heating experiments suggest that polyphenols from EVOO are effective stabilizers of α-tocopherol during olive oil heating, thus contributing to the nutritional value of cooked foods.

Keywords: *Olive oil; antioxidant activity; thermal oxidation; polyphenols; α-tocopherol; hydroxytyrosol*

INTRODUCTION

Free radicals are thought to be responsible for several pathological processes, such as cancer (*1*, *2*), atherosclerosis (*3*), and negative cellular changes associated with aging (*4*); conversely, the consumption of dietary antioxidants seems to play an important role in protecting against these degenerative events. Because of the current interest in dietary antioxidants, a number of assays have been introduced that evaluate the total antioxidant activity of food extracts (*5*) and beverages such as fruit juices (*6*), wines (*7*, *8*), and tea (*9*, *10*).

Extra virgin olive oil (EVOO), produced by mechanical pressure of the fruits (drupes) of *Olea europea* L., in addition to its high proportion of monounsaturated fatty acids, i.e., oleic acid, and the modest presence of polyunsaturated fatty acids, contains natural antioxidants such as tocopherols, carotenoids, sterols, and phenolic compounds (*11*). Among the phenolic compounds found in extra virgin olive oils, gallic, caffeic, vanillic, *p*-coumaric, syringic, ferulic, homovanillic, *p*-hydroxybenzoic, protocatecuic acids, tyrosol, and hydroxytyrosol are the most representative (*12*, *13*). Because of these characteristics, EVOO is particularly resistant to storage and more suitable for cooking than other vegetable oils (*14*). The effects of phenols and tocopherols on the oxidative stability in EVOO during storage have been extensively evaluated: it has been demonstrated that phenolic compounds are more effective than tocopherols in enhancing the stability of olive oil toward oxidation (*15*, *16*). On the contrary, it is at present not clear which are the main antioxidant components of EVOO that affect its stability during thermal degradation.

The purpose of this investigation was to study the effectiveness of different amounts of phenolic compounds on the stability of EVOO, by evaluating the loss of α-tocopherol and the total antioxidant activity (TAA) of EVOO during heating. To this aim, we first determined the antioxidant activity of the principal simple phenolic compounds present in EVOO and characterized several commercial olive oils for their TAA and their antioxidant contents; on the basis of these results, we prepared four experimental oils containing different amounts of polyphenols on which to carry out heating experiments.

MATERIALS AND METHODS

Materials. Six commercial extra virgin oils (EVOO) and three olive oils (OO) were purchased at a local supermarket. Highly refined olive oil was supplied by Sigma-Aldrich (Sigma-Aldrich s.r.l., St. Louis, MO, USA).

Chemicals. 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2′-azinobis-(3-ethylbenz-thiazoline-6-sulfonic) diammonium salt (ABTS), potassium persulfate (dipotassium peroxodisulfate), 4-hydroxy-3-methoxycinnamic (ferulic), 4-hydroxycinnamic (*p*-coumaric), 2-hydroxycinnamic (*o*-coumaric), 4-hydroxy-3-methoxybenzoic (vanillic), 4-hydroxy-3-methoxyphenylacetic (homovanillic), 3,4-dihydroxybenzoic (protocatechuic), 4-hydroxy-3,5-dimethoxybenzoic (syringic), 3,4,5-trihydroxybenzoic (gallic), 3,4-dihydroxycinnamic (caffeic), and 4-hydroxyphenylacetic acids and α-tocopherol were

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10.1021/jf001418j CCC: $20.00
Published on Web 05/04/2001

obtained from Sigma-Aldrich (Sigma-Aldrich s.r.l., St. Louis, MO, USA). Oleuropein, 4-hydroxyphenylethanol (*p*-tyrosol), and 3,4-dihydroxyphenylacetic acid were purchased from Extrasynthese (Extrasynthese, B.P. 62, Z.I. Lyon-Nord, 69726 Genay Cedex, France). 3,4 dihydroxyphenylethanol (hydroxytyrosol) was synthesized according to the procedure of Graciani Costante and Vasquez Roncero (*17*). All chemicals and solvents used were either of analytical or HPLC grade and were purchased from Carlo Erba (Milan, Italy).

Experiments were performed with a Hewlett-Packard spectrophotometer model HP 8453 (Hewlett-Packard, Strasse 8, D-76337 Waldbronn, Germany).

Preparation of Phenolic Standard Solutions in Ethanol and Refined Oil. Stock solutions of Trolox and pure phenolic compounds were prepared by dissolution in ethanol (final concentrations between 2.5 and 12.5 mM). To measure the antioxidant capacity of pure compounds dissolved in ethanol, the stock solutions were subsequently diluted in ethanol to reach final concentrations ranging from 1.0 to 15 μM. To evaluate the antioxidant capacity of pure compounds dissolved in refined oil, 100 μL of stock solution of each phenolic standard or Trolox were added to about 0.5 g of refined oil. The organic solvent was removed at room temperature using a rotary evaporator under a stream of nitrogen. The residue, i.e., the refined oil spiked with the phenolic standard, was weighed and further dissolved in *n*-hexane to reach the same final concentrations that were used for the analyses of the antioxidant capacity of the corresponding phenolic standard dissolved in ethanol.

Determination of Antioxidant Activity of Oils and Pure Compounds. The antioxidant activities of oils and pure compounds, either dissolved in ethanol or refined oil, were evaluated according to Pellegrini et al. (*18*). This method is based on the ability of antioxidants to quench the long-lived ABTS radical cation, a blue/green chromophore with characteristic absorption at 734 nm, in comparison to that of Trolox, a water-soluble vitamin E analogue. The ABTS radical cation was prepared by reacting a 7 mM aqueous solution of ABTS with 2.45 mM potassium persulfate (final concentration) and diluted in ethanol to an absorbance of 0.70 (± 0.20) at 734 nm. After addition of 1.0 mL of this diluted solution to aliquots of Trolox or samples, the absorbance reading was taken in a temperature-controlled spectrophotometer cuvette at 30 °C exactly 1 min after initial mixing. Appropriate solvent blanks were run in each assay. Addition of antioxidants to the preformed radical cation reduces it to ABTS determining a decolorization. The extent of decolorization as percentage inhibition of ABTS radical cation is determined as a function of concentration and calculated relative to the reactivity of Trolox. The activities of pure compounds were estimated at three different concentrations, within the range of the dose−response curve of Trolox, and were expressed as the Trolox equivalent antioxidant capacity (TEAC), defined as the concentration (mmol/L) of Trolox having the equivalent antioxidant activity to a 1.0 mmol/L solution of the substance under investigation. For pure compounds in refined oil, their antioxidant activity value ($TEAC_{oil}$) was obtained in the same way, after subtraction of the contribution of residual vitamin E present in the refined oil. The total antioxidant activity (TAA) of oils was evaluated directly on oils diluted in *n*-hexane at three different dilutions (between 4- and 20-fold, depending on their activity) and expressed as millimoles of Trolox per kilogram of oil.

Phenols Determination. Polyphenols were extracted from the oils as described by Favati et al. (*19*) and the phenolic content was determined by the Folin−Ciocalteu method (*20*), using gallic acid as a standard for the calibration curve. Results of duplicate analyses are expressed as milligrams of gallic acid equivalents (GAE) per kilogram of oil.

Tocopherol Determination. Oils were diluted in *n*-hexane at three different dilutions (between 50- and 100-fold) and analyzed in duplicate by normal-phase HPLC coupled with an UV detector set at 295 nm (*21*). Results are expressed as milligrams of α-tocopherol per kilogram of oil.

Extraction of Phenols from Extra Virgin Olive Oil. Phenols were recovered from a commercial EVOO by solid-phase extraction using a column packed with silica gel 60 (Sigma-Aldrich s.r.l., St. Louis, MO, USA). About 1000 g of oil were diluted with *n*-hexane (1:1, w/v) and loaded on a column previously conditioned with *n*-hexane/diethyl ether (98:2, v/v). To remove the nonpolar fraction, the column was washed with about 400 mL of *n*-hexane. Polyphenols were recovered by elution with about 300 mL of methanol and collected in a dark flask. The organic solvent was removed in a rotary evaporator under nitrogen flow at room temperature. The extract was transferred into a 200 mL volumetric flask and ethanol was added to volume. The total phenol content of extract was determined by Folin−Ciocalteu method as described before.

Preparation of Experimental Oils for Heating Experiments. A stock solution of α-tocopherol was obtained by dissolving 300 mg of standard in 25 mL of *n*-hexane, and, after appropriate dilution, the concentration was checked by spectrophotometric determination ($\epsilon_{\%} = 74$ at 292 nm). Then, 10.8 mL of α-tocopherol solution was added to 800 g of refined oil, and the organic solvent was removed using a rotary evaporator under nitrogen flow at room temperature. This gave a final α-tocopherol concentration of about 300 ppm, including the original α-tocopherol content in the refined oil.

The experimental oils containing four different levels of polyphenols (30, 100, 200, and 300 ppm) were prepared by adding an appropriate amount of the phenol mixture extracted from EVOO to four aliquots of the refined oil enriched with α-tocopherol. After removal of organic solvent, twenty-four 5-mL aliquots for each experimental oil were transferred into 20 mL glass tubes and frozen at −20 °C.

Experimental Design of Heating. The heating procedure of experimental oils was organized according to a Latin square design. To generate the design, four levels of temperature (160, 175, 185, and 190 °C), four levels of heating time (30, 60, 90, and 120 min) and four levels of polyphenols (30, 100, 200, and 300 ppm) were chosen.

Heating Experiments. Four different heating experiments were performed at given temperatures. In each experiment, 12 open tubes for each phenolic concentration were heated in a stainless steel hot plate (Liebish, German) with thermostatic control. According to the experimental design, every 30 min the corresponding tubes were taken off, and the oxidation was stopped by transferring the tubes immediately into a freezer. Samples were stored at −20 °C until analyses for their α-tocopherol and total phenol content and total antioxidant activity. Heating experiments were performed in duplicate.

Statistical Analyses. Data of heating experiments were investigated by the Latin square design analysis using a statistical package running on a PC (Statistica Statsoft Inc., Tulsa, OK); p values < 0.05 were considered to be significant. This analysis allows controlling for the influence of different levels of factors—heating time, temperature, and content of polyphenols—on selected variables such as α-tocopherol content and antioxidant activity of oils.

RESULTS AND DISCUSSION

Determination of Antioxidant Activity of Pure Compounds in Ethanol and in Refined Olive Oil. The antioxidant activities of the principal phenolic compounds present in EVOO, analyzed in ethanol (TEAC) and in refined olive oil ($TEAC_{oil}$), are reported in Table 1. As the antioxidant activity of Trolox was the same both when analyzed in ethanol and in refined oil (data not shown), all TEAC results were calculated by referring to the dose−response curve of Trolox analyzed in ethanol.

Considering the TEAC values obtained in ethanol, hydroxybenzoic acids had different abilities to scavenge the ABTS radical cation, with gallic acid being the most effective. These values are similar to those obtained

Table 1. Trolox Equivalent Antioxidant Capacity of Phenolic Compounds Analyzed in Ethanol (TEAC) and in Refined Olive Oil ($TEAC_{oil}$) Applying the ABTS Decolorization Assay

compound	TEAC (mmol/L)	$TEAC_{oil}$ (mmol/L)
Hydroxybenzoates		
gallic acid	2.45 ± 0.10 [3]	1.68 ± 0.11 [3]
vanillic acid	1.42 ± 0.03 [3]	1.32 ± 0.03 [3]
syringic acid	1.40 ± 0.03 [3]	1.26 ± 0.04 [3]
protocatechuic acid	1.13 ± 0.01 [3]	0.82 ± 0.03 [3]
Hydroxycinnamates		
p-coumaric acid	2.27 ± 0.10 [3]	1.84 ± 0.18 [3]
ferulic acid	1.87 ± 0.06 [3]	1.60 ± 0.17 [3]
o-coumaric acid	1.17 ± 0.03 [3]	0.42 ± 0.03 [3]
caffeic acid	0.99 ± 0.02 [3]	0.86 ± 0.09 [3]
Hydroxyphenylacetates		
3,4-hydroxyphenylacetic acid	2.40 ± 0.15 [3]	
homovanillic acid	2.21 ± 0.17 [3]	1.93 ± 0.10 [3]
p-hydroxyphenylacetic acid	1.39 ± 0.09 [5]	
Other Compounds		
hydroxytyrosol	1.10 ± 0.07 [3]	0.74 ± 0.08 [3]
oleuropein	1.10 ± 0.08 [3]	0.47 ± 0.06 [3]
tyrosol	1.07 ± 0.01 [3]	0.36 ± 0.01 [3]
α-tocopherol	0.99 ± 0.02 [3]	0.97 ± 0.03 [3]

previously by employing the myoglobin/ABTS assay, carried out in phosphate buffer (*22*), except for gallic acid which shows a slightly lower TEAC value. The TEAC values for hydroxycinnamates are also in agreement with previous data (*22*), although *o*-coumaric acid exhibits a higher antioxidant activity than caffeic acid. On the other hand, hydroxyphenylacetic acids exhibit TEAC values higher than those already reported (*23*). The relative TEAC values, however, are identical, with 3,4-dihydroxyphenylacetic being more effective than homovanillic and *p*-hydroxyphenylacetic acids. Tyrosol, hydroxytyrosol, and oleuropein have similarly low antioxidant activities, close to 1.1 mM. This low antioxidant activity is somewhat surprising, especially for hydroxytyrosol and oleuropein, which are demonstrated to afford linoleic acid good protection from autoxidation (*24*). However, the low TEAC values obtained for these compounds are consistent with the lack of double bonds in the branched chain, such as the -CH═CH−COOH group in cinnamic acids, that partially stabilize the phenoxy radical by resonance (*25*).

To assess whether phenolic compounds dissolved in oil exhibit the same antioxidant activity as when they are dissolved in ethanol, standards were individually added to a refined olive oil at the same concentrations used in the test carried out in ethanol. The TEAC values of pure phenolic compounds (Table 1) were generally lower when these were dissolved in oil, as compared to those estimated in ethanol, although the rankings among the different substances were identical. It is noteworthy that the TEAC value of α-tocopherol was independent of the medium, i.e., oil or ethanol, in which it was dissolved. There is not an easy explanation of the behavior of Trolox and α-tocopherol with respect to the other antioxidants. It is possible that this different behavior may be linked to their partition coefficient. However, it is not clear why α-tocopherol, a lipid-soluble antioxidant, exhibits the same behavior as Trolox, a water-soluble antioxidant, since the antioxidant activity of water-soluble phenolic compounds consistently decreases when analyzed in oil.

Table 2. Content of α-Tocopherol and Polyphenols and Total Antioxidant Activity (TAA) of Commercial Oils

oil	α-tocopherol (mg/kg)	total phenols (GAE[a], mg/kg)	TAA (mmol of Trolox/kg)
Olive Oils			
OOBE	194	24	1.06
OOSA	146	30	0.94
OOCA	121	14	0.72
Extra Virgin Olive Oils			
EVOOPR	254	265	2.69
EVOOCD	312	171	2.19
EVOOSA	251	231	2.16
EVOOBE	314	133	1.94
EVOOCA	288	117	1.76
EVOORG	369	73	1.53
Refined Olive Oil			
ROO	138	4	0.61

[a] Values are expressed as milligrams of gallic acid equivalents per kilogram of oils.

Determination of Total Antioxidant Activity of Commercial Oils. The α-tocopherol and phenolic content and the TAA of the commercial oils are shown in Table 2.

The content of α-tocopherol, the predominant tocopherol in olive oil (*26*), ranged from 121 to 194 ppm in OO and from 251 to 369 ppm in EVOO. Polyphenols, expressed as milligrams of gallic acid equivalents per kilogram, ranged from 14 to 30 ppm in OO and from 73 to 265 ppm in EVOO. The differences in antioxidant composition between OO and EVOO are a result of different manufacturing processes (*11*). In particular, EVOO, obtained by cold pressure of the paste, is much richer in phenolic compounds than refined oils, obtained by solvent extraction, that are virtually devoid of phenols. OO is a vaguely defined mixture of refined olive oil and EVOO in which the amount of EVOO may vary from 33 to 95% (*26*), thus affecting the amount of antioxidants present. As expected, the observed differences in antioxidant composition between OO and EVOO influence the TAA values. In fact, the TAA values of EVOO were higher than those of OO, as already reported by Mannino et al. (*27*).

To better understand the contribution of different antioxidants to the TAA values of oils, we calculated the TAA attributable to α-tocopherol and phenolics for each oil. The TAA due to α-tocopherol content (TAA_{toc}) was calculated by multiplying its concentration in each oil by its $TEAC_{oil}$ value (Table 1) and dividing by its molecular weight. The calculation of TAA attributable to polyphenol content (TAA_{poly}) is more complicated, as the concentration of phenolic compounds in oils depends on several factors, such as variety, origin, and ripeness of olives (*28*). As the best compromise, we chose to calculate the TAA_{poly} by using the average TEAC values of phenolic compounds when analyzed in refined oil (1.11) according to the following equation:

$$TAA_{poly} = \frac{A}{170} \times 1.11$$

where A is the amount of total phenols in mg/kg present in each oil and 170 is the molecular weight of gallic acid used as standard in evaluating the polyphenol content.

The TAA_{toc}, the TAA_{poly}, and the theoretical TAA values, obtained by the sum of TAA_{toc} and TAA_{poly}, for all analyzed oils, are shown in Table 3. The measured TAA values of oils were well correlated with the theoretical TAA values, according to the following relationship:

$$TAA\ measured = 0.951\ TAA\ theoretical + 0.390 \quad r = 0.989$$

It is noteworthy that, in all the analyzed oils, the measured TAA values (Table 2) were higher than the theoretical ones (Table 3). It might be hypothesized that these lower theoretical TAA values were due to the approximation in taking into account the contribution of polyphenols to TAA values. Hydrolyzable phenolic substances with high molecular weights have been identified in olive oil by Montedoro et al. (*29*). These compounds could exhibit higher TEAC values than those of the simple phenols analyzed by us, as already demonstrated by Hagerman et al. (*30*) in the case of tannins. However, the value of the slope in the relationship is close to the unit, and this seems to rule out the hypothesis of an underestimation in the attribution of the average TEAC value of phenolic compounds. Therefore, the presence of an intercept in the relationship demonstrates that other compounds, which do not react with the Folin−Ciocalteu reactive and are not detectable as α-tocopherol by HPLC, contribute to the measured TAA values of oils. It is noteworthy that the absolute difference between the measured and the theoretical TAA values results about the same in EVOO as well as in OO, being about 0.30 ± 0.12 and 0.38 ± 0.08 mmol of Trolox/kg, respectively. As a consequence, the relative contribution of this unknown antioxidant activity to the measured TAA is higher in OO, where the amount of known antioxidants is low, as compared to EVOO. This difference between measured and theoretical TAA values is unlikely to be only due to carotenoids and chlorophylls, which exhibit a remarkable antioxidant activity (*31*), but are present mainly in EVOO (*32*). A slight antioxidant activity of squalene, the main component of the unsaponifiable fraction of oil, has also been shown (*33*) and could contribute to the measured TAA values. Finally, the antioxidant activity of unsaturated fatty acids (*34*) such as linoleic acid, present in olive oil up to 11.5% (*11*) and probably related to the presence of double bonds, cannot be neglected.

Nevertheless, our results demonstrate the total antioxidant capacity of virgin olive oils is mainly due to their phenol and α-tocopherol contents and that the direct analysis of TAA of oils is a simple and effective method that takes into account the contribution of all antioxidants present.

Heating Experiments. The changes in the characteristics and composition of oils that occur during deep-frying have been well documented (*35*, *36*). Among vegetable oils, olive oil shows a remarkable stability during deep-frying of food, e.g., potatoes, and when heated at high temperatures (*14*, *37*, *38*). In addition to its low content in polyunsaturated fatty acids, the resistance of olive oil to deterioration at elevated temperatures might be due to the presence of natural antioxidants such as tocopherols, sterols, and phenolic compounds (*39*). Beltram Maza et al. (*40*) demonstrated that hydroxytyrosol and α-tocopherol significantly contribute to the stability of olive oil during potato frying. However, little information is available on the loss of α-tocopherol and antioxidant capacity during heating under realistic, i.e. household, conditions. Indeed, information concerning the effectiveness of antioxidants in oil undergoing domestic cooking has, to date, to be extrapolated from experiments in which the oil was heated for periods up to 72 h. The conditions—time,

Table 3. Total Antioxidant Activity Attributable to α-Tocopherol (TAA_{toc}) and Polyphenols (TAA_{poly}) Present in Commercial Oils and the Theoretical TAA Values

oil	TAA_{toc}[a] (mmol of Trolox/kg)	TAA_{poly}[b] (mmol of Trolox/kg)	$TAA_{theoretical}$[c] (mmol of Trolox/kg)
Olive Oils			
OOBE	0.44	0.16	0.59
OOSA	0.33	0.20	0.53
OOCA	0.27	0.09	0.36
Extra Virgin Olive Oils			
EVOOPR	0.57	1.73	2.30
EVOOCD	0.70	1.12	1.82
EVOOSA	0.57	1.51	2.07
EVOOBE	0.71	0.87	1.58
EVOOCA	0.65	0.76	1.41
EVOORG	0.83	0.48	1.31
Refined Olive Oil			
ROO	0.31	0.03	0.34

[a] The α-tocopherol content × its $TEAC_{oil}$ (0.97)/molecular weight (430 g/mol). [b] The total phenol content × the mean $TEAC_{oil}$ of phenolic compounds (1.11)/molecular weight of gallic acid (170 g/mol). [c] The sum of TAA_{toc} and TAA_{poly}.

Table 4. Content of α-Tocopherol and Polyphenols and Total Antioxidant Activity (TAA) of Experimental Oils

oil	α-tocopherol (mg/kg)	total phenols (GAE[a], mg/kg)	TAA (mmol of Trolox/kg)
A	315	25	1.05
B	309	125	1.54
C	306	225	2.21
D	308	326	2.97

[a] Values are expressed as milligrams of gallic acid equivalents per kilograms of oils.

Table 5. Average (n = 2) Content of α-Tocopherol and Polyphenols and Total Antioxidant Activity (TAA) of Experimental Oils after Heating According to the Experimental Design

oil	temp (°C)	time (min)	α-tocopherol (mg/kg)	total phenols (GAE[a], mg/kg)	TAA (mmol of Trolox/kg)
A	160	30	146	17	0.69
A	175	60	109	16	0.63
A	185	90	115	13	0.60
A	190	120	66	7	0.50
B	160	120	117	68	1.14
B	175	90	157	68	1.22
B	185	60	169	82	1.29
B	190	30	201	63	1.28
C	160	60	129	134	1.68
C	175	30	164	144	1.80
C	185	120	141	120	1.60
C	190	90	169	129	1.56
D	160	90	206	192	2.36
D	175	120	223	161	2.44
D	185	30	260	195	2.58
D	190	60	284	168	2.36

[a] Values are expressed as milligrams of gallic acid equivalents per kilogram of oils.

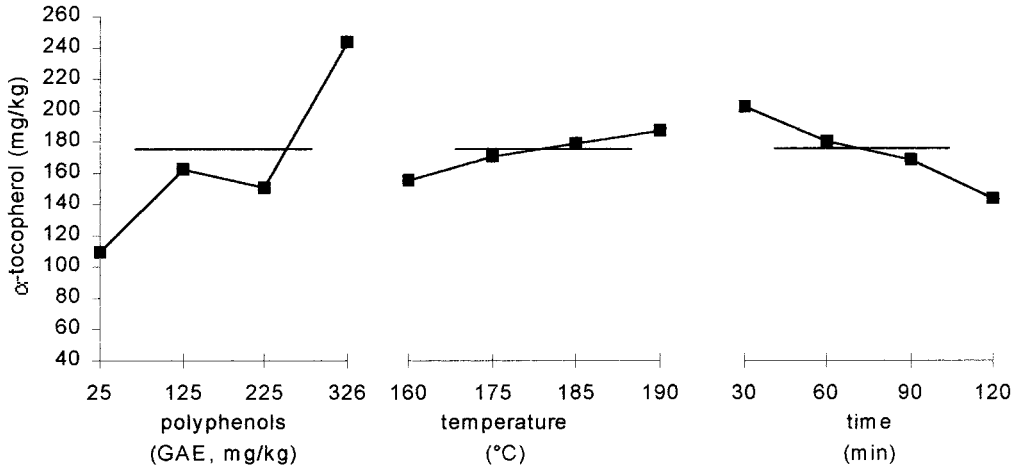

Figure 1. Changes in α-tocopherol content of experimental oils according to the level of the factors. Statistical analysis: effect of phenol content ($F = 24.86$; $p < 0.0001$), heating temperature ($F = 1.32$; $p = 0.29$), and heating time ($F = 4.32$; $p = 0.015$) on α-tocopherol content. Continue line represents the average α-tocopherol content.

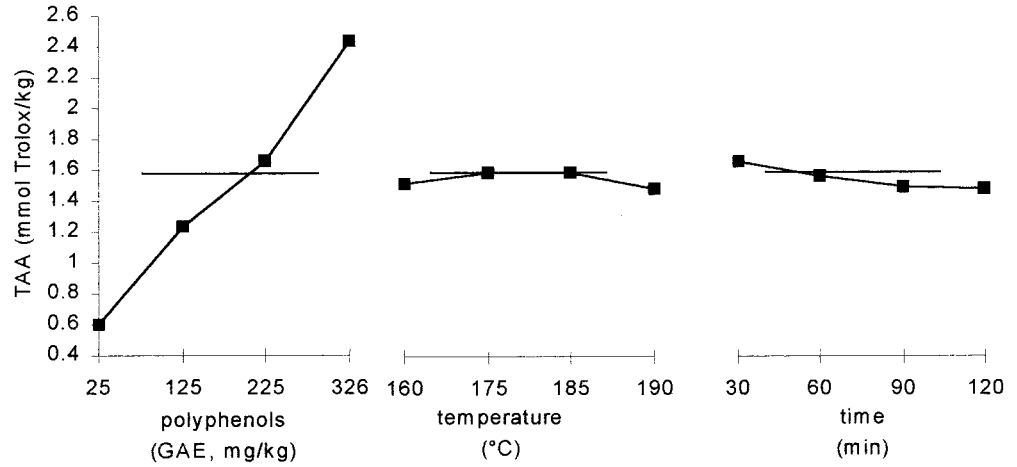

Figure 2. Changes in total antioxidant activity (TAA) of experimental oils according to the level of the factors. Statistical analysis: effect of phenol content ($F = 533.16$; $p < 0.0001$), heating temperature ($F = 1.82$; $p = 0.174$), and heating time ($F = 5.16$; $p = 0.007$) on the total antioxidant activity (TAA). Continue line represents the average TAA.

temperature, polyphenol and α-tocopherol content—of our experimental design, set up to evaluate the antioxidant characteristics of oils during heating, have been chosen to closely approximate the actual composition of commercial oils and habitual domestic cooking conditions. In particular, the antioxidant content of experimental oils spanned from that present in a first quality EVOO to that of a fair quality OO (Table 4). The range of temperature was quite strict, ranging from 160 to 190 °C; this interval of temperature is the most commonly used for deep-frying (*14*). Finally, the heating time, from 30 to 120 min, was chosen to give a time interval that resembles that of domestic cooking practices. The TAA values, total phenol and α-tocopherol content in experimental oils, after heating according to the experimental design, are reported in Table 5. The residual α-tocopherol content in oil was significantly related to the phenol content ($F = 24.86$; $p < 0.0001$) and heating time ($F = 4.32$; $p = 0.015$), but not with heating temperature ($F = 1.32$; $p = 0.29$) (Figure 1). Similarly, the residual TAA was significantly related with phenol content ($F = 533.16$; $p < 0.0001$) and heating time ($F = 5.16$; $p = 0.007$), but not with heating temperature ($F = 1.82$; $p = 0.174$) (Figure 2).

Even though many researchers have studied the effect of polyphenols on oil stability, scant data on the loss of α-tocopherol and total antioxidant capacity in oils during cooking have been reported to date. Gordon and Kourimska (*41*) found a sparing effect of polyphenols from rosemary extracts toward tocopherols degradation in refined rapeseed oil during repeated experimental heating cycles at 162 °C.

Our data suggest that polyphenols from EVOO are effective stabilizers of α-tocopherol during olive oil heating, thus contributing to the nutritional value of cooked foods. In addition, this is, in our knowledge, the first report to elucidate the contribution of polyphenols to the prevention of antioxidant activity decay in olive oil during realistic heating conditions. A similar hypothesis has been so far formulated only from data on thermal stability, measured by indices of fat degradation, directly linked to organoleptic quality (*39*). As the loss of organoleptic quality in oils is secondary to the loss of their antioxidant activity, the latter parameter could be more suitable to effectively measure the nutritional quality of oils with different composition and origin.

ABBREVIATIONS USED:

EVOO, extra virgin olive oil; TEAC, Trolox equivalent antioxidant capacity; TAA, total antioxidant activity;

OO, olive oil; Trolox, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; ABTS, 2,2′-azinobis-(3-ethylbenzthiazoline-6-sulfonic) acid; $TEAC_{oil}$, Trolox equivalent antioxidant capacity determined in refined olive oil; GAE, gallic acid equivalents.

ACKNOWLEDGMENT

We thank Tiziana Cammarata Spataro and Mirko Olmi for their skillful technical assistance. This work was presented in part to the Euro Food Chem X, Budapest; 22−24 September, 1999.

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Received for review November 28, 2000. Revised manuscript received February 21, 2001. Accepted February 22, 2001.

JF001418J