JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

How Heating Affects Extra Virgin Olive Oil Quality Indexes and Chemical Composition

YOSRA ALLOUCHE,*,† ANTONIO JIMÉNEZ,† JOSÉ JUAN GAFORIO,‡ MARINO UCEDA,† AND GABRIEL BELTRÁN†

Estación de Olivicultura y Elaiotecnia, CIFA "Venta del Llano", Instituto Andaluz de Investigación y Formación Agraria, Pesquera, Alimentaría y de la Producción Ecológica (IFAPA), P.O. Box 50, Mengibar, Jaén E-23620, Spain, and Área de Inmunología, Departamento de Ciencias de la Salud, Facultad de Ciencias Experimentales y de la Salud, Universidad de Jaén, 23071 Jaén, Spain

Two monovarietal extra virgin olive oils from Arbequina and Picual cultivars were subjected to heating at 180 °C for 36 h. Oxidation progress was monitored by measuring oil quality changes (peroxide value and conjugated dienes and trienes), fatty acid composition, and minor compound content. Tocopherols and polyphenols were the most affected by the thermal treatment and showed the highest degradation rate although their behavior was different for each cultivar. α-Tocopherol loss was more important in Arbequina oil whereas, total phenol content loss was greater in Picual oil. The later showed an important decrease in hydroxytyrosol (3,4-DHPEA) and its secoiridoid derivatives (3,4-DHPEA-EDA and 3,4-DHPEA-EA), while lignans decrease was lesser. For Arbequina oil these compounds remained stable, and a lowering tendency was observed for tyrosol (p-HPEA) and its derivatives (p-HPEA-EDA and p-HPEA-EA). In general, flavone content showed a decrease during heating, being higher for Arbequina oil. On the other hand, oleic acid, sterols, squalene, and triterpenic alcohols (erythrodiol and uvaol) and acids (oleanolic and maslinic) were quite constant, exhibiting a high stability against oxidation. From these results, we can conclude that despite the heating conditions, VOO maintained most of its minor compounds and, therefore, most of its nutritional properties.

KEYWORDS: Heating; fatty acids; minor compounds; oxidation; quality indexes; virgin olive oil (VOO)

INTRODUCTION

Virgin olive oil (VOO), one of the major fat sources of the Mediterranean diet, has been widely associated with the prevention of several pathologies like cancer (*1*), heart disease (*2*), and aging by inhibiting oxidative stress (*3*). These beneficial properties are mainly attributed to its composition, a high percentage of monounsaturated acids (oleic acid: C18:1, n 9) and significant amounts of minor components with strong antioxidant activity. Therefore, VOO exhibits high resistance to oxidation in comparison with other vegetable oils.

Among the minor components, the total tocopherol fraction, with vitamin E activity, constitutes between 100 and 300 mg/kg (*4*). Chemically, α-tocopherol is the most important antioxidant form of vitamin E since it reacts with fatty acid peroxyl radicals, the primary products of lipid peroxidation, and intercepts the chain reaction (*5*). This antioxidant capacity can be beneficial above all in degenerative diseases associated with cellular aging such as heart disease, arthritis, senility, and diabetes (*6*).

The range of phenolic compounds, which are responsible for some virgin olive oil organoleptic properties (*7*, *8*), is between 200 and 1500 mg/kg (*9*). These compounds can inhibit oxidation by a variety of mechanisms based on radical scavenging, hydrogen atom transfer, and metal-chelating attributes (*10*–*13*). Furthermore, several studies have demonstrated that these compounds possess antioxidant (*14*), anti-inflammatory (*15*), and antithrombotic (*16*) activities in cell cultures and in vitro studies. Moreover, they display an important effect on cancer chemoprevention and therapy and on lowering the oxidative modifications of low density lipoprotein and on increasing the antioxidant capacity of plasma (*17*, *18*).

Squalene is another minor compound present in olive oil in quantities as high as 1000–8000 mg/kg (*19*) that may also exhibit effects on the oxidative stability of VOO by quenching of singlet oxygen and scavenging free radicals (*20*). Antitumor activity against colon (*21*) and skin (*22*) cancer has been attributed to this compound.

In VOO, the amount of sterols is about 113–265 mg/100 g of oil (*23*, *24*). These compounds are important dietary components for lowering low density lipoprotein (LDL) cholesterol and maintaining good heart health. Also they possess anticancer, anti-inflammatory, antiatherogenicity, and antioxidative activities (*25*).

* To whom correspondence should be addressed. Tel: 0034 953366366. Fax: 0034 953366380. E-mail: yosra.allouche.ext@juntadeandalucia.es.

† IFAPA.

‡ Universidad de Jaén.

10.1021/jf070628u CCC: $37.00
Published on Web 10/13/2007

Table 1. Initial Characteristics of the Unheated VOO from Arbequina and Picual Cultivars[a]

parameter	Arbequina	Picual
PV (mequiv O_2/kg)	10.10 ± 0.23	10.74 ± 0.33
K_{232}	1.96 ± 0.04	1.67 ± 0.03
K_{270}	0.09 ± 0.01	0.12 ± 0.006
fatty acid concn (% peak area)		
C16:0	14.34 ± 0.07	10.63 ± 0.26
C16:1	2.43 ± 0.32	1.55 ± 0.08
C18:0	1.63 ± 0.10	2.51 ± 0.04
C18:1	64.73 ± 0.23	77.04 ± 0.44
C18:2	12.80 ± 0.23	4.90 ± 0.04
C18:3	0.98 ± 0.02	0.80 ± 0.06
C20:0	0.58 ± 0.04	0.49 ± 0.04
C20:1	0.78 ± 0.10	0.73 ± 0.03
C22:0	0.21 ± 0.05	0.16 ± 0.03
O/L[b]	5.06 ± 0.08	15.72 ± 0.21
MUFAs/PUFAs[c]	4.94 ± 0.07	13.94 ± 0.33
squalene (mg/100 g)	427.17 ± 44.61	1345.60 ± 159.22
total sterols (mg/kg)	1554.5 ± 9.19[d]	1159.5 ± 84.15[d]
cholesterol (mg/kg)	3.97 ± 1.01[d]	6.41 ± 1.28[d]
brassicasterol (mg/kg)	0.78 ± 1.10[d]	2.32 ± 0.17[d]
campesterol (mg/kg)	61.40 ± 0.30[d]	40.70 ± 6.22[d]
estigmasterol (mg/kg)	12.05 ± 0.18[d]	11.83 ± 7.40[d]
β-sitosterol (mg/kg)	1459 ± 6.10[d]	1083 ± 64.70[d]
Δ-7-estigmasterol (mg/kg)	2.95 ± 0.42[d]	2.87 ± 0.61[d]
total phenols (mg/kg caffeic acid)	81.67 ± 2.08	406.00 ± 7.81
hydroxytyrosol (mg/kg)	0.04 ± 0.006	0.24 ± 0.02
DHPEA-EDA (mg/kg)	0.95 ± 0.04	3.27 ± 0.09
DHPEA-EA (mg/kg)	0.88 ± 0.11	15.39 ± 0.31
tyrosol (mg/kg)	0.24 ± 0.02	0.46 ± 0.01
pHPEA-EDA (mg/kg)	4.97 ± 0.26	7.46 ± 0.19
pHPEA-EA (mg/kg)	0.95 ± 0.03	8.85 ± 0.16
1-acetoxypinoresinol (mg/kg)	6.02 ± 0.30	7.22 ± 2.28
pinoresinol (mg/kg)	0.07 ± 0.003	0.90 ± 0.78
luteolin (mg/kg)	1.25 ± 0.12	2.41 ± 0.04
apigenin (mg/kg)	0.82 ± 0.04	1.02 ± 0.04
total tocopherols (mg/kg)	171.67 ± 1.53	192.33 ± 2.08
α-tocopherol (mg/kg)	170.00 ± 1.73	178.67 ± 1.53
β-tocopherol (mg/kg)	2.00 ± 0.58	3.33 ± 0.58
γ-tocopherol (mg/kg)	0.00 ± 0.00	10.33 ± 0.58
erythrodiol (mg/kg)	12.79 ± 1.01[d]	9.61 ± 2.28[d]
uvaol (mg/kg)	3.97 ± 0.56[d]	5.90 ± 0.12[d]
oleanolic (mg/kg)	395.01 ± 6.42[d]	398.14 ± 76.69[d]
ursolic (mg/kg)	5.67 ± 3.32[d]	4.34 ± 0.41[d]
maslinic (mg/kg)	46.62 ± 2.81[d]	17.53 ± 1.03[d]
oxidative stability (hours at 98 °C)	36.59 ± 0.007[d]	102.65 ± 1.22[d]

[a] Each value is the mean of three replicates ± standard deviation (SD) unless noted otherwise. [b] O/L: oleic/linoleic. [c] MUFA/PUFA: Monounsaturated/polyunsaturated. [d] Mean of two replicates ± standard deviation (SD).

The most abundant triterpenes in VOO are two acids (oleanolic and maslinic) and two alcohols (erythrodiol and uvaol), their concentrations range between 15 and 100 mg/kg (*26*) and 6–20 mg/kg (*6*), respectively. These compounds have been reported to have essentially an anti-inflammatory activity and a possible protector effect against cardiovascular risk such as hypertension (*6*).

VOO is considered as an olive fruit juice since it is obtained by physical methods, and because of its sensory characteristics and nutritional properties it can be consumed raw in salads and other foodstuffs, although normally it is subjected to culinary processes such as frying, boiling, and microwave heating (*27*).

These cooking techniques include a variety of chemical reactions which can be categorized as hydrolysis, oxidation, and polymerization and, as a consequence, lead to the loss of its organoleptic peculiarities and nutritional value. Therefore and because of the increase of the consumption of fried foods, we investigated the quality indexes, the compositional changes, and the oxidative stability of two extra virgin olive oils subjected to a heating operation at 180 °C simulating the "frying process" with special interest in its nutritional compounds.

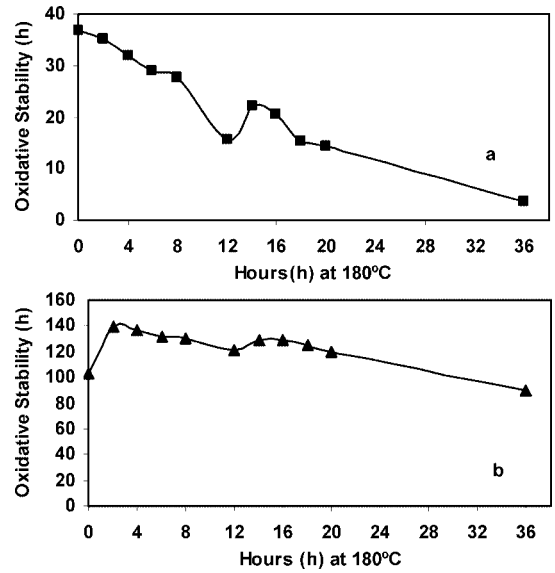

Figure 1. Changes in oxidative stability (measured at 98 °C) in extra virgin oils from Arbequina (**a**) and Picual (**b**) cultivars subjected to heating at 180 °C.

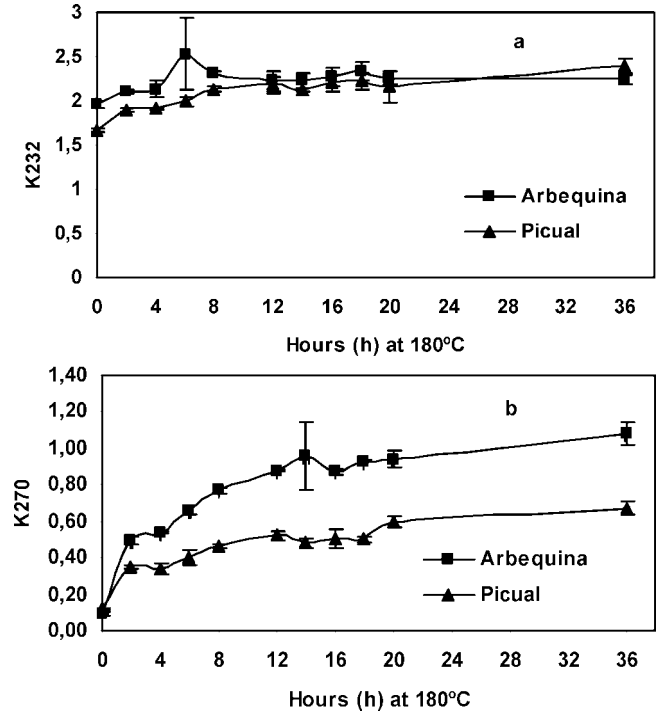

Figure 2. Changes in the ultraviolet absorption at 232 (**a**) and 270 nm (**b**) in VOO from Arbequina and Picual cultivars subjected to heating at 180 °C.

MATERIALS AND METHODS

Olive Oil Samples. Two extra virgin olive oils (VOO) from Arbequina and Picual cultivars were used in this experiment.

Heating Procedure. Ten samples of each VOO were placed in open beakers and heated in a hot air oven apparatus at 180 °C for 2, 4, 6, 8, 12, 14, 16, 18, 20, and 36 h. One beaker was taken from the oven for analysis at scheduled times (*28, 29*).

Analytical Determinations. Peroxide values (PVs) and absorption at 232 and 270 nm (K_{232} and K_{270}) were measured following the analytical methods described in the European Regulations EEC 2568/

Table 2. Changes in the Content of Palmitic, Palmitoleic, Stearic, Oleic, Linoleic, and Linolenic Acids in VOO from Arbequina and Picual Cultivars during Heating at 180 °C[a]

heating time (h)	C16:0		C16:1		C18:0		C18:1		C18:2		C18:3	
	Arbequina	Picual	Arbequina	Picual	Arbequina	Picual	Arbequina	Picual	Arbequina	Picual	Arbequina	Picual
2	13.31 ± 0.22	11.17 ± 0.21	2.52 ± 0.13	1.65 ± 0.13	1.59 ± 0.02	2.77 ± 0.02	66.52 ± 0.39	76.10 ± 0.30	12.76 ± 0.04	4.91 ± 0.16	0.79 ± 0.03	0.78 ± 0.06
4	14.44 ± 0.43	10.84 ± 0.16	2.53 ± 0.11	1.52 ± 0.16	1.54 ± 0.03	2.73 ± 0.07	65.55 ± 0.20	76.62 ± 0.21	12.58 ± 0.10	4.94 ± 0.10	0.77 ± 0.04	0.76 ± 0.06
6	16.66 ± 0.47	11.74 ± 0.10	1.85 ± 0.07	1.06 ± 0.01	1.80 ± 0.03	3.07 ± 0.03	67.02 ± 0.35	78.48 ± 0.07	10.29 ± 0.18	2.80 ± 0.04	0.54 ± 0.02	0.64 ± 0.006
8	16.19 ± 0.29	12.74 ± 1.4	1.81 ± 0.02	1.05 ± 0.09	1.82 ± 0.05	3.09 ± 0.06	67.3 ± 0.53	77.56 ± 1.01	10.25 ± 0.28	2.64 ± 0.07	0.54 ± 0.02	0.61 ± 0.04
12	15.74 ± 0.37	12.09 ± 0.09	1.81 ± 0.006	1.00 ± 0.03	1.79 ± 0.04	3.04 ± 0.02	67.66 ± 0.07	78.49 ± 0.18	10.23 ± 0.10	2.70 ± 0.06	0.54 ± 0.02	0.60 ± 0.02
14	16.05 ± 0.21	12.81 ± 0.05	1.76 ± 0.05	1.00 ± 0.03	1.76 ± 0.01	3.09 ± 0.04	67.35 ± 0.22	77.59 ± 0.11	10.38 ± 0.05	2.72 ± 0.11	0.55 ± 0.01	0.58 ± 0.02
16	16.92 ± 0.17	12.61 ± 0.14	1.79 ± 0.03	1.03 ± 0.05	1.78 ± 0.01	3.09 ± 0.02	66.83 ± 0.08	77.85 ± 0.35	10.03 ± 0.03	2.74 ± 0.07	0.54 ± 0.02	0.60 ± 0.03
18	16.3 ± 0.50	12.02 ± 0.25	1.78 ± 0.04	1.01 ± 0.04	1.81 ± 0.04	3.09 ± 0.06	67.57 ± 0.69	78.28 ± 0.23	9.80 ± 0.30	2.74 ± 0.03	0.54 ± 0.04	0.61 ± 0.03
20	16.21 ± 0.15	12.16 ± 0.16	1.81 ± 0.06	1.02 ± 0.03	1.80 ± 0.04	3.06 ± 0.01	67.31 ± 0.06	78.25 ± 0.05	10.17 ± 0.04	2.72 ± 0.08	0.54 ± 0.05	0.59 ± 0.02
36	16.65 ± 0.13	12.15 ± 0.14	1.81 ± 0.02	1.05 ± 0.06	1.84 ± 0.03	3.10 ± 0.02	67.72 ± 0.17	78.06 ± 0.19	9.44 ± 0.08	2.64 ± 0.11	0.49 ± 0.01	0.56 ± 0.03

[a] Each value is the mean of three determinations ± standard deviation (SD).

Table 3. Changes in Sterol Composition (mg/kg) in VOO from Arbequina and Picual Cultivars during Heating at 180 °C[a]

heating time (h)	total sterols		cholesterol		brassicasterol		campesterol		estigmasterol		β-sitosterol		Δ7-estigmasterol	
	Arbequina	Picual	Arbequina	Picual	Arbequina	Picual	Arbequina	Picual	Arbequina	Picual	Arbequina	Picual	Arbequina	Picual
2	1458.50 ± 36.06	1098.00 ± 26.87	6.31 ± 3.66	6.41 ± 1.28	0.50 ± 0.71	0.00 ± 0.00	59.90 ± 3.44	36.35 ± 1.67	12.41 ± 1.34	6.48 ± 0.47	1360.70 ± 25.40	1037.80 ± 20.12	3.66 ± 1.12	2.30 ± 0.10
4	1495.00 ± 9.90	1059.00 ± 16.97	5.83 ± 0.25	6.56 ± 2.41	1.50 ± 2.12	2.64 ± 0.71	63.63 ± 5.60	36.81 ± 1.71	13.46 ± 2.20	6.88 ± 0.64	1391.80 ± 7.70	994.55 ± 14.67	3.74 ± 1.08	2.75 ± 0.55
6	1431.50 ± 116.67	1036.00 ± 9.90	4.19 ± 1.35	6.38 ± 3.10	0.00 ± 0.00	2.07 ± 0.02	57.46 ± 4.38	36.04 ± 1.71	11.45 ± 0.93	6.79 ± 0.28	1343.30 ± 106.46	972.92 ± 11.35	2.19 ± 1.19	3.57 ± 0.48
8	1494.00 ± 113.14	1062.50 ± 9.19	4.70 ± 2.25	5.69 ± 1.41	0.21 ± 0.30	6.86 ± 9.71	60.04 ± 4.13	36.13 ± 0.31	12.66 ± 0.09	7.44 ± 0.06	1399.30 ± 109.12	996.07 ± 3.36	2.72 ± 0.61	4.79 ± 2.30
12	1401.50 ± 20.51	1149.00 ± 66.47	6.19 ± 3.46	6.38 ± 1.56	0.35 ± 0.49	3.45 ± 0.20	55.21 ± 0.02	38.47 ± 1.41	11.21 ± 0.16	7.45 ± 0.38	1314.90 ± 16.17	959.45 ± 101.85	2.52 ± 0.04	2.85 ± 0.65
14	1392.00 ± 12.73	1023.00 ± 73.54	4.18 ± 0.04	6.94 ± 2.02	0.69 ± 0.98	1.99 ± 0.07	55.68 ± 0.51	33.38 ± 3.27	11.14 ± 0.10	7.33 ± 0.34	1305.70 ± 13.91	962.83 ± 64.52	2.78 ± 0.03	2.26 ± 0.45
16	1390.50 ± 10.61	1173.00 ± 73.54	5.08 ± 0.73	5.81 ± 1.36	0.90 ± 0.29	1.73 ± 0.72	56.32 ± 1.41	38.71 ± 2.43	12.24 ± 0.49	7.65 ± 1.31	1301.50 ± 5.99	1104.40 ± 68.41	3.48 ± 1.01	2.35 ± 0.15
18	1343.50 ± 2.12	1079.50 ± 146.37	4.03 ± 1.89	7.60 ± 0.35	0.40 ± 0.57	2.41 ± 0.73	53.74 ± 0.08	39.80 ± 11.14	11.42 ± 0.97	4.49 ± 0.55	1259.50 ± 2.94	1011.10 ± 133.23	2.69 ± 0.004	3.19 ± 0.32
20	1444.50 ± 0.71	1132.50 ± 60.10	4.33 ± 0.20	5.28 ± 2.91	0.00 ± 0.00	2.03 ± 0.21	55.83 ± 0.07	37.33 ± 2.38	11.12 ± 0.21	7.75 ± 0.01	1356.50 ± 1.28	1070.50 ± 57.21	2.09 ± 0.10	2.49 ± 0.03
36	1476.00 ± 15.56	1205.50 ± 2.12	7.38 ± 0.08	4.19 ± 0.22	0.00 ± 0.00	1.20 ± 1.70	58.16 ± 0.82	38.58 ± 0.07	12.84 ± 0.49	9.04 ± 0.87	1379.60 ± 15.58	1116.30 ± 7.08	2.07 ± 0.19	4.82 ± 0.008

[a] Each value is the mean of two determinations ± standard deviation (SD).

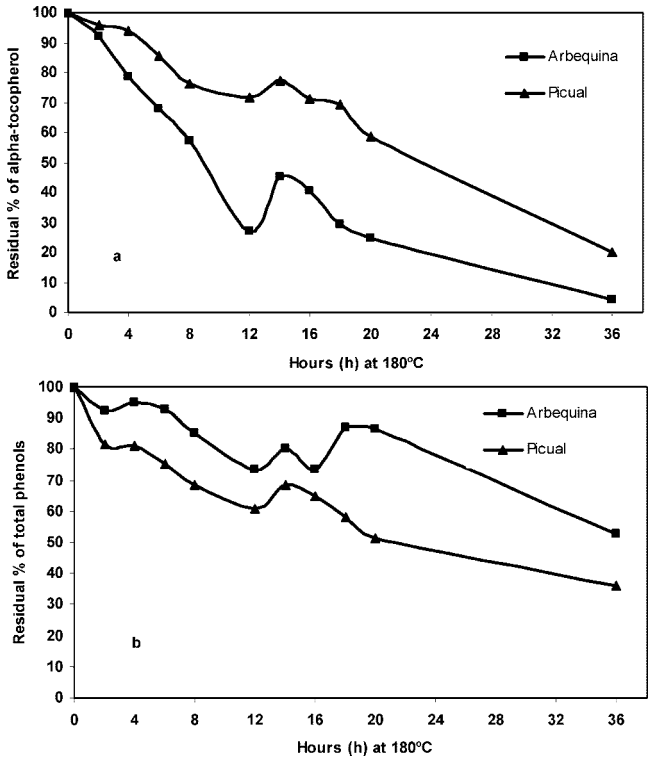

Figure 3. Residual content of α-tocopherol (**a**) and total phenols (**b**) in VOO from Arbequina and Picual cultivars subjected to heating at 180 °C.

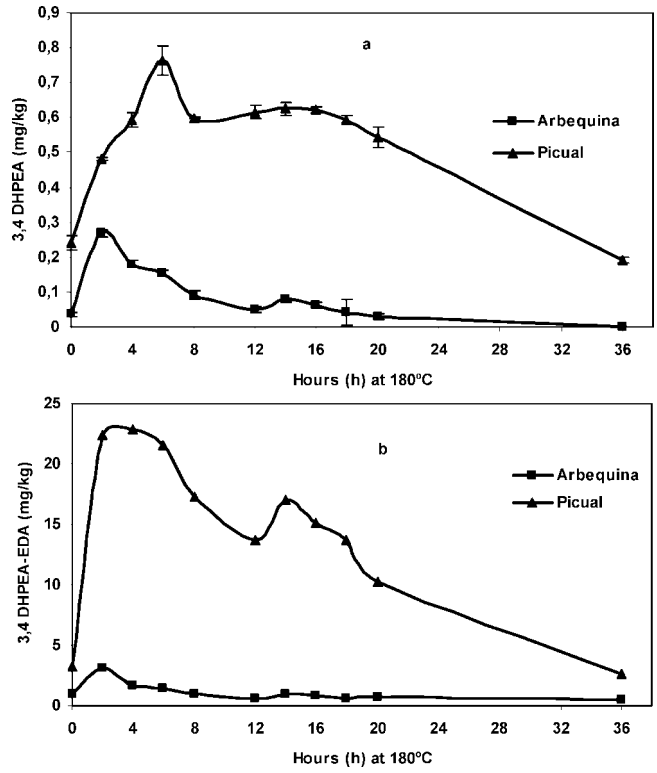

Figure 4. Evolution of 3,4-DHPEA (**a**) and 3,4-DHPEA-EDA (**b**) in VOO from Arbequina and Picual cultivars during heating at 180 °C.

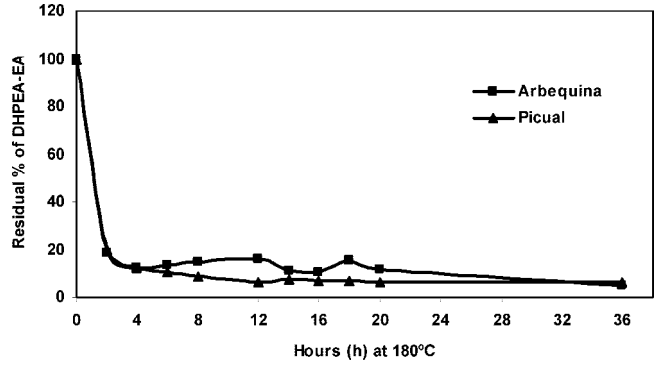

Figure 5. Residual percentage of DHPEA-EA in VOO from Arbequina and Picual cultivars subjected to heating at 180 °C.

91 (*30*). PVs were expressed as milliequivalents of active oxygen per kilogram of oil (mequiv O_2/kg); K_{232} and K_{270} extinction coefficients were calculated from absorption at 232 and 270 nm, respectively.

Sterols composition was carried out according to the EU Regulation 2568/91 (*30*). The chromatographic separation was performed in a Perkin-Elmer Autosystem gas chromatograph using a SE54 of 30 m length, 0,25 mm de internal diameter and 0,25 μm film thickness (Supelco, Bellefonte, PA). The oven temperature was held at 260 °C and helium was used as carrier gas. Injector and detector temperature were 280 °C and 290 °C respectively. Results were expressed as mg/kg.

Fatty acid composition of the oils was determined according to the EU Regulation 2568/91 (*30*). The chromatographic separation was performed in a Perkin-Elmer Autosystem gas chromatograph with a split/splitless injector and a FID detector, equipped with a BPX 70 capillary column of 50 m length, 0.25 mm i.d., and 0.25 μm film thickness (SGE Scientific, Australia). The oven temperature was held at 198 °C, and helium was used as carrier gas. The injector and detector temperatures were 235 and 245 °C, respectively. The volume injected was 0.5 μL, and the time of analysis was 45 min. The results are expressed as peak area percent.

Squalene was determined by adding 1 mL of squalan (1 mL/mg), as internal standard, to the samples prepared to FA determination (*31*). The results were expressed as mg/100 g of squalene.

Tocopherol composition was analyzed by HPLC, applying the IUPAC method 2432 (*32*). Detection and quantification were carried out in a Perkin-Elmer HPLC equipped with an isocratic pump Lc 200 and a UV–vis detector, Lc 295, set at 295 nm. The tocopherol concentration was expressed as mg/kg of oil.

Polyphenol content was carried out according to the method described by Vázquez-Roncero et al. (*33*) using the Folin-Ciocalteau reagent and absorbance measurement at 725 nm. The results were expressed as mg/kg of caffeic acid.

HPLC Analysis of Phenolic Compounds. *Extraction of Phenolic Compounds.* A sample of virgin olive oil was weighed (1.5 g) and 100 μL of a standard solution (0.002 g of syringic acid/100 mL of methanol) was added. The oil was dissolved in *n*-hexane (1 mL), the phenolics were extracted with 1.25 mL of methanol/water (60:40 v/v) two times, and the extracts were washed with *n*-hexane rising to a final volume of 2.5 mL (*34*).

RP-HPLC Determination of Phenols. HPLC analysis was performed using a HP1100 system equipped with an autosampler, quaternary pump, and diode array detector. A reversed-phase C18 Pecosphere column (83 × 4.6 mm i.d., 3 μm particle size, Brown Lee Columns) was used with an injection volume of 20 μL and a flow rate of 0.45 mL/min. The mobile phase was a mixture of water/acetic acid (98:2 v/v) (solvent A) and methanol/acetic acid (98:2 v/v) (solvent B). The total run time was 70 min, the solvent gradient changed according to the following conditions: 90% A–10% B for 10 min, 80% A–20% B in 8 min then remained for 2 min, 60% A–40% B in 10 min, 50% A–50% B in 10 min, and 100% B in 10 min until the end of the run. Phenolic compounds were quantified at 280 nm using syringic acid as internal standard and the response factors determined by Mateos et al. (*35*).

Hydroxypentacyclic acids (HPTAs) were determined according to the method described by Pérez-Camino and Cert (*26*), the chromatographic analysis was performed using a Perkin-Elmer gas chromatograph, Autosystem model, fitted with a flame ionization detector and a split injection system (split ratio 1:0.25). Separation was carried

Table 4. Changes in Tyrosol (pHPEA), pHPEA-EDA, and pHPEA-EA in VOOs from Arbequina and Picual Cultivars during Heating at 180 °C[a]

	pHPEA (mg/kg)		pHPEA-EDA (mg/kg)		pHPEA-EA (mg/kg)	
heating time (h)	Arbequina	Picual	Arbequina	Picual	Arbequina	Picual
2	0.78 ± 0.13	0.76 ± 0.01	7.89 ± 0.50	41.52 ± 0.30	0.45 ± 0.009	1.28 ± 0.002
4	0.84 ± 0.01	1.06 ± 0.21	7.05 ± 0.20	43.64 ± 0.50	0.42 ± 0.04	1.48 ± 0.07
6	0.89 ± 0.07	1.17 ± 0.10	6.93 ± 0.62	44.67 ± 3.14	0.72 ± 0.30	1.69 ± 0.30
8	0.77 ± 0.02	0.92 ± 0.03	6.76 ± 0.14	41.23 ± 0.67	0.34 ± 0.24	2.17 ± 0.37
12	0.60 ± 0.01	0.89 ± 0.04	5.82 ± 0.33	39.22 ± 0.27	0.33 ± 0.42	2.16 ± 0.14
14	0.64 ± 0.01	0.59 ± 0.24	6.51 ± 0.25	42.44 ± 1.92	0.10 ± 0.02	2.06 ± 0.12
16	0.72 ± 0.003	0.93 ± 0.10	6.22 ± 0.08	40.38 ± 0.61	0.13 ± 0.01	2.12 ± 0.03
18	0.60 ± 0.07	0.85 ± 0.08	3.70 ± 2.03	40.33 ± 0.47	0.22 ± 0.04	2.27 ± 0.11
20	0.49 ± 0.05	0.90 ± 0.03	5.68 ± 0.08	39.15 ± 0.57	0.13 ± 0.03	2.60 ± 0.07
36	0.25 ± 0.02	0.77 ± 0.04	2.29 ± 0.29	31.40 ± 0.09	0.42 ± 0.56	3.02 ± 0.08

[a] Each determination is the mean of three determinations ± standard deviation (SD).

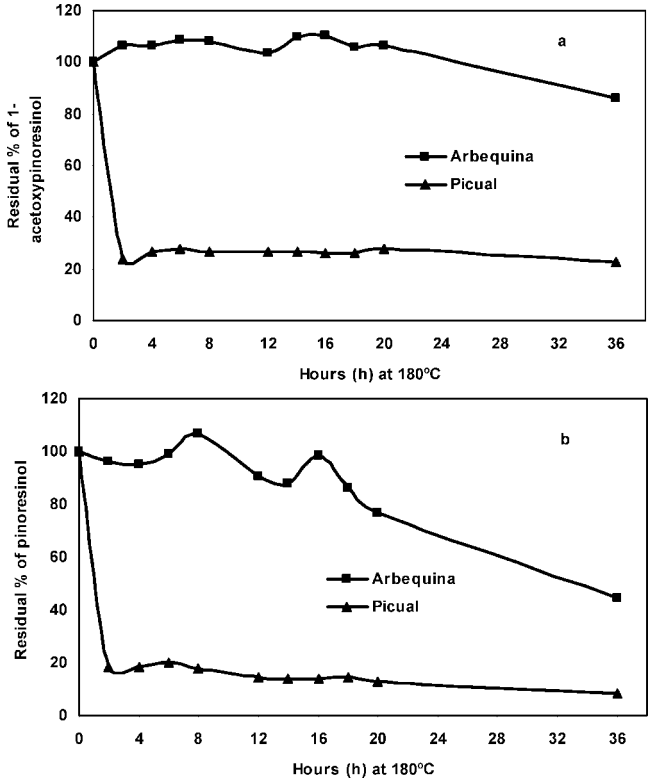

Figure 6. Residual percentage of 1-acetoxypinoresinol (**a**) and pinoresinol (**b**) in VOO from Arbequina and Picual cultivars subjected to heating at 180 °C.

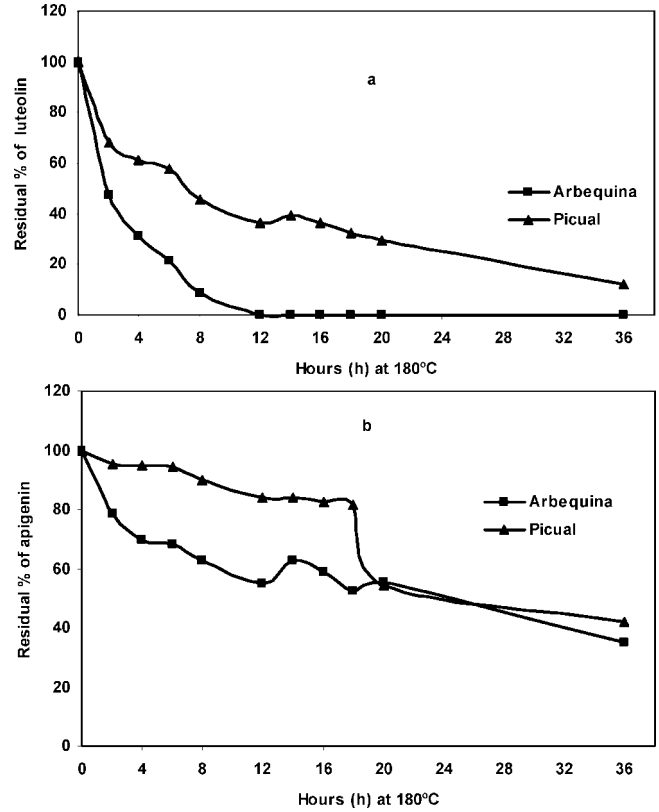

Figure 7. Residual percentage of luteolin (**a**) and apigenin (**b**) in VOO from Arbequina and Picual cultivars subjected to heating at 180 °C.

out on a HP-5 capillary column (30 m, 0.32 mm i.d., 0.25 μm of thickness). The operating conditions were as follows: oven temperature 260 °C for 5 min and then increased at 4 °C/min up to 320 °C; injector and detector at 320 °C. Helium was used as carrier gas at a column head pressure of 25 psi.

HPTAs were quantified assuming the same response factor for all triterpenic acids, and the results are expressed as mg/kg of betulinic acid.

Oxidative stability was measured as the induction time in a Rancimat 743 (Metrohm, Switzerland) at 98 °C and an air flow of 10–12 L/h (*36*). The measurements were determined in duplicate for each sample, and the results are given as induction time (hours).

Statistical analysis. Statistical analyses (mean and standard deviation) were carried out using the program Statistix, Version 8.0.

RESULTS AND DISCUSSION

The initial characterizations of Arbequina and Picual oils were based on the determination of the peroxide value, absorption at 232 and 270 nm, fatty acids, squalene, sterols, phenolic compounds, tocopherols, and triterpenic alcohols and acids. Data are summarized in **Table 1**.

By measurment of the quality parameters (PV, K_{232}, and K_{270}), the VOOs used in the experiment were classified into the "extra virgin" category as established by the EU regulation: PV (maximum values = 20 mequiv O_2/kg), K_{232} (maximum value = 2.50), and K_{270} (maximum value = 0.20).

Sterol composition, total sterols, and the sum of erythrodiol and uvaol of both oils met also the limits established by the EU regulation (*30*). Furthermore, fatty acid composition, phenolic compounds, and tocopherols showed values similar to those described for these olive cultivars (*37*). However, the values obtained for the pentacyclic hydroxytriterpenic acids (oleanolic, ursolic, and maslinic) were higher than those described by Pérez-Camino and Cert (*26*) for Arbequina and Picual cultivars. These differences might be explained by the olive growing conditions, fruit ripeness, and oil extraction system (*26*).

VOO is characterized by a higher oxidative stability compared to other edible oils (*38*) due to the high ratio of monounsaturated/

Table 5. Changes in Triterpenic Alcohols and Acids (mg/kg) in VOOs from Arbequina and Picual Cultivars during Heating at 180 °C[a]

heating time (h)	erythrodiol		uvaol		oleanolic acid		ursolic acid		maslinic acid	
	Arbequina	Picual	Arbequina	Picual	Arbequina	Picual	Arbequina	Picual	Arbequina	Picual
2	12.33 ± 0.62	7.69 ± 0.11	4.97 ± 0.93	5.91 ± 1.02	348.38 ± 25.87	365.89 ± 29.68	3.28 ± 0.78	6.57 ± 2.12	46.74 ± 0.54	22.96 ± 0.11
4	12.33 ± 0.33	7.79 ± 0.66	3.52 ± 1.02	5.31 ± 0.04	401.37 ± 15.22	353.93 ± 1.68	5.79 ± 0.64	7.72 ± 3.42	43.69 ± 0.49	17.28 ± 2.70
6	12.33 ± 1.20	8.59 ± 0.54	4.18 ± 0.35	4.52 ± 0.73	365.74 ± 127.28	383.87 ± 56.71	4.42 ± 3.85	7.44 ± 4.07	43.80 ± 0.47	22.65 ± 0.26
8	14.95 ± 3.16	9.95 ± 2.01	3.62 ± 2.10	5.41 ± 0.25	421.13 ± 30.28	323.36 ± 40.38	2.06 ± 0.08	4.20 ± 2.55	51.66 ± 3.28	18.42 ± 0.99
12	12.87 ± 1.88	7.88 ± 0.18	2.93 ± 1.94	6.32 ± 0.24	426.13 ± 42.74	345.40 ± 11.04	6.63 ± 0.93	7.71 ± 3.62	35.54 ± 0.28	21.10 ± 1.99
14	11.28 ± 0.09	7.62 ± 1.22	3.08 ± 0.97	2.99 ± 0.52	433.50 ± 54.53	507.81 ± 90.60	18.13 ± 17.47	8.41 ± 2.11	54.21 ± 2.60	23.79 ± 10.01
16	12.62 ± 1.72	9.35 ± 0.71	3.44 ± 1.08	5.40 ± 0.35	229.32 ± 286.88	477.00 ± 29.97	5.65 ± 2.33	4.78 ± 1.19	46.37 ± 0.26	23.87 ± 0.06
18	11.76 ± 0.35	8.43 ± 0.84	4.50 ± 0.42	4.93 ± 2.61	422.01 ± 13.70	515.17 ± 12.08	4.15 ± 0.81	3.87 ± 0.63	44.35 ± 5.70	25.56 ± 3.26
20	12.00 ± 0.39	9.29 ± 1.17	2.76 ± 1.31	4.21 ± 2.31	475.61 ± 26.91	388.09 ± 39.88	8.61 ± 5.18	7.81 ± 1.79	48.99 ± 1.78	18.91 ± 3.81
36	13.69 ± 0.83	10.14 ± 0.52	5.31 ± 0.41	5.02 ± 0.16	408.58 ± 17.85	417.00 ± 92.91	16.55 ± 2.36	15.91 ± 12.13	33.93 ± 4.90	16.50 ± 3.68

[a] Each determination is the mean of two determinations ± standard deviation (SD).

polyunsaturated fatty acid (MUFAs/PUFAs). As established by other authors, the contribution of several VOO components to its oxidative stability is as follows: phenolic compounds (50%), oleic/linoleic ratio O/L (27%), *o*-diphenols and monophenols 21 and 10%, respectively. The contribution of α-tocopherol was evaluated around 9%, and no effect on olive oil stability was found for *β*- and *γ*-tocopherol (*39*).

In our study, the different oxidative stability for Arbequina and Picual oils is the combined result of their fatty acid composition, particularly MUFAs/PUFAs and O/L, and minor compounds. The relatively similar content of sterols, tocopherols, and triterpenic alcohols and acids in both oils could explain that these compounds had a smaller contribution to the oxidative stability than the phenolic compounds and squalene and, therefore could explain the considerably higher stability of Picual oils.

During heating at 180 °C, a decrease on the oxidative stability was observed for both oils anticipating that the antioxidative system is being depleted, and as expected, this decrease was more pronounced for Arbequina oils (**Figures 1a** and **1b**).

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 (*40*). As expected, both indexes have shown a great increase during the heating experiment (**Figures 2a** and **2b**); the higher values were obtained for Arbequina oils. This increase indicated the formation of conjugated dienes or peroxides and trienes or unsaturated aldehydes and ketones (*41–43*).

Many authors have related the levels of peroxides, products of the lipid oxidation, with the quality of edible oils and changes by high temperature (*44*); therefore, in our experiment, the peroxides values should reach and even exceed the legal limit established by the EU for extra virgin olive oil (≤20 mequiv of O_2/kg), however the peroxide values during the heating experiment were lower than in the reference oil. This great decrease observed at 2 h of heating coincided with an important increase of the ultraviolet absorption at 270 nm and could be explained by the transformation of the peroxides into other products like aldehydes and ketones, as has been previously described by others authors (*45*).

Changes in fatty acid composition during the heating experiment are shown in **Table 2**. In both olive oils, palmitoleic acid (C16:1) has shown a decrease after 4 and 6 h of heating remaining constant until the end of the heating operation (36 h).

Oleic acid, the most abundant fatty acid in olive oil, did not change with the heating time (**Table 2**). However, linoleic (C18:2) and linolenic (C18:3) acids content were reduced after 6 h of heating and then remained stable. At 6 h of heating, the rate of decrease of linoleic acid was higher for Picual oils than that for Arbequina oils (42.86% and 19.61%, respectively). However the reduction rate of linolenic acid was higher for Arbequina than that for Picual (50.00% against 20.00%).

Our results are in agreement with those reported in the literature, since thermal degradation is closely related to the unsaturation of fatty acids (*46*). A significant reduction of oleic and linoleic acids has been reported during frying (*47*), and thermooxidation of oils (*48*). However, in our study no decrease of oleic acid was detected, but a slight increase after 6 h of heating was registered and then remained constant, probably as the result of linoleic acid decrement.

The heating effect on squalene was different for each VOO: for Arbequina oil no significant changes were observed; for Picual oil squalene content was reduced by 25% at the end of heating assay. Under normal (*49*) and accelerated conditions of storage (*50*), a slight loss of squalene was observed suggesting that this hydrocarbon is rather stable during autooxidation. Psomiadou and Tsimidou (*49*) have found that this hydrocarbon has a slight antioxidant activity which was concentration dependent. This result may explain the different tendency observed for both oils, since unheated Picual oil had 1400 mg/100 g, while Arbequina had 400 mg/100 g.

There are no data on the behavior of squalene when olive oil is subjected to high temperatures (180 °C) or during frying, but in the case of rapeseed oils, it has been found that squalene plays a more significant role in oil protection during frying temperatures (*51*), probably due to its higher unsaturation rate.

The major sterol present in olive oil is *β*-sitosterol, constituting up to 90–95% of total sterols, followed by campesterol and stigmasterol which make up about 3% and 1%, respectively. During the heating experiment, these compounds have shown a high stability and no degradation was detected (**Table 3**). These results were similar to those obtained by Rastrelli et al. (*52*) in various storage conditions of two Italian VOOs for 12 months but different from those reported by Boskou (*53*) who describe the formation of oxidation products under unfavorable conditions such as high temperature and the presence of air.

In olive oils, tocopherols are mainly represented by the α-tocopherol, which is biologically the most active. For both VOOs, a considerable loss was observed during the heating suggesting that α-tocopherol is being used to protect the oils against thermal damage. However the degradation rate was different for each olive oil; α-tocopherol content in Arbequina oils is lost more rapidly than that in Picual oils (**Figure 3a**). Our results are similar to those described by Brenes et al. (*27*) for the same cultivars. The rapid decrease of α-tocopherol with the heating time has also been reported by Pellegrini et al. (*54*).

Although there was no difference in the initial content of α-tocopherol for the unheated VOO, the difference observed

in the rate of degradation should be partially attributed to the difference in the amount of phenolic compounds. As phenol content in Arbequina oils is very low (only 81.67 ppm), α-tocopherol protects the oils from oxidation by decreasing quickly (**Figure 3a**). Conversely, in the case of Picual oils, phenolic substances seem to be responsible of the oil protection, and therefore the rate of degradation of the phenolic compounds was faster than that for α-tocopherol (**Figure 3b**). At the end of the heating experiment, the content of polyphenols in Picual and Arbequina oils was reduced by 64% and 47%, remaining 146 ppm and 43 ppm, respectively.

In olive oils, the α-tocopherol is also accompanied by a small amount of the γ-isomer, this compound was absent in the unheated Arbequina oils. However, during the heating appeared some peaks which are probably products of oxidation and therefore they were not considered.

For Picual oils, the trend of γ-tocopherol to decrease was slower than α-tocopherol (data not shown). Some studies have shown that the antioxidant activity of tocopherols depends on the temperature and that at higher temperatures the order is inverted $\gamma > \beta > \alpha$ (*55–57*).

As shown in **Figure 3b**, the degradation of total phenol content was different for each oil being more pronounced in Picual than in Arbequina. Many authors considered phenolic compounds as the most effective antioxidants in virgin olive oil. Aparicio et al. (*39*) and Gutiérrez et al. (*58*) have estimated that the contribution of these compounds to the oxidative stability is approximately about 50%. These antioxidants interrupt the initiation and propagation stages of the oxidative chain reaction since they react with lipid radicals to form more stable products (*59*). The measurement of the total phenolic content by colorimetric assay (using the Folin-Ciocalteu) is insufficient because it does not show the compounds really implicated in the antioxidant activity of the total phenolic fraction.

The main components of the phenolic fraction are hydroxytyrosol (3,4-DHPEA), tyrosol (p-HPEA), and their derivatives linked to the aldehydic and dialdehydic forms of elenolic acid (3,4-DHPEA-EA, 3,4- DHPEA-EDA, p-HPEA-EA, p-HPEA-EDA), which are described as secoiridoids (*35, 60, 61*). Moreover, significant amounts of lignans, pinoresinol and 1-acetoxypinoresinol, (*35, 60, 62*), and flavones, luteolin and apigenin (*63*) are present.

The initial concentration of hydroxytyrosol (3,4-DHPEA) and its derivatives 3,4-DHPEA-EDA and 3,4-DHPEA-EA was significantly higher in Picual oils than in Arbequina oils (**Table 1**). For both oils, the trends for 3,4-DHPEA and 3,4-DHPEA-EDA were similar during the heating operation (**Figures 4a** and **4b**), observing an increase at the first hours and then a lowering tendency until the end of the experiment.

The higher content of hydroxytyrosol at the first hours of heating is a result of an increase in hydrolytic activities on complex phenols, probably caused by the high temperatures. After that, a drop in the complex fraction results in a final reduction in hydroxytyrosol and even its almost complete disappearance in the case of Arbequina oils.

The increase of the 3,4-DHPEA-EDA after approximately 2 h of heating at 180 °C and the later decrease have also been found for Picual oils by Brenes et al. (*27*). These authors did not detect an increase for Arbequina at the second hour, contrary to our results. The increase of 3,4-DHPEA-EDA might be explained by a coelution of 3,4-DHPEA-EDA oxidized form as proposed by Brenes et al. (*27*) for p-HPEA-EDA, although further studies should be performed to confirm this point.

Approximately, 80% of 3,4-DHPEA-EA, an isomer of oleuropein aglycon, was completely depleted after the first 2 h of heating and then the rate of degradation remained relatively stable (**Figure 5**). The loss was more important in Picual oils than in Arbequina oils, probably because of its greater initial content, more than 17 times (**Table 1**). This important loss at the beginning of the heating suggests that this isomer of oleuropein aglycon is rapidly consumed by the oil as a response of the high temperature. Similar results were also obtained for the same cultivars and at the same conditions by Brenes et al. (*27*).

Similarly, tyrosol (p-HPEA) and tyrosol-like species, such as the dialdehydic form of elenolic acid linked to tyrosol (p-HPEA-EDA) and ligustroside aglycon (p-HPEA-EA) showed the same trend observed for 3,4-DHPEA, 3,4-DHPEA-EDA (increase in the initial stage of oxidation), and 3,4-DHPEA-EA (decrease in the initial stage of oxidation) (**Table 4**), but during subsequent heating their concentrations remained relatively constant with a lowering tendency for both oils, being more stable than the *o*-diphenolic aglycons. The increase of p-HPEA-EDA was also found by Brenes et al. (*27*) who attribute the peak formed to the coelution of an oxidized compound, probably p-HPEA-EDA-oxidized, with this component.

The low degradation rate of tyrosol and its aldehydic and dialdehydic was also observed under heating conditions by Brenes et al. (*27*), but also in conditions of storage at room temperature (*64*), accelerated storage conditions (*50*), or frying conditions (*65*).

These results are consistent with the much higher antioxidant capacity of hydroxytyrosol and its derivatives, as compared to that of the tyrosol family, in virgin olive oil as reported by other authors (*66–68*).

The lignans pinoresinol and 1-acetoxypinoresinol were described as the major components of the phenolic fraction of VOOs (*69*). These compounds possess in vitro antioxidant activity (*70*), and they may be able to inhibit lipid peroxidation in vivo if they are absorbed (*71*). Recently, much attention has been paid to these compounds because of their beneficial effects as phytoestrogen substances (*72*).

The behavior of these compounds was similar to those observed for the aldehydic derivative of hydroxytyrosol and tyrosol in the case of Picual oils. Both compounds decrease drastically at the beginning of the heating operation (after 2 h at 180 °C), and a lower reduction was observed in the subsequent hours of heating. Similar results were reported in frying conditions (*65*). However, Brenes et al. (*27*) observed few losses for these compounds in Picual oils, suggesting less stability against oxidation. On the other hand, for Arbequina oils the rate of degradation of pinoresinol was very low and at the end of heating (36 h) only about 56% was lost. On the contrary, 1-acetoxypinoresinol has shown an increase trend and its concentration only decreases at the end of heating by 14% (**Figures 6a** and **6b**).

The flavone luteolin decreased rapidly with the heating time being more pronounced in Arbequina oils. After 12 h of heating, luteolin was completely depleted (**Figure 7a**). However, apigenin showed a slow degradation, and the rate of disappearance was higher in Arbequina oils than in Picual oils (**Figure 7b**).

Therefore, apigenin showed higher stability than luteolin. This difference may be attributed to the structure of these compounds, since their antioxidant activity is correlated with the number of phenolic hydroxyls in the molecule (*73, 74*).

Like sterols, triterpenic alcohols (erythrodiol and uvaol) and acids (oleanolic and maslinic) were quite stable during the

thermal treatment. Ursolic acid, another triterpene present in olive oil but in less quantities, has shown a tendency to increase during heating, probably as the consequence of the formation of oxidation products (**Table 5**). No data are available about the antioxidant activity of triterpenic acids on lipid autoxidation and heating conditions.

From this study we can conclude that under heating conditions important losses of α-tocopherol and *o*-diphenols were observed, although this decrease was cultivar dependent. Hydroxytyrosol, 3,4-DHPEA-EDA, 3,4-DHPEA-EA and flavonoids were the most affected by the thermal treatment, whereas lignans, tyrosol, p-HPEA-EDA, and p-HPEA-EA were lesser affected. Furthermore, most of the nutritional components such as phytosterols, triterpenic alcohols and acids, and squalene remained at levels with nutritional activities. These findings are very interesting since these compounds present healthy benefits.

LITERATURE CITED

(1) Escrich, E.; Ramirez-Tortosa, M. C.; Sánchez-Rovira, P.; Colomer, R.; Solanas, M.; Gaforio, J. J. Olive oil in cancer prevention and progression. *Nutr. Rev.* **2006**, *64*, 40–52.

(2) Estruch, R.; Martínez-González, M. A.; Corella, D.; Salas-Salvadó, J.; Ruiz-Gutierrez, V.; Covas, M. I.; Fiol, M.; Gómez-Garcia, E.; López-Sabater, M. C.; Vinyoles, E.; Arós, F.; Conde, M.; Lahoz, C.; Lapetra, J.; Sáez, G.; Ros, E. Effects of a Mediterranean style diet on cardiovascular risk factors. A randomized trial. *Ann. Intern. Med.* **2006**, *145* (1), 1–11.

(3) Quiles, J. L.; Ochoa, J. J.; Ramirez-Tortosa, M. C.; Huertas, J. R.; Mataix, J. Age related mitochondrial DNA deletion in rat liver depends on dietary fat unsaturation. *J. Gerontol.: Biol. Sci.* **2006**, *61*, 107–114.

(4) Boskou, D. Olive oil. *World Rev. Nutr. Diet.* **2000**, *87*, 56–77.

(5) Schneider, C. Chemistry and biology of vitamin E. *Mol. Nutr. Food Res.* **2005**, *49*, 7–30.

(6) Ruiz Gutiérrez, V. The unsaponificable components of olive oil. Mesa B. *Evidencias científicas de los efectos beneficiosos de los componentes minoritarios del aceite de oliva*; Congreso Internacional sobre Aceite de oliva y Salud (CIAS): Jaén, Spain, 2004; pp 39–44.

(7) Gutierrez, R.; Gonzales-Quijano, R.; Janer del Valle, C.; Janer del Valle, M. L.; Gutierrez Rosales, F.; Vazquez Roncero, A. Relación entre los polifenoles y la calidad y estabilidad del aceite de olive virgen. *Grasas Aceites* **1977**, *28*, 101–106.

(8) Vazquez-Roncero, A. Les polyphenols de l'huile d'olive et leur influence sur les caractéristiques de l'huile. *Rev. Fr. Corps Gras* **1978**, *25*, 21–26.

(9) Hrncirik, K.; Fritsche, S. Comparability and reliability of different techniques for the determination of phenolic compounds in virgin olive oil. *Eur. J. Lipid Sci. Technol.* **2004**, *106*, 540–549.

(10) Decker, E. A.; Warner, K.; Richards, M. P.; Shahidi, F. Measuring antioxidant effectiveness in food. *J. Agric. Food Chem.* **2005**, *53*, 4303–4310.

(11) Huang, D.; Ou, B.; Prior, R. L. The chemistry behind antioxidant capacity assays. *J. Agric. Food Chem.* **2005**, *53*, 4290–4302.

(12) Prior, R.; Wu, X.; Schich, K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *J. Agric. Food Chem.* **2005**, *53*, 4290–4302.

(13) Roginsky, V.; Lissi, E. A. Review of methods to determine chain-breaking antioxidant activity in food. *Food Chem.* **2005**, *92*, 235–254.

(14) Moreno, J. A.; Lopez-Miranda, J.; Gomez, P.; Benkhalti, F.; El Boustani, E. S.; Perez-Jimenez, F. Effect of phenolic compounds of virgin olive oil on LDL oxidation resistance. *Med. Clin.* **2003**, *120*, 128–131.

(15) Carluccio, M. A.; Siculella, L.; Ancora, M. A. Olive oil and red wine antioxidant polyphenols inhibit endothelial activation: antiatherogenic properties of Mediterranean diet phytochemicals. *Arterioscler. Thromb. Vasc. Biol.* **2003**, *23*, 622–629.

(16) Petroni, A.; Blasevich, M.; Salami, M.; Papini, N.; Montedero, G. F.; Galli, C. Inhibition of platelet agregation and eicosanoid production by phenolic components of olive oil. *Throm. Res.* **1995**, *78*, 151–160.

(17) Fito, M.; Covas, M. I.; Lamuela-Raventos, R. M.; Vila, J.; De la Torre, C.; Marrugat, J. Olive oil and inhibition of low density lipoprotein oxidation. Role of phenolic compounds. *Med. Clin.* 2000*115*, 166–169.

(18) Visioli, F.; Galli, C.; Bornet, F. Olive oil phenolics are dose-dependently absorbed in humans. *FEBS Lett.* **2000**, *468*, 159–160.

(19) Mateos, R.; Dominguez, M. M.; Espartero, J. L.; Cert, A. Antioxidant effect of phenolic compounds, alpha-tocopherol, and other minor components in virgin olive oil. *J. Agric. Food Chem.* **2003**, *51*, 7170–7175.

(20) Kohno, Y.; Egania, Y.; Itoh, S. Kinetic study of quenching reaction of free radicals by squalene in *n*-butanol. *Biochim. Biophys. Acta* **1995**, *1256*, 52–56.

(21) Gaforio, J. J. Bioactive compounds from olive oil as potential useful therapeutic agents in cancer. Mesa A. *El aceite de oliva en la prevención y progresión del cáncer*. Congreso Internacional sobre aceite de oliva y salud (CIAS): Jaén, Spain, 2004; pp 13–16.

(22) Rao, C. V.; Newmark, H. L.; Reddy, B. S. Chemopreventive effect of squalene on colon cancer. *Carcinogenesis* **1998**, *19*, 287–290.

(23) Gutierrez, F.; Jimenez, B.; Ruiz, A.; Albi, M. A. Effect of olive ripeness on the oxidative stability of virgin olive oil extracted from the varieties Picual and Hojiblanca and on the different components involved. *J. Agric. Food Chem.* **1999**, *47*, 121–127.

(24) Kiritsakis, A.; Markakis, P. Olive oil: A review. *Adv. Food Res.* **1987**, *31*, 453–482.

(25) Berger, A.; Jones, P. J. H.; Abumweis, S. S. Plant sterols: factors affecting their efficacy and safety as functional food ingredients. *Lipids Health Dis.* **2004**, 3–5.

(26) Pérez-Camino, M. C.; Cert, A. Quantitative determination of hydroxy pentacyclic triterpene acids in vegetable oils. *J. Agric. Food Chem.* **1999**, *47*, 1558–1562.

(27) Brenes, M.; García, A. M.; Dobarganes, M. C.; Velasco, J.; Romero, C. Influence of thermal treatment simulating cooking processes on the polyphenol content in virgin olive oil. *J. Agric. Food Chem.* **2002**, *50*, 5962–5967.

(28) Gómez-Alonso, S.; Mancebo-Campos, V.; Salvador, Ma D.; Fregapane, G. Oxidation kinetics in olive oil triacylglycerols under accelerated shelf-life testing (25–75°C). *Eur. J. Lipid Sci. Technol.* **2004**, *106*, 369–375.

(29) Márquez-Ruiz, G.; Dobarganes, C. Short-chain fatty acid formation during thermooxidation and frying. *J. Sci. Food Agric.* **1996**, *70*, 120–126.

(30) European Community regulation EEC/ 2568/91 of 11 July 1991 on the characteristics of olive and pomace oils and on their analytical methods. *Off. J. Eur. Communities*. **1991**, *L248*, 1–82.

(31) Lanzón, A.; Guinda, A.; Albi, T.; De la Osa, C. A rapid method for squalene determination in vegetable oils. *Grasas Aceites* **1995**, *46*, 276–278.

(32) IUPAC (International Union of Pure and Applied Chemistry). Determination of tocopherols and tocotrienols in vegetables fats by HPLC. Method No. 2.432. *Standard methods of analyses of oils, fats and derivatives*. Blackwell Scientific Publications: Oxford, U.K., 1992.

(33) Vázquez-Roncero, A.; Janer del Valle, C.; Janer del Valle, M. L. Determinación de polifenoles totales del aceite de oliva. *Grasas Aceites* **1973**, *24*, 350–357.

(34) Beltrán, G.; Jimenez, A.; Aguilera, M. P. Uceda, M. Análisis mediante HPLC de la fracción fenólica del aceite de olive virgen de la variedad 'Arbequina'. Relación con la medida del amargor K_{225} y la estabilidad. *Grasas Aceites* **2000**, *51*, 320–324.

(35) Mateos, R.; Espartero, J. L.; Trujillo, I.; 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.

(36) Gutiérrez, F. Determinación de la estabilidad oxidativa de aceites de oliva vírgenes: comparación entre el Método del Oxigeno Activo (A.O.M.) y el método Rancimat. *Grasas Aceites* **1989**, *40* (1), 1–5.
(37) Uceda, M.; Hermoso, M.; Aguilera, Ma P. La calidad del aceite de oliva. In: *El cultivo del olivo*, 4th ed.; Barranco, D., Fernández-Escobar, R., Rallo, L. Eds.; Mundi-Prensa: Madrid, Spain, 2004 pp 589–614.
(38) Velasco, J.; Dobarganes, C. Oxidative stability of virgin olive oil. *Eur. Lipid Sci. Technol.* **2002**, *104*, 661–676.
(39) Aparicio, R.; Roda, L.; Albi, M. A.; Gutierrez, F. Effect of various compounds on virgin olive oil stability measured by Rancimat. *J. Agric. Food Chem.* **1999**, *47*, 4150–4155.
(40) Ancín, M. C.; Martinez, M. T. Estudio de la degradación de los aceites de olive sometidos a fritura. I. Determinación del parámetro que mejor cuantifica esta degradación. *Grasas Aceites* **1991**, *1*, 22–31.
(41) Albi, T.; Lanzón, A.; Guinda, A.; Léon, M.; Pérez-Camino, M. C. Microwave and conventional heating effects on thermooxidative degradation of edible fats. *J. Agric. Food Chem.* **1997**, *45*, 3795–3798.
(42) Du Plesis, L. M.; Van Twisk, P.; Nierkerk, P. J.; Steyn, M. Evaluation of peanut and cottonseed oils for deep-frying. *J. A.O.C.S.* **1981**, *58*, 575–578.
(43) Lopez-varela, S.; Sanchez-Muniz, F. J.; Garrido-Polonio, C.; Arroyo, R.; Cuesta, C. Relationship between chemical and physical indexes and column and HPSE chromatography methods for evaluating frying oil. *Zeitschrift Fuer Ernaehrungswissenschaf.* **1997**, *34*, 308–313.
(44) Melton, S. L.; Jafar, S.; Sykes, D.; Trigiano, M. K. Review of stability measurement for frying oils and fried food flavour. *J. Am. Oil Chem. Soc.* **1994**, *71*, 1301–1308.
(45) Benzie, I. Lipid peroxidation: a review of causes, consequences, measurement and dietary influences. *Int. J. Food Sci. Nutr.* **1996**, *46*, 233.
(46) Fedeli, E. The behaviour of olive oil during cooking and frying. In *Frying of foods*; Varela, G., Bender, A. E., Morton, I. D., Eds.; Ellis Harwood Ltd.: Chichester, England, 1988; pp 52–81.
(47) Romero, A.; Cuesta, C.; Sanchez-Muniz, F. J. Effect of replenishment during deep-fat frying of frozen foods in a sunflower oil and high-oleic acid sunflower oil. *J. Am. Oil Chem. Soc.* **1998**, *75*, 161–167.
(48) Márquez-Ruiz, G.; Dobarganes, C. Short fatty acid formation during thermoxidation and frying. *J. Sci. Food Agric.* **1996**, *70*, 120–126.
(49) Psomiadou, E.; Tsimidou, M. On the role of squalene in olive oil stability. *J. Agric. Food Chem.* **1999**, *47*, 4025–4032.
(50) Hrncirik, K.; Fritsche, S. Relation between the endogenous antioxidant system and the quality of extra virgin olive oil under accelerated storage conditions. *J. Agric. Food Chem.* **2005**, *53*, 2103–2110.
(51) Malecka, M. The effect of squalene on the thermostability of rapeseed oil. *Nahrung* **1994**, *38*, 135–140.
(52) Rastrelli, L.; Passi., S.; Ippolito, F.; Vacca, G.; De Simona, F. Rate of degradation of α-tocopherol, squalene, phenolic, and polyunsaturated fatty acids in olive oil during different storage conditions. *J. Agric. Food Chem.* **2002**, *50*, 5566–5570.
(53) Boskou, D. Frying temperature and minor constituents of oils and fats. *Grasas Aceites* **1998**, *49*, 326–330.
(54) Pellegrini, N.; Visioli, F.; Buratti, S.; Brighenti, F. Direct analysis of total antioxidant activity of olive oil and studies on the influence of heating. *J. Agric. Food Chem.* **2001**, *49*, 2532–2538.
(55) Kochhar, S. P. Stabilization of frying oils with natural antioxidative components. *Eur. J. Lipid Sci. Technol.* **2000**, *102*, 552–559.
(56) Poncrazc, G.; Weiser, H.; Matzinger, D. Tocopherols-antioxidants in nature. *Technol. Fat Sci. Technol.* **1995**, *97*, 90–104.
(57) Gordon, M. H.; Kourimska, L. The effects of antioxidants on changes in oils during heating and deep frying. *J. Sci. Food Agric.* **1995**, *68*, 347–353.
(58) Gutiérrez, F.; Arnaud, T.; Garrido, A. Contribution of polyphenols to the oxidative stability of virgin olive oil. *J. Sci. Food Agric.* **2001**, *81*, 1463–1470.
(59) Frankel, E. N. Methods to determine extent of oxidation. In *Lipid Oxidation*; Frankel, E. N., Ed.; The Oily Press: Glasgow, U.K., 1998; pp 79–98.
(60) Gómez-Alonso, S.; Salvador, M. D.; Fregapane, G. Phenolic compounds profile of Cornicabra virgin olive oil. *J. Agric. Food Chem.* **2002**, *50*, 6812–6817.
(61) Montedoro, G. F.; Servili, M.; Baldioli, M.; Selvaggini, R.; Miniati, E.; Macccchioni, A. Simple and hydrolysable compounds in virgin olive oil. 3. Spectroscopic characterizations of the secoiridoid derivatives. *J. Agric. Food Chem.* **1993**, *41*, 2228–2234.
(62) Brenes, M.; Hidalgo, F. J.; Garcia, 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.
(63) Vázquez-Roncero, A.; Janer, C.; Janer, M. L. Phenolic components in olive fruits. III. Polyphenols in olive oil. *Grasas Aceites.* **1976**, *27*, 185–191.
(64) Brenes, A.; García, P.; Garrido, A. Acid hydrolysis of secoiridoid aglycons during storage of virgin olive oil. *J. Agric. Food Chem.* **2001**, *49*, 5609–5614.
(65) Gómez-Alonso, S.; Fregapane, G.; Salvador, M. D.; Gordon, M. H. Changes in phenolic composition and antioxidant activity of virgin olive oil during frying. *J. Agric. Food Chem.* **2003**, *51*, 667–672.
(66) Baldioli, M.; Servili, M.; Perreti, G.; Montedero, G. F. Antioxidant activity of tocopherols and phenolic compounds of virgin olive oil. *J. Am. Oil Chem. Soc.* **1996**, *73*, 1589–1593.
(67) Papadopoulos, G.; Boskou, D. Antioxidant effect of natural phenols on olive oil. *J. Am. Oil Chem. Soc.* **1991**, *68*, 669–671.
(68) Romani, A.; Lapucci, C.; Cantini, C.; Ieri, F.; Mulinacci, N.; Visioli, F. Evolution of minor polar compounds and antioxidant capacity during storage of bottled extra virgin olive oil. *J. Agric. Food Chem.* **2007**, *55*, 1315–1320.
(69) Owen, R. W.; Mier, W.; Giascosa, 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.
(70) Owen, R. W.; Mier, W.; Giascosa, 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.
(71) Kang, M. H.; Mait, M.; Tsujihara, N.; Osawa, T. Sesamolin inhibits lipid peroxidation in rat liver and kidney. *J. Nutr.* **1998**, *128*, 1018–1022.
(72) Schouw, Y. T.; Kleijn, M. J. J.; Peeters, P. H. M.; Grobbee, D. E. Phyto-oestrogens and cardiovascular disease risk. *Nutr., Metab. Cardiovasc. Dis.* **2000**, *10*, 154–167.
(73) Cao, G.; Sofic, E.; Prior, R. L. Antioxidant and prooxidant behaviour of flavonoids: structure activity relationships. *Free Radical Biol. Med.* **1997**, *22*, 749–760.
(74) Rice-Evans, C. A.; Miller, N. J.; Paganga, G. Structure antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biol. Med.* **1996**, *20*, 933–956.

Received for review March 5, 2007. Revised manuscript received August 2, 2007. Accepted September 6, 2007.

JF070628U