Journal of Bioenergetics and Biomembranes, Vol. 34, No. 2, April 2002 (© 2002)

Feeding Fried Oil Changes Antioxidant and Fatty Acid Pattern of Rat and Affects Rat Liver Mitochondrial Respiratory Chain Components

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Received August 6, 2001; accepted October 15, 2001

Fat frying is a popular food preparation method but several components like antioxidant vitamins could be lost due to oxidation and some others with toxic effects could appear. Because of such large consumption of frying oils, the effect of high temperatures on the oils is of major concern both for product quality and nutrition, taking into account that dietary fat source deeply influences several biochemical parameters, especially of mitochondrial membranes. Virgin olive oil possesses specific features for modulating the damages occurred by endogenous and exogenous oxidative stress being particularly rich in antioxidant molecules. We evaluated the extent of modifications suffered by virgin olive oil following a short-time deep fat frying procedure: vitamin E and phenolic compound as well as total antioxidant capacity (measured by ESR) decreased, while polar compounds increased. The intake of such an altered oil mainly affected the hydroperoxide and TBARS contents of mitochondrial membranes which were enhanced after the dietary treatments. Also, several mitochondrial respiratory chain components (Coenzyme Q, cytochrome b, $c + c_1$, and $a + a_3$) were affected.

KEY WORDS: Coenzyme Q; mitochondrial cytochromes; virgin olive oil; fried oil; hydroperoxides.

INTRODUCTION

Recent consumer interest in "healthy eating" has raised awareness to limit the consumption of fat and fatty foods. What are the relative nutritional advantages and disadvantages of consuming fried foods? Are all fried foods bad for you? Fats and oils are heated at high temperatures during baking, grilling, and pan-frying; however, deep fat frying is the most common method of high temperature treatment (Warner *et al*., 1999). Deep fat frying is a popular food preparation method because it produces desirable fried food flavor, golden brown color, and crisp texture. Frying has little or no impact on the protein or mineral content of fried food, whereas the dietary fiber content of potatoes is increased after frying due to the formation of resistant starch. Moreover, the high temperature and short transit time of the frying process cause less loss of heat labile vitamins than other types of cooking. For example, vitamin C concentration of French fried potatoes is as high as in raw potatoes, and thiamine is well retained in fried potato products as well as in fried pork meat (Fillion and Henry, 1998).

Although some unsaturated fatty acids and antioxidant vitamins are lost due to oxidation, fried foods are generally a good source of vitamin E (Fillion and Henry, 1998). Because of such large consumption of frying oils and fats, the effect of high temperatures on these oils and fats is of major concern both for product quality and nutrition (Fillion and Henry, 1998; Paul and Mittal, 1997; Warner, 1999). On the other hand, it is well known that dietary fat sources deeply influence several biochemical

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0145-479X/02/0400-0127/0

parameters both in plasma and in mitochondrial membranes (e.g., Mataix *et al.*, 1998; Quiles *et al.*, 1999a). In the last decade we outlined the specific properties of virgin olive oil in this sense, stressing its properties for modulating the damages occurred by endogenous and exogenous oxidative stress (Quiles *et al.*, 1999b; Svegliati Baroni *et al.*, 1999). Adaptations of the electron transport system in relation to dietary fat type have been widely reported (Huertas *et al.*, 1991a,b; Quiles *et al.*, 2001). The importance of fatty acids resides in the finding that mitochondrial membrane adapts its lipid composition to dietary fat (Charnock *et al.*, 1992; Quiles *et al.*, 1999a; Yamaoka *et al.*, 1988). It has been reported how fatty acids alter mitochondrial function in different organisms and tissues (Barzanti *et al.*, 1994; McMillin *et al.*, 1992; Stillwell *et al.*, 1997). In addition, an interaction between fatty acids and genes has recently been credited with determining mitochondrial function (Kim and Berdanier, 1998). Moreover, virgin olive oil is particularly rich in antioxidant molecules and it is one of the most frequently used fat sources in Europe and studies are needed to clarify some of the properties of this product that plays a pivotal role in the so-called Mediterranean Diet.

In light of the previous studies we investigated the effects of dietary fried virgin olive oil with respect to nonfried virgin olive oil on rat liver mitochondrial composition and function. In addition, several plasma biochemical parameters were also investigated as well as the possible oxidative modifications suffered by virgin olive oil during the frying process.

MATERIALS AND METHODS

Frying Procedure

A domestic deep-fat fryer with 3, 5-L aluminium vessel (260 × 192 × 348 mm of capacity) was used for frying. The fryer was filled with 3 L of oil and heated until 180°C for 15 min. Four cycles of 15 min of frying were performed with a 2-h rest period between each frying cycle. For the analytical determinations in the oils, samples were preserved after each frying cycle (15, 30, 45, and 60 min fried oils) and also a sample from nonfried oil was preserved. Frying procedures were repeated with six oil samples. For the animal study, the diet was made using nonfried oil for the nonfried diet group and oil fried for 60 min for the fried-oil diet group. All the oil samples were filtered and stored in a freezer at −20°C before analysis. All chemicals and solvents used were from Sigma (St. Louis MO, U.S.A.) and Merck (Darmstadt, Germany).

Analytical Determinations in the Oil Samples

Extra virgin olive oil (EEC, 1991) was purchased in a local supermarket. Fatty acid profile of oil samples was measured by gas–liquid chromatography as described by Lepage and Roy (1986). A gas–liquid chromatograph Model HP-5890 Series II (Hewlett Packard, Palo Alto, CA, U.S.A.) equipped with a flame ionization detector was used to analyze fatty acids as methyl esters. Chromatography was performed using a 60-m long capillary column, 32-mm id, and 20-mm thickness impregnated with Sp 2330^{TM} FS (Supelco Inc. Bellefonte, Palo Alto, CA, U.S.A.). The injector and the detector were maintained at 275 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, hold 12 min, 14°C/min to 160°C.

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 Water (Milford, MA) 717 plus auto sampler, and a column Beckman Ultrasphere 5 μm silica (250 mm × 4.6 mm). The elutes 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 peak was identified by predetermining the retention times of individual tocopherol standards and results were expressed as tocopherol equivalents.

Total phenolics were extracted from the oils according to the method described by Vázquez-Roncero *et al.* (1973). The concentration of total phenols was estimated with the Folin-Ciocalteu reagent using caffeic acid as standard.

Total antioxidant capacity of the oils tested to protect against oxidation of free radical was measured by ESR. Four milliliters 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-stabilized, synthetic radical. The amount of radical remaining at 5 min after mixing (by which time reaction was complete) was determined by double integration of the ESR spectrum and by comparison with a control reaction in which ethanol was substituted for the sample solution in accord with

Gardener *et al*. (1998). 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.

Total polar components (TPC) were determined according to IUPAC recommendations (IUPAC, 1992) with the modification introduced by Dobarganes *et al*. (1984).

Each value obtained is the mean ± SEM of six samples. Differences among periods of time were submitted to a one-way ANOVA. Duncan's test was performed a post hoc to evaluate differences among groups. Previously, all variables were tested for normal and homogeneous variance by Levene test. When a variable was found not normal, it was log-transformed and reanalyzed. *P* value of less than 0.05 was considered significant. Data were analyzed using SPSS statistical software package (SPSS for Windows, 9.0.1, 1999, SPSS Inc. Chicago, IL, U.S.A.).

Analytical Determinations in the Rats

Sixteen male Wistar rats (*Ratus novergicus*) initially weighing 80–90 g (supplied by the Laboratory Animal Service of the University of Granada), were allocated in two groups of eight per cage and maintained on a 12-h light/12-h darkness cycle with free access to food and drinking water. The study lasted 8 weeks during which, the rats were fed a semisynthetic and isoenergetic diet with the following composition (in grams per 100 g of diet): 26.7 casein, 13.53 starch, 45.29 sucrose, 8.0 edible oil, 3.68 mineral supplement, 1.0 vitamin supplement, 1.84 cellulose, 0.09 choline, and 0.30 methionine. Mineral and vitamin supplements were designed following the AIN criteria (AIN76 supplements) (American Institute of Nutrition, 1977). Groups only differed in the type of edible oil used to feed the rats on. A group was fed on nonfried oil and fried oil group was fed on the four cycles fried oil.

At the end of the experiment, the rats were killed by decapitation at the same time of the day in all cases (between 12:00 and 13:00 h) to avoid circadian fluctuations. The protocols were approved by the Ethical Committee of the Interministerial Commission of Science and Technology. Animals were handled according to the guidelines for care and use of laboratory animals of the Spanish Society for Laboratory Animal Sciences.

Blood was collected in EDTA-coated tubes, and the plasma was centrifuged at 1750*g* for 10 min. All samples were stored at −80°C until analyzed. Liver mitochondria were isolated following Fleischer *et al.* (1979) procedure.

Triglycerides and total cholesterol in plasma were determined by enzymatic methods, using Boehringer-Mannheim kits (Munich, Germany). Mitochondrial membrane cholesterol was determined according to Röschlau *et al.* (1974). The protein concentrations of mitochondrial samples were determined according to the method of Lowry *et al.* (1951), using bovine serum albumin as a standard.

Fatty acid profile of plasma and liver mitochondrial membranes was measured by gas–liquid chromatography according to Lepage and Roy (1986) as described above for the oil samples.

The ferrous-oxide xylenol orange (FOX2) method was used for determining hydroperoxides (HP) in mitochondrial membrane. HP levels were assayed according to the principle of the rapid peroxide-mediated oxidation of Fe^{2+} to Fe^{3+} under acidic conditions (Nourouz-Zadeh *et al.*, 1994) using tryphenylphosphine (TPP), an agent that avoids artifactual color generation in samples that might contain substantial quantities of loosely available iron. Briefly, mitochondria (0.1 mg) were incubated at 37°C for 30 min with and without 1 mM TPP. Then FOX2 reagent was added to each sample and incubated again at 37°C for 30 min in a water-shaking bath. After centrifugation (2000*g* for 5 min) the supernatants were monitored at 560 nm. TBARS in mitochondrial membrane were determined according to Orrenius *et al.* (1977).

Analyzes of coenzyme Q_9 and α-tocopherol in mitochondrial membranes were carried out according to Lang and Packer (1987), by high-performance liquid chromatography with in-line Diode Array Detector, model 168 (Beckman Instruments, Inc. Fullerton, CA). Plasma coenzyme Q_9 and α-tocopherol were also assayed by HPLC according to MacCrehan (1990).

The concentration of mitochondrial cytochrome $c + c_1$, b, and $a + a_3$ was evaluated by differential spectra in a λ16-Perkin Elmer double-beam spectrophotometer according to Vanneste (1966) and Nicholls (1976). Exactly 200 μL of sodium deoxycholate 10% (w/v) plus the sample (the equivalent volume to 2 mg of mitochondrial protein), together with 7 mM KH_2PO_4 until a final volume of 1.7 mL, were gently mixed in a spectrophotometer cuvette. After 10 μL of 20 mM potassium ferricyanide were added to the mix, to allow the total oxidation of the cytochromes, the oxidized spectra between 650 and 500 nm was recorded. Afterward, 20 μg of sodium dithionite were added to reduce the cytochromes completely, acquiring again the spectra between 650 and 500 nm. The differential spectra (reduced minus oxidized) were recorded to calculate the concentration of cytochromes, using the following extinction coefficients: $\varepsilon_{c+c1}(A_{550}-A_{540}) = 20\ mM^{-1} \cdot cm^{-1}$;

$\varepsilon_b(A_{561}–A_{575}) = 25\ mM^{-1} \cdot cm^{-1}$; $\varepsilon_{a+a3}(A_{605}–A_{630}) = 24\ mM^{-1} \cdot cm^{-1}$.

Cytochrome *c* oxidase activity was assayed at 25°C using cytochrome *c* (90 μM; in 10 mM Tris) reduced by sodium dithionite. After reduction, cytochrome *c* was purified in a Sephadex G-25 column (Battino *et al.*, 1986; Degli Esposti and Lenaz, 1982), so that the ratio between the extinction at 500 and 565 nm was between 8 and 10. Cytochrome *c* was then mixed with 10 mM Tris, 50 mM KCl, 1 mM EDTA, and added with 0.3 mg/mL of antimicine A. Samples were poured into the cuvette and mixed, monitoring the absorbance decrease of cytochrome *c* upon oxidation at 417–409 nm every 10 s for 2 min; the extinction coefficient used for cytochrome *c* was 40.7 $mM^{-1} \cdot cm^{-1}$ (Battino *et al.*, 1986).

The results represent the mean ± SEM of eight animals. Significant ($P < 0.05$) interaction terms were evaluated by a Student *t* test. Data were analyzed using the SPSS/PC statistical software package (SPSS for Windows, 9.0.1, 1999, SPSS Inc. Chicago, IL, U.S.A.).

RESULTS

Oil Determinations

Nonfried oil showed a typical fatty acid composition (Mataix *et al.*, 1998), and we did not find changes in the fatty acids profile of the oils after frying (Table I). Figure 1 shows the vitamin E content present in the oil submitted to different frying times. Nonfried oil showed an amount of 400 mg/kg of vitamin E, which is normal for this type of edible oil. The frying procedure led to a decrease in the levels of vitamin E after 60 min frying period but not for 15, 30, or 45 min.

Table I. Fatty Acid Composition of Experimental Oils

Fatty acid	Nonfried oil	60-min fried group
16:0	9.8 ± 0.0	10.2 ± 0.3
18:0	4.0 ± 0.1	4.2 ± 0.2
18:1 ($n-9$)	76.8 ± 0.1	76.1 ± 0.1
18:2 ($n-6$)	6.8 ± 0.0	6.7 ± 0.1
18:3 ($n-6$)	0.4 ± 0.0	0.5 ± 0.0
20:1 ($n-9$)	0.3 ± 0.0	0.3 ± 0.0
20:2 ($n-6$)	0.2 ± 0.0	0.2 ± 0.0
20:4 ($n-6$)	0.1 ± 0.0	0.1 ± 0.0
24:0	0.2 ± 0.0	0.2 ± 0.0
SFA	14.0 ± 0.1	14.7 ± 0.3
MUFA	77.7 ± 0.2	77.1 ± 0.1
PUFA	8.3 ± 0.1	8.3 ± 0.2

Note. Results are expressed as mean ± SE. SFA: Saturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids.

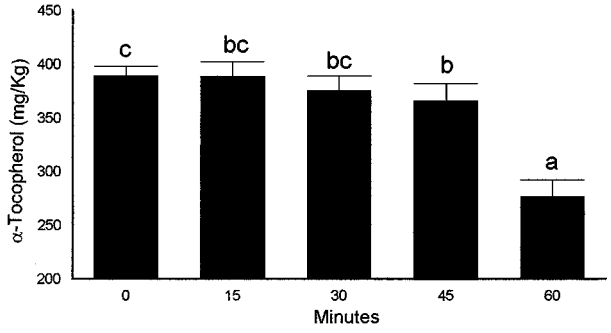

Fig. 1. Effect of frying on vitamin E content in virgin olive oil. Values are means ± SE for six oil samples. Each value is significantly different from all other values not showing the same letter on the top of the bar ($P < 0.05$), i.e., the same letter on the top of two or more bars means no statistically significant differences between the corresponding values.

The total content in phenolic compounds is shown in Fig. 2. Initial level of nonfried oil is around 1500 mg/kg and this amount is progressively decreasing with the frying. The lowest amount is found for the 60-min fried oil, which has around 740 mg/kg. Figure 3 represents the antioxidant capacity of the oils measured by ESR. This parameter shows that frying decreases the antioxidant capacity of the oil by about 50% at the end of the study. The decrease in the antioxidant capacity is progressive.

Finally, Fig. 4 shows the percentage of total polar material, which is a specific marker of damage in the oil produced by frying. The content in polar materials start to increase in our model after 45 min of frying and remain constant for the 60-min fried oil.

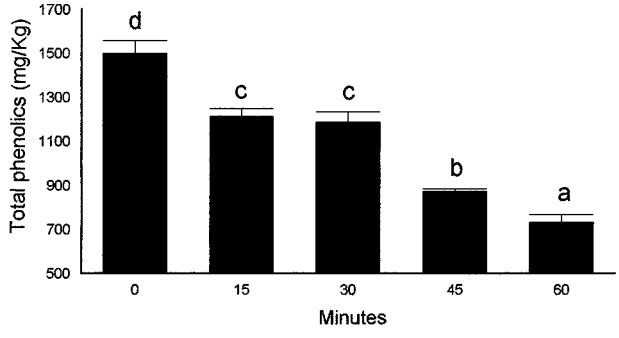

Fig. 2. Effect of frying on total phenolic in virgin olive oil. Values are means ± SE for six oil samples. Each value is significantly different from all other values not showing the same letter on the top of the bar ($P < 0.05$), i.e., the same letter on the top of two or more bars means no statistically significant differences between the corresponding values.

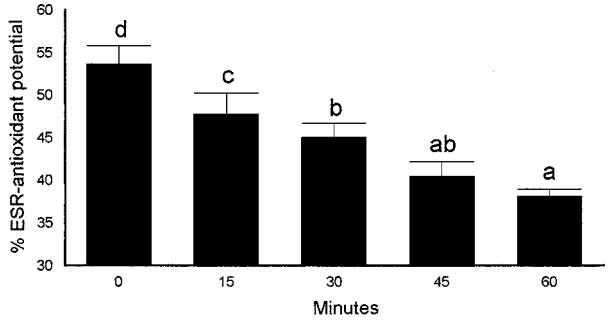

Fig. 3. Effect of frying on the antioxidant capacidy measured by ESR in virgin olive oil. Values are means ± SE for six oil samples. Each value is significantly different from all other values not showing the same letter on the top of the bar ($P < 0.05$), i.e., the same letter on the top of two or more bars means no statistically significant differences between the corresponding values.

Food Intake, Rat Weight, and Liver Weight

Dietary intake did not vary significantly among groups. Body weight was similar for all groups throughout the whole study and the weight of the liver was not affected by experimental treatments (data not shown).

Rat Plasma Determinations

Table II shows the determinations done on plasma of rats fed for 8 weeks on fried or nonfried olive oil. Concerning total cholesterol and triglycerides, normal values were found in both groups with no differences between them. The level of the antioxidants vitamin E and coenzyme Q_9 were significantly lower in fried-oil fed group. Concerning the fatty acid profile of plasma, no differences were found for total monounsaturated fatty acids (MUFA),

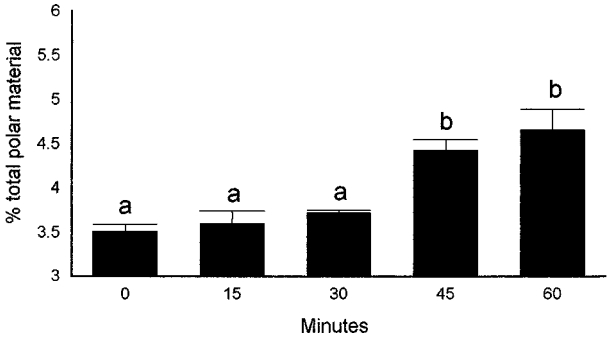

Fig. 4. Effect of frying on the formation of total polar components in virgin olive oil. Values are means ± SE for six oil samples. Each value is significantly different from all other values not showing the same letter on the top of the bar ($P < 0.05$), i.e., the same letter on the top of two or more bars means no statistically significant differences between the corresponding values.

Table II. Analytical Parameters in Plasma of Rats Fed on Nonfried and 60 min Fried Virgin Olive Oil for 8 Weeks

Analytical parameter	Nonfried group	Fried group
Total cholesterol (mg/100 mL)	86.9 ± 3.9	82.7 ± 4.7
Triglycerides (mg/100 mL)	324.7 ± 25.8	338.8 ± 52.4
Coenzyme Q_9 (μmol/L)	2.8 ± 0.2	1.9 ± 0.2[a]
Vitamin E (μmol/L)	48.5 ± 5.2	30 ± 2.3[a]
Saturated fatty acids (SFA) (%)	28.8 ± 0.5	31.29 ± 0.7[a]
Unsaturated fatty acids (UFA) (%)	71.2 ± 0.5	68.7 ± 0.7[a]
Monounsaturated fatty acids (MUFA) (%)	52.1 ± 1.4	49.6 ± 1.6
Diunsaturated fatty acids (DUFA) (%)	6.3 ± 0.3	6.2 ± 0.2
SFA:MUFA	0.5 ± 0.1	0.6 ± 0.1
PUFA ($n-6$)	15.6 ± 0.9	15.8 ± 0.9
PUFA ($n-3$)	1.3 ± 0.1	1.3 ± 0.1
Unsaturation index (UI)	4.1 ± 0.1	3.6 ± 0.1[a]

Note. Results are expressed as mean ± SE.
[a] Nonfried group versus fried group, significantly different for $P < 0.05$.

diunsaturated fatty acids (DUFA), polyunsaturated fatty acids $n-6$ (PUFA $n-6$), PUFA $n-3$ or the ratio between saturated fatty acids and monounsaturated fatty acids (SFA:MUFA). However, the level of total SFA was higher in fried-oil fed animals and the level of total unsaturated fatty acids (UFA) and unsaturation index (UI) were lower in fried-oil fed animals.

Rat Liver Mitochondrial Determinations

The results on the mitochondrial membrane of rats are showed in Table III. No differences were found in mitochondrial membrane content of cholesterol. The levels of coenzyme Q_9 were higher in fried-oil fed animals and no differences were found between two groups for vitamin E. Concerning lipid peroxidation markers, both lipid hydroperoxides and TBARS were higher in the group of animals on the diet formulated with fried-oil fed for 8 weeks.

Concerning the lipid profile in the mitochondrial membranes, similar changes to those described for plasma fatty acids were found, i.e., lower UFA, lower UI, and higher SFA in fried-oil fed rats than in nonfried-oil fed rats. The rest of the fatty acid parameters (MUFA, DUFA, SFA:MUFA, PUFA $n-6$, and PUFA $n-3$) did not show differences between both groups.

Figure 5 shows the levels of cytochromes b, $c+c_1$, and $a+a_3$ as well as the cytochrome c oxidase activity in liver mitochondrial membranes. No differences were found for cytochrome b after frying. However, the levels of cytochrome c, $a+a_3$, and the cytochrome c oxidase activity was higher in rats fed on fried oil compared with those fed on nonfried oil.

Table III. Analytical Parameters in Liver Mitochondrial Membranes of Rats Fed on Nonfried and 60 min Fried Virgin Olive Oil for 8 Weeks

Analytical parameter	Nonfried group	Fried group
Total cholesterol (mg/100 mL)	90.4 ± 3.6	95.9 ± 8.7
Coenzyme Q_9 (μmol/L)	23.6 ± 0.9	38.7 ± 6.1[a]
Vitamin E (μmol/L)	30.5 ± 2.5	40.5 ± 6.7
TBARS (nmol/μmol Pi)	12.5 ± 1.3	23.1 ± 1.9[a]
Hydroperoxides (nmol/μmol Pi)	1.1 ± 0.1	1.9 ± 0.2[a]
Saturated fatty acids (SFA) (%)	36.7 ± 0.3	39.2 ± 1.1[a]
Unsaturated fatty acids (UFA) (%)	63.3 ± 0.3	60.8 ± 1.1[a]
Monounsaturated fatty acids (MUFA) (%)	32.9 ± 0.7	29.8 ± 2.2
Diunsaturated fatty acids (DUFA) (%)	5.8 ± 0.2	6.1 ± 0.3
SFA:MUFA	1.1 ± 0.1	1.4 ± 0.2
PUFA ($n-6$)	23.5 ± 0.6	24.3 ± 0.9
PUFA ($n-3$)	3.6 ± 0.1	3.8 ± 0.2
Unsaturation index (UI)	4.1 ± 0.1	3.8 ± 0.1[a]

Note. Results are expressed as mean ± SE.
[a] Nonfried group versus fried group, significantly different for $P < 0.05$.

DISCUSSION

Oxidative injury is assumed to play a crucial role in the development of several chronic diseases, e.g., coronary heart disease (CHD) and cancer, and the possibility that dietary antioxidants may protect against LDL oxidation and oxidative injury has received growing attention in the past few years (Halliwell and Gutteridge, 1999).

Dietary fatty acids can influence the susceptibility of cells to oxidative stress, probably also by changing cell membrane fatty acid composition (Battino *et al.*, 1999). Cells enriched with MUFA have been shown to be less susceptible to oxidative damage, whereas $n-6$ PUFA increased the susceptibility to oxidative damage. Moreover, since some aldehydic products are damaging to human health (Grootveld *et al.*, 1998), the results of several investigations indicate that the dietary ingestion of thermally stressed PUFA-rich culinary oils promotes the induction, development, and progression of CHD. Despite the availability of much epidemiological and experimental evidence related to the dietary consumption of virgin olive oil in limiting the development and progression of several pathologies, the toxicological hazards associated with the ingestion of unheated and/or thermally stressed virgin olive oil is partially lacking. This is particularly interesting if we considered that, as we recently demonstrated (Quiles *et al.*, 2001), the dietary fat type may modulate the composition and function of mitochondrial respiratory chain components.

The data we are discussing indicate that the virgin olive oil used in the rat diet had not suffered any changes in its fatty acid pattern after frying (Table I). This has to be ascribed to the short-time deep fat frying procedure employed. Nevertheless, the vitamin E and total phenolic compound contents were deeply affected by thermal procedure. In fact, a 50% loss of both vitamin E and phenolic compounds can be found after 60 min of frying treatment.

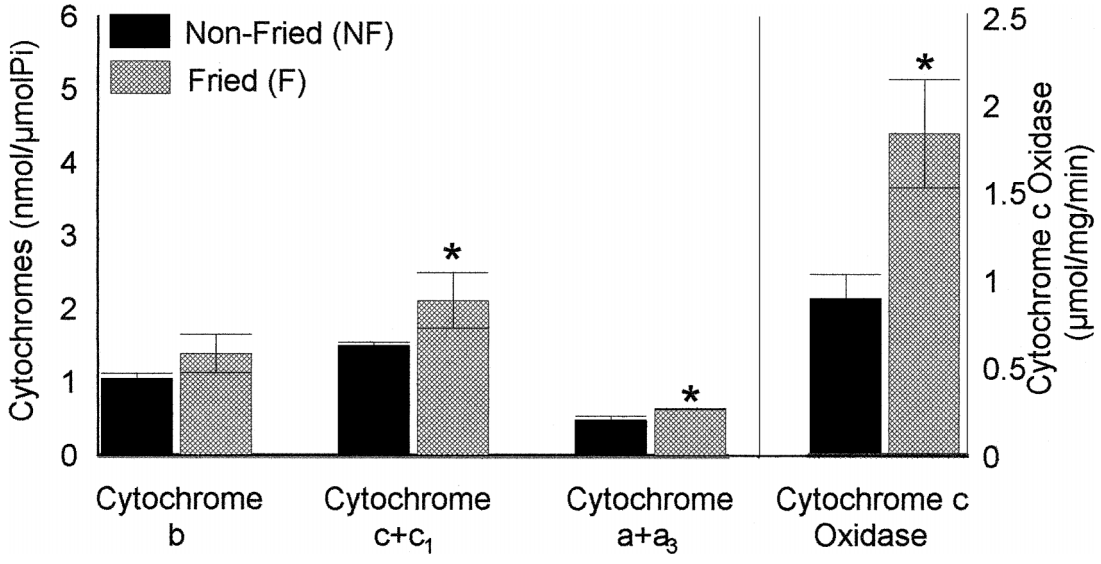

Fig. 5. Effect of feeding rats for 8 weeks with fried or nonfried virgin olive oil on the levels of cytochrome b, $c + c_1$ and $a + a_3$ and on the cytochrome c oxidase activity in liver mitochondrial membranes. Results are mean ± SEM ($n = 8$). *represents difference between fried and nonfried oil fed animals for each variable ($P < 0.05$).

The frying procedure decreased the content of phenolics and vitamin E probably as the result of a thermal destruction of these molecules or because of their use in the protection of the oils against the oxidative insult. An idea as to just how labile phenols are comes from studies where authors reported that these compounds are lost in the oil merely by the effect of time, as a consequence of hydrolytic or oxidative processes during the storage (Cinquanta *et al*., 1997; Cortesi *et al.*, 1995).

The above mentioned changes in the fried olive oil had, as a consequence, a sharp decrease in total oil antioxidant potential measured by a very sensitive ESR method. Finally, total polar component measurements are a representative index of the total alterations of the stressed oil. In fried oils, total polar components are the breakdown products from the frying process (Melton *et al*., 1994). Actually, total polar components drastically increased in the oil that underwent thermal oxidation.

Therefore the evaluation of the possible effects that the thermally oxidized products present in fried olive oil may have on biological membrane after feeding is very interesting.

The effects have been quite homogeneous and results suggest that dietary intake of fried oil may lead to severe risks of producing jeopardized mitochondrial membranes. In fact, both indexes of peroxidation (HP and TBARS) increased together with concomitant loss of unsaturated fatty acids, which suffered, in part, the effect of the oxidative process. It is noteworthy to specify that the fatty acid profile in the oil after frying did not change, that the changes found in plasma and mitochondrial membranes concerning fatty acids and lipid peroxidation came from the alterations that oil suffered in terms of loss of antioxidants or enhancement of toxic compounds.

The behavior of lipophylic antioxidant (i.e., CoQ and Vitamin E) deserves specific comments considering the pivotal role played by CoQ in the mitochondrial membrane. The twofold activity of CoQ (i.e., redox carrier and antioxidant) has been widely investigated and demonstrated (Kagan and Quinn, 2001) and such behavior is of deep importance in the maintenance of mitochondrial welfare. Increased CoQ contents in mitochondria following different kind of limited oxidative stress (e.g., dietary treatment, exercise, xenobiotic administration, and ageing) should not be surprising (Beyer *et al*., 1984; Huertas *et al*., 1991a; Quiles *et al*., 2001). In fact, it has been found (Quiles *et al*., 1994, 1999b) that during oxidative insults, CoQ is mobilized from the plasma compartment (which would behave as a wide antioxidant "reservoir") toward the sites that mostly need CoQ presence for bioenergetics as well as for antioxidant reasons. In other words, a recall of antioxidant molecules by damaged tissue from blood would take place. In this study such hypothesis is supported by the concomitant sharp decrease of both CoQ and vitamin E in plasma after fried oil dietary administration.

As far as other mitochondrial components are concerned, the cytochrome contents were enhanced following fried oil uptake as it occurred for complex IV activity. Again, these data appear to be the results produced by the thermally oxidized products taken with the diet. A control on the cytochrome oxidase activity is strongly determined also by the lipid environment induced by the diet as indicated by Huertas *et al*. (1991b). Both dietary fats and xenobiotics administration may modulate and induce the cytochrome oxidase activity as well as the actual cytochrome $a + a_3$ contents (Huertas *et al.*, 1991b). More recently, we have confirmed that different degrees of endogenous peroxidation produced by dietary fat and exercise may lead to a generalized enhancement of cytochrome content in rat liver mitochondria and that also cytochrome oxidase activity is influenced in the same way (Quiles *et al*., 2001). The fine molecular mechanism responsible for this induction phenomenon is still unclear, however, it is widely accepted that while high levels of oxidative stress lead to significant modifications and damage, low levels of oxidative stress are paradoxically stimulatory (Burdon *et al.*, 1989; Chung *et al.*, 1993) and are able to enhance, for example, cytochrome *c* content in different tissues (Beyer *et al.*, 1984).

The overall data discussed suggest that even a very stable fat source such as virgin olive oil could undergo important oxidative modifications following a short-time deep frying procedure. Such modifications may lead to alterations of both structure and function of liver mitochondria after dietary intake of these thermally oxidized oils.

ACKNOWLEDGMENTS

This work was supported by the CICYT project ALI91-1113-C03-01. M. Battino was visiting scientist at Granada University, thanks to Ministerio de Educación y Ciencia, Madrid. J. L. Quiles and M. C. Ramirez-Tortosa received a short-term fellowship from Ancona University. The authors are grateful to Monica Glebocki for text elaboration.

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