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Role of vitamin E and phenolic compounds in the antioxidant capacity, measured by ESR, of virgin olive, olive and sunflower oils after frying

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Abstract

The effect of lipid profile, vitamin E and total phenolic content was studied in relation to the antioxidant capacity (measured by ESR) of three edible oils (virgin olive, sunflower and olive oils), using short-time deep fat frying as a model. Physico-chemical changes in the oils were also studied. Samples were analysed before and after 15, 30, 45 and 60 min fryings. Determination of free radicals, by electron spin resonance spectroscopy, revealed the highest antioxidant capacity in virgin olive oil and sunflower oil. This parameter was mainly influenced by vitamin E content, followed by lipid profile and phenolic content. The frying procedure decreased the antioxidant capacity in all tested oils. Sunflower oil underwent more chemical changes by frying than did olive and virgin olive oils. Antioxidant capacity of the edible oils was correlated with polar components and ultraviolet indices but not with peroxide index or acidity value. The use of ESR, as a rapid and very sensitive method for determining antioxidant capacity of edible oils, is suggested. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Vitamin E; Phenolic compounds; ESR; Frying; Edible oils

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1. Introduction

It is important to assess the oxidative degradation of fats and oils in the food industry, because free radical-initiated oxidation is one of the main causes of rancidity. Free radicals are known to be responsible for the oxidation of food components, resulting in alterations of the major quality-control parameters, such as colour, flavour, aroma and nutritional value of foodstuffs (Donelli & Robinson, 1995).

Excessive free radical formation, contributing to the onset of certain pathologies, may demand a high dietary intake of fruits, which are rich in antioxidant vitamins and phenolics (Owen, Giacosa, Hull, Haubner, Spiegelhalder, & Bartsch, 2000). That is a good reason to assess the amounts of these compounds in dietary oils and how different technological processes, such as frying, affect their availability.

There are numerous studies that report changes in fats and oils after heating or frying procedures (Che Man & Jaswir, 2000; Gertz, 1996; Takeoka, Fuli, & Dao, 1997). Most of them conclude that such changes depend on the temperature, the heating cycles, the surface/volume and food/oil ratios, the fatty acid and the antioxidant composition of the oils (Melton, Jafar, Sykes, & Trigiano, 1994). On the other hand, the methods developed to assess the effects of frying on oils are not always consistent, making interpretation of the phenomena difficult (Barrera-Arellano, Márquez-Ruiz, & Dobarganes, 1997). Thus, studies of thermal oxidation of oils are far from completed.

All chemical changes of fats and oils and their natural contaminants at elevated temperatures originate in oxidation, hydrolysis, polymerisation, isomerisation or

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cyclisation reactions. All these reactions may be promoted by oxygen, moisture, traces of metal and free radicals (Gertz, 1996). These processes may reduce the amount of antioxidants in the oil, decrease its stability and produce new products which are responsible for loss of the nutritional value and quality of the oil (odour, flavour, absorption, etc).

The aim of the present study is to evaluate the role of phenolics and vitamin E, present in the unsaponifiable fraction of some edible oils, in the antioxidant capacity of virgin olive, olive (commercial mixture of virgin and refined olive oils) and sunflower oils being thermally oxidised. A short-time deep-fat frying procedure is used as an experimental model of oil oxidation. Total antioxidant capacity of edible oils is evaluated by electron spin resonance (ESR) spectroscopy, which represents a novel use of this method in lipid oxidation research.

2. Materials and methods

2.1. Materials

Extra virgin olive oil (EEC, 1991), olive oil (mix of virgin plus refined olive oils) and sunflower oil were purchased in a local supermarket. All chemicals and solvents used were of maximal quality.

2.2. Deep-fat frying procedure

Four domestic deep-fat fryers with a 3.5 l aluminium vessel (260×192×348 mm of capacity) were used for frying. For every experimental oil, i.e. virgin olive oil, olive oil or sunflower oil, the four fryers were filled with 3 l of the same oil and heated to 180 °C. Then, 300 g of potato slices (cut into regular pieces of approximately 40–50 mm×10 mm×10 mm) were added to each deep-fat fryer. After 15 min, fryer number one was switched off and its oil was cooled to room temperature (15 min fried oil). Fifteen minutes later (30 min from the beginning of the frying period) fryer number two was switched off and the oil allowed to cool to room temperature (30 min fried oil). This process was repeated with fryers number three (45 min fried oil) and four (60 min fried oil). At the end of the experimental process, there were five different types of oil according to the frying process for each tested oil: non-fried oil (non-fried original oil), 15 min fried oil, 30 min fried oil, 45 min fried oil and 60 min fried oil. Potato batches were added and removed every 7.5 min while fryers were working. The proportion of food to frying oil in the repeated frying was kept at 500 g/3 l. This procedure was carried out to avoid differences related to changes in the oil volume, exposure time of the potatoes to the oil, and changes in the surface/volume ratio. All the oil samples were filtered and stored in a freezer at −20 °C before analysis. Frying procedures were repeated six times for each of the three experimental oils.

2.3. Determination of fatty acid composition

To obtain the total fatty acid composition of non-fried and fried oils, fatty acid methyl esters were formed according to the method of Lepage and Roy (1986). A gas–liquid chromatograph Model HP-5890 Series II (Hewlett Packard, Palo Alto, CA, USA), equipped with a flame ionisation detector, was used to analyse fatty acids. Chromatography was performed using a 60-m long capillary column; 32 mm id and 20 mm thickness, impregnated with Sp^{TM} 2330 FS (Supelco Inc. Bellefonte, Palo Alto, CA, USA). The injector and the detector were maintained at 250 and 275 °C, respectively; nitrogen was used as carrier gas, and the split ratio was 29:1. Temperature programming (for a total time of 40 min) was as follows: initial temperature, 160 °C for 5 min, 6 °C/min to 195 °C, 4 °C/min to 220 °C, 2 °C/min to 230 °C, holding for 12 min, then 14 °C/min to 160 °C.

2.4. Vitamin E determination

The concentration of vitamin E in the samples was determined by high-performance liquid chromatography (HPLC) with a Beckman in-line Diode Array Detector, model 168 (Fullerton, CA), a Waters (Milford, MA) 717 plus auto sampler and a column Beckman Ultrasphere 5 μm silica (250 mm×4.6 mm). The eluates were detected at 292 nm. The flow rate was 2 ml/min and the eluent was methanol/water (99:1, v/v). Samples were treated according to Ueda and Igarashi (1990). The tocopherol peaks were identified by predetermining the retention times of individual tocopherol standards and results were expressed as tocopherol equivalents.

2.5. Total phenolic content

Total phenolics were extracted from the oils according to the method described by Vázquez-Roncero, Janer del Valle, and Janer del Valle (1973). The concentration of total phenols was estimated with the Folin–Ciocalteu reagent, using caffeic acid as standard.

2.6. Determination of the antioxidant capacity by ESR

Total antioxidant capacity of the oils tested to protect against oxidation of free radical was measured by ESR. Four millilitres of ethanol were added to 1 ml oil. After centrifugation at 1750×***g*** for 5 min, the ethanolic solutions were taken and antioxidant potential was assessed from the ability of ethanolic solution to reduce an equivalent volume of a 0.5 mM ethanolic solution of galvinoxyl (2,6-di-*tert*-butyl-*a*-(3,5,di-*tert*-butyl 4-oxo 2,5,cyclohexadien-1-ylidene)-*p*-tolyloxy), a sterically protected, resonance-stabilised, synthetic radical. The amount of radical remaining 5 min, after mixing (by which time reaction was complete), was determined by double integration of the ESR spectrum and comparison with a control reaction in which ethanol was substituted for the sample solution according to Gardener, McPhail, and Duthie (1997).

Spectra were obtained at 21 °C on a Bruker ECS 106 spectrometer working at 9.5 Ghz (X-band frequency) and equipped with a cylindrical (TM_{110} mode) cavity. Microwave power was 10.1 mW and modulation amplitude was 0.142 mT.

2.7. Physico-chemical parameters

Acidity percentage was obtained by the AOCS Ac5-41 method (AOCS, 1993). Peroxide index was obtained by the AOCS CD8-53 method (AOCS, 1993). The absorbance at 232 or 270 nm (K_{232} and K_{270}) was determined according to recommendations in the Regulations of the European Community, Annexe IX (EEC, 1991).

Total polar components (TPC) were determined according to IUPAC reccomendations (IUPAC, 1992) with the modification introduced by Dobarganes, Pérez, and Gutierrez (1984).

2.8. Statistical analysis of experimental data

Each value obtained is the mean ± S.E.M. of six samples. The differences among oils for each time period, as well as within the same oil for the different periods of time, were submitted to a one-way ANOVA. Duncan's test was performed post hoc to evaluate differences among groups. A two-way ANOVA analysis was performed to determine the effects of oil and time on each variable. Pearson's product–moment linear correlation test was used among all the variables. Previously, all variables were tested for normal and homogeneous variance by the Levene test. When a variable was found not normal, it was log-transformed and reanalysed. A *P* value of less than 0.05 was considered significant. Data were analysed using a SPSS statistical software package (SPSS for Windows, 6.1, 1994, SPSS Inc. Chicago, IL, USA).

3. Results and discussion

3.1. Effect of the oil type and the frying process on fatty acids, vitamin E and total phenolic content

Many authors have described changes in different lipid fractions after heating or frying experiments (Che Man & Jaswir, 2000; Melton et al., 1994; Pérez-Camino, Márquez-Ruiz, & Dobarganes, 1987; Sánchez-Muniz, Cuesta, & Garrido-Polonio, 1993). These changes have been related to the formation of non-volatile decomposition products by thermal oxidation and polymerisation of the unsaturated fatty acids. Dobarganes, Pérez-Camino, Gutierrez, and Repetto (1985) have reported that theses processes are proportional to the amount and type of unsaturated fatty acids. In the present study, only the type of oil but not the time or the interaction between time and oil affected any of the studied fatty acids or indices (Table 1).

Non-fried oils showed a typical composition (Gunstone, Harwood, & Padley, 1994),and there were changes in the fatty acid profiles of the oils after frying (Table 1), despite the high concentration of polyunsaturated fatty acids (PUFA) in sunflower oil

Table 1
Fatty acid composition (%) of non-fried (0 minutes fried) and 60 minutes fried oils and *two-way* ANOVA analyses for the effects of oil and time[a]

Fatty acid	Virgin olive oil		Olive oil		Sunflower oil		Two-way ANOVA[b] (*P*)		
	0 min	60 min	0 min	60 min	0 min	60 min	Oil	Time	Oil×Time
16:0	9.81±0.04b	10.22±0.33b	9.66±0.17b	9.97±0.18b	6.35±0.01a	6.43±0.06a	0.000	0.196	0.637
16:1*n*-1	0.63±0.00b	0.63±0.00b	0.73±0.00c	0.74±0.01c	0.12±0.00a	0.13±0.00a	0.000	0.432	0.391
18:0	3.96±0.02a	4.17±0.17a	3.73±0.10a	3.83±0.02a	4.37±0.00a	4.66±0.27a	0.003	0.263	0.938
18:1*n*-9	76.8±0.05b	76.1±0.50b	78.1±0.10c	78.1±0.14c	31.2±0.04a	31.1±0.17a	0.000	0.266	0.728
18:2*n*-6	6.81±0.01b	6.71±0.05b	5.43±0.02a	5.35±0.02a	55.9±0.03c	55.5±0.27c	0.000	0.685	0.354
18:3	1.11±0.00b	1.11±0.02b	1.15±0.02b	1.12±0.00b	0.38±0.00a	0.40±0.02a	0.000	0.552	0.862
20:1*n*-9	0.28±0.00b	0.29±0.00b	0.27±0.00b	0.26±0.00b	0.17±0.00a	0.17±0.00a	0.000	0.467	0.228
20:2*n*-6	0.19±0.02b	0.22±0.02b	0.21±0.38b	0.23±0.01b	0.09±0.01a	0.10±0.01a	0.006	0.567	0.553
20:4*n*-6	0.12±0.00a	0.12±0.01a	0.11±0.01a	0.11±0.00a	0.75±0.00b	0.81±0.06b	0.000	0.385	0.924
20:5*n*-3	0.03±0.01a	0.02±0.00a	0.02±0.00a	0.02±0.00a	0.03±0.00a	0.04±0.00a	0.023	0.580	0.399
22:4*n*-6	0.05±0.00a	0.06±0.00a	0.05±0.00a	0.05±0.00a	0.28±0.00b	0.31±0.02b	0.000	0.179	0.853
24:0	0.16±0.02b	0.19±0.02b	0.20±0.02b	0.19±0.01b	0.09±0.02a	0.11±0.00a	0.000	0.420	0.536

[a] Results are expressed as mean ± S.E. For each fatty acid, means with different letters are significantly different for a one-way ANOVA analysis followed by a Duncan post hoc test ($P < 0.05$).

[b] Significant effects are done by $P < 0.05$.

(Fig. 1A). These results agree with the designed model of frying in which the role of different components of the unsaponifiable fraction (vitamin E and phenolics) was studied, trying to avoid typical changes in the lipid profile of deeply fried oils.

The values of vitamin E reported in this study (Fig. 1B; Table 2) are higher than those found by other authors (Barrera-Arellano et al., 1997; Chase, Akoh, & Eiten Miller, 1994; Dobarganes et al., 1985; Manzi, Panfili, Esti, & Pizzoferrato, 1998; Severini, Romani, Dall'Aglo, Provene, Conte, & Lerici, 1997). However, Cinquata, Esti, and La Notte (1997) have reported values of 320 mg vit E/kg and Baldioli, Servilli, Perretti, and Montedoro (1996) have reported values close to 300 mg vit E/kg in virgin olive oil. The high variability in the amount of vitamin E in vegetable oils has been widely reported and depends on several factors, such as genetic, agronomic, environmental, extraction procedural, and others (Cimato, 1990; Mousa, Gerasopoulos, Metzidakis, & Kiristakis, 1996).

Both the time and the type of oil affected the levels of vitamin E (Table 2). There was a good correlation ($r = 0.9803$; $P < 0.001$) between PUFA and vitamin E in the oils. Kalmal Eldin and Anderson (1997) have described a positive correlation between linoleic acid an α-tocopherol. These authors suggested that, as tocopherol is very important in protecting PUFA against oxidation, there could be a biochemical link between the tocopherol levels and the degree of unsaturation in vegetable oils, the main source of dietary PUFA and vitamin E.

Vitamin E levels significantly decreased in all the oils after 60 min of frying. However, the response was different, depending on the oil. Sunflower lost, in absolute value, 201 mg kg^{-1}, followed by virgin olive oil (127 mg kg^{-1}) and olive oil (77.1 mg kg^{-1}). These results accord with other studies (Barrera-Arellano et al., 1997) and suggest that vitamin E is being used to protect the oils against the thermal damage. It is interesting to see how the decrease in tocopherol with the frying time is different, depending on the type of oil. Thus, sunflower oil lost tocopherol after 15 min and then the quantity of this antioxidant remained constant until the end of the study. In the case of virgin olive and olive oils, the loss of tocopherol started after 45 or 60 min of frying. These differences, together with the fact that sunflower oil lost, in absolute terms, a higher amount of vitamin E, may be important, since it seems that olive and virgin olive oils resist the frying procedure better. The importance of tocopherol as an antioxidant will be discussed below.

Table 2
Two-way ANOVA analyses for the effects of oil and time effects on the parameters studied

Parameter	Two-way ANOVA[a]		
	P		
	Oil	Time	Oil×Time
Acidity percentage	0.000	0.396	0.837
K_{232}	0.000	0.000	0.000
K_{270}	0.000	0.000	0.029
Peroxide index	0.000	0.006	0.000
Total polar material (TPC)	0.000	0.000	0.000
ESR	0.000	0.000	0.004
Vitamin E	0.000	0.000	0.014
Total phenolics	0.000	0.000	0.000
PUFA[b]	0.000	0.762	0.534

[a] Significant effects are done by $P < 0.05$.

[b] Polyunsaturated fatty acids.

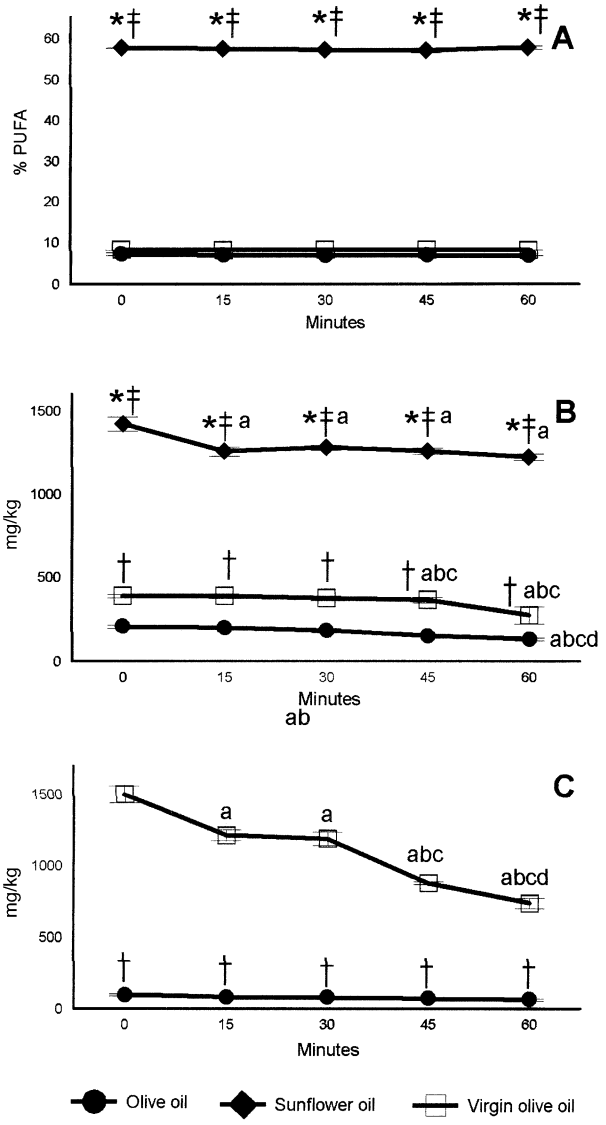

Fig. 1. Polyunsaturated fatty acids (PUFA) (A), vitamin E (B) and total phenolic compounds (C) in virgin olive oil, sunflower oil and olive oil before (time 0) and after 15, 30, 45 and 60 min of frying. Statistical differences: * = Virgin olive vs. sunflower; † = Virgin olive vs. Olive; ‡ = Sunflower vs. Olive; a = 15, 30, 45 or 60 min vs. 0 min; b = 30, 45 or 60 min vs. 15 min; c = 45 or 60 min vs. 30 min; d = 60 min vs. 45 min.

Phenolic compounds form part of the socalled "minor constituents" of olive oil. These compounds are of great importance for several characteristics of the olive oil, such as flavour, shelf-life and resistance against oxidation (Gutfinger, 1981; Tsimidou, Lytridou, Bosou, Pappa-Louisi, Kotsifaki, & Petrakis 1996; Vázquez Roncero, Janer del Valle, & Janer del Valle, 1975). However, the levels of phenolic compounds differ widely among varieties, locations, maturity, and other factors (Amiot, Fleuriet, & Macheix, 1986; Cinquanta et al., 1997). Fig. 1C and Table 2 show the total concentrations of phenolics (expressed as mg of caffeic acid per oil kg) in the experimental oils. The two way ANOVA analysis (Table 2) shows that time, oil and the interaction between time and oil had effects on the total phenolic content of the oils. The highest amount was found in non-fried virgin olive oil (1501 ± 59.6 mg kg^{-1}). Montedoro, Servilli, Baldioli, and Miniati (1992) have reported data around 1000 mg kg^{-1} in olive oil although, in general, the values found in the literature are lower (Cinquanta et al., 1997; Gutfinger, 1981). Although the refining process of the virgin olive oil eliminates almost all the phenolic compounds (Akasbi, Shoeman, & Saari, 1993), phenolics are found in the olive oil group because olive oil is a commercial mixture of virgin and refined olive oils (EEC, 1991).

The frying procedure reduced the content of phenolics, probably as the result of thermal destruction of these molecules or because they are used in the protection of the oils against the oxidative insult. These compounds are lost in the oil over time, as a consequence of hydrolytic or oxidative processes during storage (Cinquanta et al., 1997; Cortesi, Azzolini, Rovellini, & Fedeli, 1995).

3.2. Antioxidant capacity of the oils, the role of minor components and effect of the frying procedure

Many studies have been developed to elucidate the oxidative stability of edible oils as a function of chemical composition. In general, it is assumed that high levels of polyunsaturated fatty acids, together with low concentrations of antioxidants, promote susceptibility of oils to oxidation. Many methods have been used to evaluate the oxidative stability of oils and to compare the antioxidant activities of several compounds (Baldioli et al., 1996). However, as far as we know, nobody has used electron spin resonance spectroscopy (ESR) to study the total antioxidant activity of edible oils or their stability after frying procedures.

The use of ESR confers a series of advantages over spectrophotometric and other methods employed to measure antioxidant properties. The free radical used was galvinoxyl, which does not indiscriminately abstract H-atoms from a wide range of substrates. Consequently, reactions are only likely to be significant with good H-atom donors (a condition essential to consider a molecule an antioxidant). Additionally, the galvinoxyl radical has a well-defined spectrum and better stability than other radical intermediates formed during oxidation processes. Indeed, the technique is very sensitive, allowing detection at the sub-micromolar level, and can be used on turbid or highly-coloured solutions (Gardener et al., 1997).

In the present study, we found that time, oil and the interaction between oil and time affected the antioxidant capacity of the oils (Table 2). The oils with the maximal total antioxidant capacity (Fig. 2), before and after different frying times, were virgin olive oil and sunflower oils, with approximately twice more capacity than olive oil. The frying procedure decreased the antioxidant capacity in all the studied oils. It is very interesting to observe that two oils (virgin olive and sunflower oils) with a very different fatty acid composition had practically the same response through the time. However, virgin olive oil and olive oil, with no difference in fatty acids, showed different responses in the ESR assay. ESR values for sunflower oil remained constant through time, which is quite interesting. Probably the use of tocopherol (this oil had the highest decrease in this parameter) allowed this oil to maintain its antioxidant capacity through the study.

Oils with the highest amounts of vitamin E and/or phenolic compounds showed the highest resistance to oxidation. Additionally, it is very important that, although fatty acids were not modified by the frying, this process decreased the antioxidant capacity of the oils, due to a loss of vitamin E in sunflower oil or of phenolic compounds in virgin olive oil.

Although it is very difficult to know the specific weight of each oil component, it was found that ESR results had the best correlation with the vitamin E content ($r=0.7375$; $P<0.001$), followed by PUFA composition ($r=-0.5120$; $P<0.001$) and total phenolics

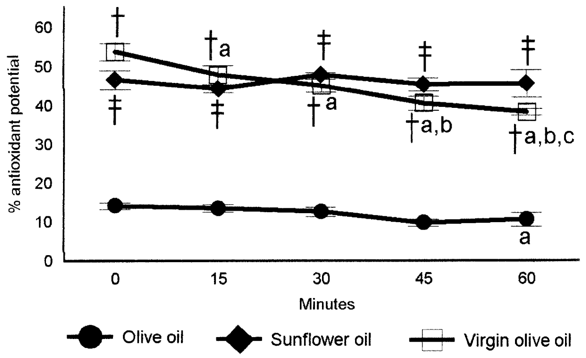

Fig. 2. ESR-total antioxidant capacity in virgin olive oil, sunflower oil and olive oil before (time 0) and after 15, 30, 45 and 60 min of frying. Statistical differences: * = Virgin olive vs. sunflower; † = Virgin olive vs. Olive; ‡ = Sunflower vs. Olive; a = 15, 30, 45 or 60 min vs. 0 min; b = 30, 45 or 60 min vs. 15 min; c = 45 or 60 min vs. 30 min; d = 60 min vs. 45 min.

($r = 0.4472$; $P < 0.001$). From these results we could conclude that resistance of oils to oxidation depends on the interaction between antioxidants and fatty acids.

In addition, the results found here, suggest that ESR can be used to test the resistance of edible oils to oxidation since this method is very sensitive and fast and is a direct observation of the real antioxidant capacity of the oils.

3.3. Changes in physico-chemical parameters of the oils after frying

It is interesting to draw a distinction between oxidative degradation after the frying process and the changes in the oils related to physico-chemical properties. In the present study, typical parameters analysed were the percentage of free fatty acids (acidity percentage), peroxide index, ultraviolet absorbance and total polar components. Could a short-time deep-fat frying process modify these parameters and what is the relative importance of the unsaponifiable fraction in these changes?

In fats and oils, hydrolysis results in the formation of free fatty acids, mono- and di-glycerides and glycerol. Thus, acidity percentage, an index of total free fatty acids present in frying oils, has been used to study the hydrolytic modifications of the fried oils (Gertz, 1996). Some authors have reported changes in the acidity value of different oils after frying or heating (Dobarganes et al., 1985). However, these changes were different, depending on the initial value for this parameter and on the frying time. All these variations depend on the volatility of the free fatty acid that can be formed and evaporated into and from the oils at the same time. In our study, the acidity value (Fig. 3A; Table 2) did not change for any of the studied oils from the start until the end of the frying procedure. These results are in accord with the absence of changes found in the fatty acid profile of the oils and the proposed experimental model.

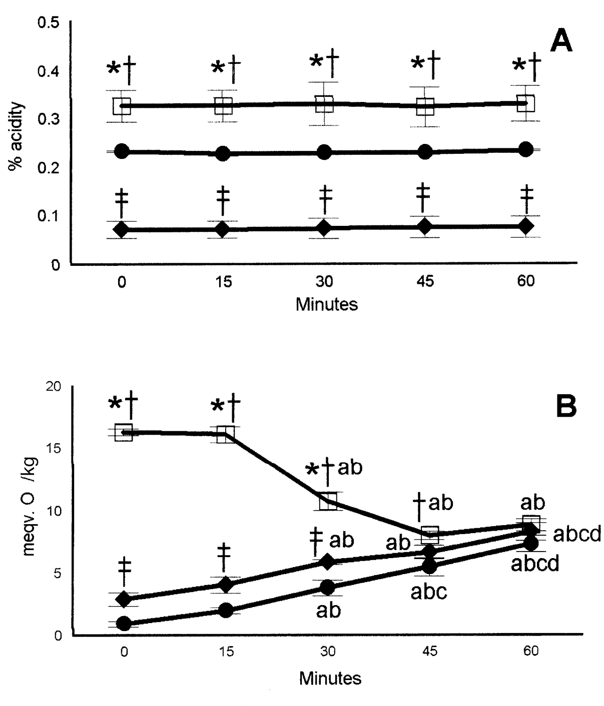

Fig. 3. Percentage of acidity (A) and peroxide value (B) in virgin olive oil, sunflower oil and olive oil before (time 0) and after 15, 30, 45 and 60 min of frying. Statistical differences: * = Virgin olive vs. sunflower; † = Virgin olive vs. Olive; ‡ = Sunflower vs. Olive; a = 15, 30, 45 or 60 min vs. 0 min; b = 30, 45 or 60 min vs. 15 min; c = 45 or 60 min vs. 30 min.; d = 60 min vs. 45 min.

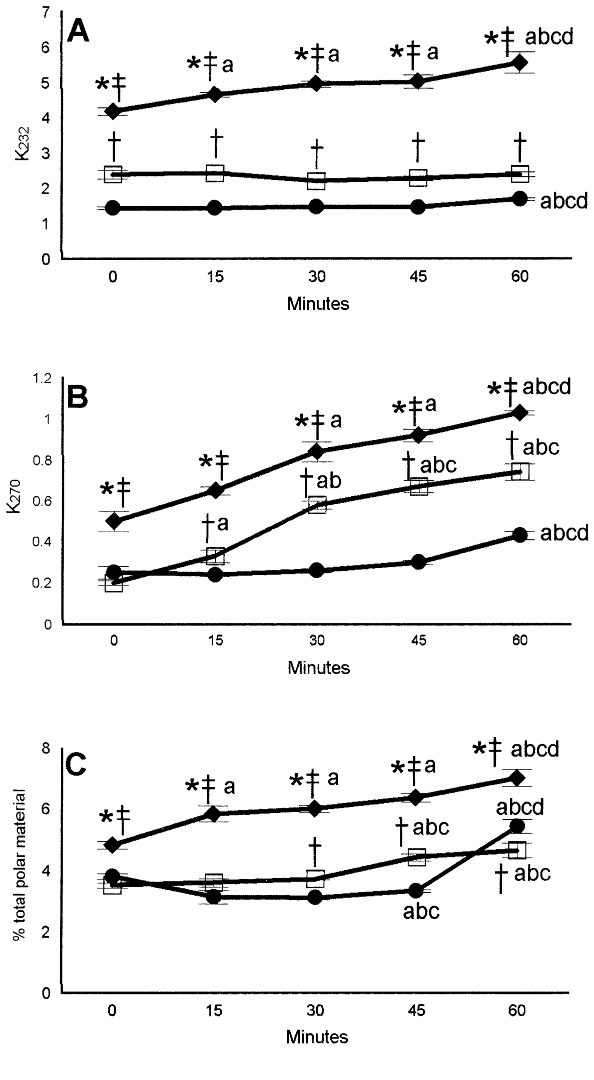

Fig. 4. K_{232} (A), K_{270} (B) and total polar components (TPC) (C) in virgin olive oil, sunflower oil and olive oil before (time 0) and after 15, 30, 45 and 60 min of frying. Statistical differences: * = Virgin olive vs. sunflower; † = Virgin olive vs. Olive; ‡ = Sunflower vs. Olive; a = 15, 30, 45 or 60 min vs. 0 min; b = 30, 45 or 60 min vs. 15min; c = 45 or 60 min vs. 30 min; d = 60 min vs. 45 min.

Many authors have related the levels of peroxides, products of the lipid oxidation, with the quality of edible oils and with changes by high temperature processes (e.g. Melton et al., 1994). In the present research, Table 2 shows that the type of oil, the time of frying and the interaction between time and oil, all affected the peroxide index in the samples. The maximal amount of peroxides among the non-fried oils was found in virgin olive oil (Fig. 3B). Similar results have been previously reported (Pérez and Permanyer, 1986). The reason for this observation stems from the fact that virgin olive oil is the only non refined oil used and the refining process removes the peroxides present in non-fried oils. However, from 15 min to 45 min of frying, due to the volatility of these compounds, loss of peroxides in virgin olive oil, started; after this, the ratio of generation of peroxides was higher than the ratio of evaporation and the total amount increased. At the end of the process all the oils had similar levels of peroxides.

K_{232} is related to the formation of hydroperoxides, conjugated dienes, carboxylic compounds and conjugated trienes. K_{270} depends on secondary oxidation products formed from the initial compounds detected at 232 nm (Ancin & Martínez, 1991). Both indices have been used to assess the changes after frying by many authors (e.g. Takeoka et al., 1997). Data from the present work showed that oil, time and the interaction between oil and time affected these parameters (Table 2). Fig. 4A,B, reveal an increase with the frying time in both parameters in all the experimental oils, with the lowest level for olive oil and the highest for sunflower oil. There was a positive correlation between the parameters ($r = 0.8064$; $P < 0.001$), but there was no correlation with the peroxide index.

Total polar components (TPC) is a representative measurement of the total alteration of the oil. In fried oils, there are breakdown products from the frying process (Melton et al., 1994). For this reason it is generally considered as a good index of the alterations caused by this process. In the present study, TPC increased in all the experimental groups after frying (Fig. 4C; Table 2). The highest absolute value and increment were found in sunflower oil. According to Takeoka et al. (1997), oils rich in unsaturated fatty acids produce more polar compounds than saturated oils. Although the mentioned situation was true for the unfried oils, it changed after 60 min of frying, resulting in olive oil, with the same degree of unsaturation and fatty acid composition as virgin olive oil (Table 1), having a higher TPC.

This orginates in differences in the unsaponifiable fractions between the two types of oils, as has been above described. The effectiveness of antioxidants in retarding oil degradation has been reported in many papers (e.g. Gordon & Kourimska, 1995). Barrera-Arellano et al. (1997) found different productions of TPC between two different sunflower oils with different composition of tocopherol, as found in our study between olive and virgin olive oils.

No correlation was found between TPC and peroxide index or acidity. However, there was a correlation of 0.8270 ($P < 0.001$) with K_{232} and of 0.8072 ($P < 0.001$) with K_{270}. Additionally, ESR correlated better with K_{232} ($r = 0.6529$; $P < 0.001$) and K_{270} ($r = 0.5301$; $P < 0.001$) than with TPC ($r = 0.3913$; $P < 0.001$). These results suggest that K_{232}, K_{270} and TPC are good markers of the changes in the oil composition after a short time of frying.

In summary, the frying procedure, used here as a model, to test the antioxidant capacity of edible oils, decreased the antioxidant capacity in all tested oils. Sunflower oil underwent more physico-chemical changes by frying than olive and virgin olive oils, which is an additional finding in this study. It is interesting to note that antioxidant capacity of the edible oils, was mainly correlated with the amount of antioxidant present in the oils, with polar components and ultraviolet indices but not with peroxide index or acidity value. Finally, the use of ESR as a rapid and very sensitive method to determine antioxidant capacity of edible oils is suggested.

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