Evaluation of phenolic compounds degradation in virgin olive oil during storage and heating

DESPINA DASKALAKI – GRIGORIA KEFI – KALI KOTSIOU – MARIA TASIOULA-MARGARI

Summary

Virgin olive oil is oil with a high biological value, due to its polyphenol content. Virgin olive oils were subjected to heat treatment simulating common domestic processing, including boiling, frying and storage. These processes can affect the phenolic compounds content of oils to a certain degree, depending on each one treatment. Thermal oxidation of oils at 180 °C (frying) caused a significant decrease ($p < 0.005$) in hydroxytyrosol derivatives (60% reduction after 30 min and 90% reduction after 60 min) and, to a lower degree, in tyrosol derivatives. No changes were observed in the content of lignans ($p > 0.005$). On the other hand, thermal oxidation of oils at 100 °C (boiling) for 2 h caused a decrease by less than 20% in all classes of phenolic compounds. The reduction of phenolic compounds during storage under environmental conditions was correlated with the peroxide value. When the peroxide value did not exceed the level of 20 meq.kg^{-1}, as was recorded for low linoleic acid oils and low oxygen availability at the bottles' headspace, a degree of reduction of approximately 30% in hydroxytyrosol derivatives and 10% in tyrosol derivatives was observed, while lignans remained unchanged. Finally, during both heating and storage under environmental conditions, the evolution of oxidized phenolic compounds was observed. The structures of these oxidized products were confirmed by HPLC-MS.

Keywords

virgin olive oil; phenolic compounds degradation; heating; storage; stability; oxidized phenols, HPLC-MS.

Virgin olive oil is one of the edible oils most consumed in the Mediterranean area. It can be used without any refining, when it retains its natural flavour and aroma [1]. Virgin olive oil is of a high biological value due to monounsaturated oleic acid and to its antioxidant contents. The main antioxidants of virgin olive oil are the phenolic compounds and α-tocopherol. These compounds are important for the sensory quality [1] and shelf life of oil [2]. Phenolic compounds play also a major role in preventing certain chronic human diseases. Specific health-promoting effects of olive oil phenolics include inhibition of the oxidation of low-density lipoproteins (LDLs) thought to be involved in the onset of atherosclerosis [3]. These substances have also anticarcinogenic activities [4].

The most important phenolic compounds in olive oil are: the aldehydic form of oleuropein aglycon (AFOA), dialdehydic form of decarboxylated oleuropein aglycon (DAFOA), aldehydic form of ligstroside aglycon (AFLA), dialdehydic form of decarboxylated ligstroside aglycon (DAFLA), simple phenols (hydroxytyrosol, tyrosol, vanillic acid, *p*-coumaric acid, ferullic acid), flavonoids (luteolin, apigenin) and lignans (pinoresinol, 1-acetoxypinoresinol), [5, 6]. The concentration of phenolic compounds in olive oil depends on the olive tree variety, the harvest and extraction processes [7, 8].

Virgin olive oil is used as a salad oil, often even after storage for a period of one year under environmental conditions. Several factors can influence the oxidative processes that extend or shorten the self-life of olive oil. The characteristic resistance to oxidation of virgin olive oil is related to its unique fatty acid composition in addition to minor components such as phenolic compounds and tocopherols, which may degrade to a certain degree [9–12].

Virgin olive oil is also consumed after domestic heating, such as frying, boiling and oven heating. These processes reduce the amount of phenolic compounds in the oil to a different degree, depending on different composition of virgin olive oil and different steps of treatment [13–16].

Despina Daskalaki, Grigoria Kefi, Kali Kotsiou, Maria Tasioula-Margari, Department of Chemistry, Section of Industrial and Food Chemistry, University of Ioannina, Ioannina 45110, Greece.

Correspondence author:
Maria Tasioula-Margari, tel.: 0030 2651098345, fax: 0030 2651098795, e-mail: mtasioul@cc.uoi.gr

However, hydroxytyrosol derivatives are less stable whereas tyrosol derivatives are more stable phenolic compounds [13]. The reduction of polyphenols is responsible for the reduction of the nutritional value and quality of the oil. Moreover, the evolution of oxidation products of phenolic compounds in stored, thermooxidized [12, 17, 18] and olive oil used for frying have been reported [15, 16, 19].

In a previous work, the loss of phenolic compounds during storage [9] and heating [13] of Lianolia cultivar was studied. The aim of the present study is to extend this study to other heating treatments and to the Koroneiki cultivar, because it is a wide-spread and the most important variety cultivated in Greece. The degradation of different classes of phenolic compounds from virgin olive oil after heating, at boiling and frying temperatures, as well as during storage under environmental conditions was studied.

MATERIALS AND METHODS

Olive oil

Samples of virgin olive oil from Lianolia variety from the region of Preveza, and from Koroneiki variety from the region of Chania (Crete) and Kalamata (Peloponnesus), Greece, were used. These cultivars were chosen as being representative of Greek olive oil production and because they have different fatty acid composition. The ratio of oleic : linoleic acids is higher in Koroneiki variety (12.66) than in Lianolia variety (6.59). The olives were collected during the period 2002/03 to 2004/05 and they were processed using a three-phase centrifugation system. Various local industrial olive oil mills supplied the samples. The samples were filtered and stored under N_2 below –20 °C prior to analysis.

Reagents and standards

Acetonitrile, methanol, isopropanol (2-propanol) and water were all of HPLC grade and were purchased from Merck (Darmstadt, Germany). Methanol, acetonitrile, and hexane for phenols extraction were pro-analysis grade and purchased from Merck. The standards used for identification and quantification of phenolic compounds were: tyrosol (for expression of tyrosol derivatives) purchased from Sigma-Aldrich (Steinheim, Germany), oleuropein-7-glycoside (for expression of hydroxytyrosol derivatives) from Extrasynthese (Genay, France), vanillic acid, *p*-coumaric acid, ferullic acid, luteolin and apigenin purchased from Merck-Schuchardt (Hohenbrunn, Germany) and pinoresinol (for expression of 1-acetoxipinoresinol) was purchased from Separation Research (Turku, Finland).

Oil Treatment

Storage

Virgin olive oil samples were filtered and stored in glass bottles (25 ml) with 1% and 3% headspace. The bottles were sealed and stored in the dark for 15 months, to simulate home storage conditions (20 °C ± 4 °C).

Boiling

Samples of 50 g of oil were poured into glass beakers (250 ml) and were heated at 100 °C**.** A water bath was used to heat oil samples at 100 °C for different periods of time (1–4 h). The experiment was carried out in triplicate. Samples were heated in an oven at 100 °C for 24 h and 72 h, in order to increase the oxidized products concentration for identification purposes by HPLC-MS.

Frying

Samples of 50 g of oil were poured into glass beakers (250 ml) and were heated on a heating plate at 180 °C for different periods of time (10–60 min). A period of 10 min was needed to reach the temperature of 180 °C. The experiment was carried out in triplicate.

Determination of quality indices

The peroxide index, spectroscopic indices, free acidity and fatty acid composition analyses were carried out according to the methods described by European Community Regulation, standard methods [20].

Liquid-liquid extraction of phenolic compounds

An amount of 5.0 g of virgin olive oil was extracted using 3 × 5 ml of methanol. The combined extracts were brought to dryness and the residue was taken up in 5 ml acetonitrile. Washing with hexane (3 × 5 ml) was performed and the resulting acetonitrile solution was evaporated, giving a residue that was dissolved in 3 ml of a mixture of methanol and water (1:1, v/v).

GC apparatus

GC analysis was performed in a Fisons GC-9000 Series system (Milano, Italy) with a FID detector and a capillary column Carbowax (30 m × 0.32 mm × 0.32 μm; Altech Associates, Deerfield, Illinois, USA).

HPLC apparatus

HPLC analysis was performed in a JASCO liquid chromatography system (Tokyo, Japan)

equipped with a PU-980 pump, a UV 970 (UV/vis) detector and a Rheodyne injection valve (20 μl loop). The columns used were Lichrospher 100RP-18 column (4.0 mm id × 250 mm, particle size = 5 μm; Merck) and Luna RP-C18 (4.6 mm id × 250 mm, particle size = 5 μm; Phenomenex, Macclesfield, United Kingdom).

LC-MS

LC-DAD-MS was performed in an Agilent 1100 Series LC/MSD Trap, Model SL (Waldbronn, Germany) equipped with a thermostated column compartment 1100, diode array detector 1100 and a standard autosampler 1100. The mobile phase and the solvent gradient were the same as used in HPLC (see below). All of the analyses used the ion-spray source in the negative mode with the following settings: nebulizer gas (N_2) 40.0 psi, drying gas 12 l·min^{-1} and drying gas temperature 350 °C. Target mass was 350 m/z. Full scan data were acquired by scanning from m/z 50 to 800.

HPLC conditions

Detection was performed at 280 nm and 340 nm. The elution solvents used were A (2% acetic acid in water) and B (mixture of methanol and acetonitrile, 1:1, v/v). The samples were eluted by the following gradient: 95% A and 5% B as initial conditions, 70% A and 30% B for 25 min, 65% A and 35% B for 25 min, 30% A and 70% B for 15 min, 0% A and 100% B for 5 min and, finally, 95% A and 5% B for 5 min. The flow rate was 1 ml.min^{-1} and run time 75 min. The analyses were run at room temperature. The sample injection volume was 20 μl.

Phenolic compounds were quantified as follows: Simple phenols were quantified using available commercial standards. Tyrosol was used for tyrosol derivatives quantification, multiplied by their molecular weight ratio (304:138 and 362:138 for DAFLA and AFLA respectively). Oleuropein glycoside was used for hydroxytyrosol derivatives quantification, multiplied by their molecular weight ratio (154:540, 320:540 and 378:540 for hydroxytyrosol, DAFOA and AFOA, respectively). Pinoresinol was used for 1-acetoxypinoresinol quantification, multiplied by their molecular weight ratio 358:416.

Statistical analysis

The chemical data were analysed using Statistica 6.0 statistical software (Statsoft, Tulsa, Oklahoma, USA). The significance of the differences of the means at a 5% level was determined using t-test.

RESULTS AND DISCUSSION

Tab. 1 shows the quality characteristics (average values and ranges) of the analysed samples. The results for acid value, extinction coefficients, K_{232}, K_{270} and peroxide values are within the limits stated in European Community Regulations [20],

Tab. 1. Quality characteristics of olive oil samples from Lianolia and Koroneiki variety.

	Lianolia (n = 20)		Koroneiki (n = 20)	
	Average	Range	Average	Range
Acidity [%] (expressed as % of oleic acid)	0.40	0.12–0.57	0.34	0.14–0.79
Peroxide Value [meq.kg^{-1}]	6.67	3.42–10.91	11.47	9.24–13.00
K_{232}	1.99	1.42–2.28	1.67	1.31–1.94
K_{270}	0.14	0.09–0.18	0.13	0.09–0.17
Fatty acid composition [%]				
C 16:0	13.52	12.35–15.27	12.53	11.36–13.38
C 16:1	1.44	0.90–1.78	1.02	0.70–1.89
C 18:0	2.06	1.75–2.66	2.70	2.38–3.08
C 18:1	70.41	67.33–74.43	76.22	75.12–78.48
C 18:2	10.69	7.81–12.55	6.02	4.98–7.13
C 18:3	0.90	0.71–1.05	0.73	0.27–1.12
Phenolic Compounds[a] [mg·kg^{-1}]				
Hydroxytyrosol derivatives[b]	124.53	18.67–414.45	109.7	27.49–410.85
Tyrosol derivatives[c]	118.57	51.47–450.54	114.69	67.33–304.23
Lignans[d]	7.92	4.16–10.09	15.73	9.8–33.74
Flavonoids[e]	2.87	1.14–3.99	2.00	0.16–4.58
Simple phenols[f]	0.42	0.00–1.00	0.16	0.00–0.38

a – concentrations were expressed as mg standard compound in kg of olive oil, *b* – hydroxytyrosol, DAFOA, AFOA, *c* – tyrosol, DAFLA, AFLA, *d* – 1-acetoxypinoresinol, *e* – apigenin, luteolin, *f* – *p*-coumaric acid, ferullic acid, vanillic acid.

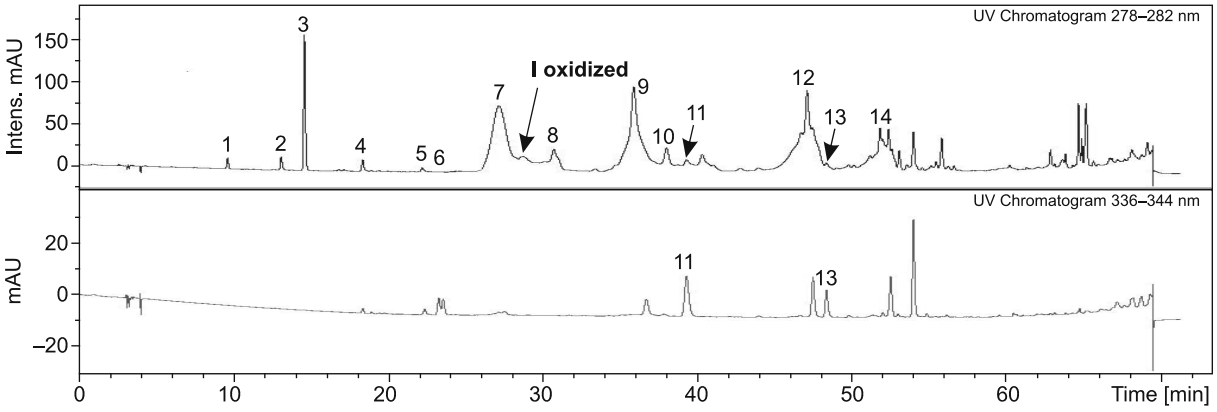

Fig. 1. Separation of the phenolic fraction from Koroneiki variety olive oil by reversed-phase HPLC.

Peaks: 1 – hydroxytyrosol, 2 – tyrosol, 3 – internal standard (*p*-hydroxyphenyl-acetic acid), 4 – vanillic acid, 5 – *p*-coumaric acid, 6 – ferullic acid, 7 – DAFOA, 8 – isomeric form of DAFOA, 9 – DAFLA, 10 – 1-acetoxypinoresinol, 11 – luteolin, 12 – AFOA, 13 – apigenin, 14 – AFLA. I – oxidized product I.

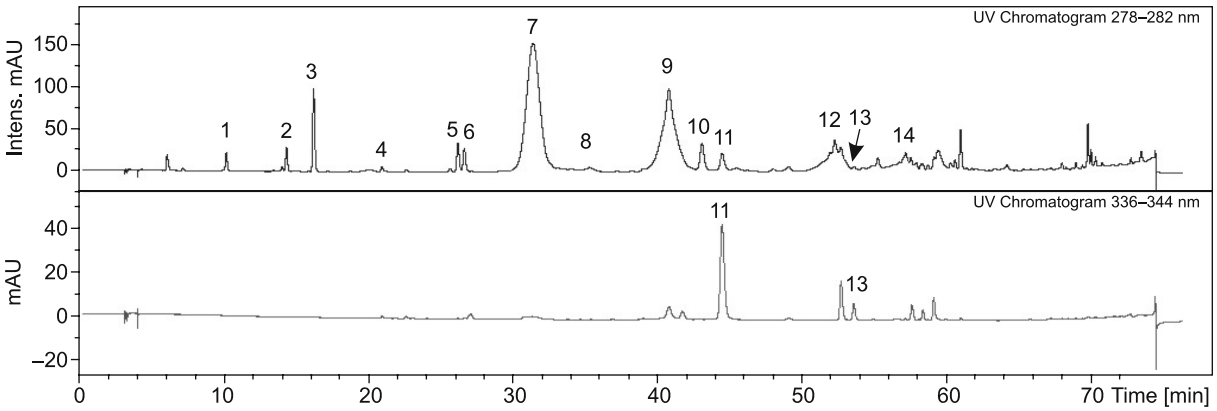

Fig. 2. Separation of the phenolic fraction of Lianolia variety olive oil by reversed-phase HPLC.

Peaks: 1 – hydroxytyrosol, 2 – tyrosol, 3 – internal standard (*p*-hydroxyphenyl-acetic acid), 4 – vanillic acid, 5 – *p*-coumaric acid, 6 – ferullic acid, 7 – DAFOA, 8 – isomeric form of DAFOA, 9 – DAFLA, 10 – 1-acetoxypinoresinol, 11 – luteolin, 12 – AFOA, 13 – apigenin, 14 – AFLA.

Tab. 2. Quality characteristics for olive oil samples before and after heating.

	Time [min]	Free acidity* [%]	Peroxide Value [meq.kg^{-1}]	K_{232}	K_{270}
Sample 1: Lianolia variety olive oil					
Initial	0	0.33 ± 0.01	9.73 ± 0.2	1.81 ± 0.03	0.18 ± 0.02
Frying 180 °C	10	0.33 ± 0.01	12.07 ± 0.3	2.13 ± 0.03	0.50 ± 0.02
	30	0.33 ± 0.01	11.91 ± 0.3	3.34 ± 0.05	1.03 ± 0.03
	60	0.60 ± 0.01	15.25 ± 0.3	6.35 ± 0.08	1.38 ± 0.04
Boiling 100 °C	120	0.35 ± 0.01	10.01 ± 0.3	2.40 ± 0.03	0.28 ± 0.02
	240	0.35 ± 0.01	11.32 ± 0.3	4.01 ± 0.03	0.40 ± 0.02
Sample 2: Koroneiki variety olive oil					
Initial	0	0.65 ± 0.01	7.89 ± 0.2	1.85 ± 0.03	0.12 ± 0.02
Frying 180 °C	10	0.65 ± 0.01	9.25 ± 0.3	2.15 ± 0.03	0.42 ± 0.02
	30	0.70 ± 0.01	12.36 ± 0.3	4.65 ± 0.06	0.81 ± 0.02
	60	0.70 ± 0.01	16.21 ± 0.3	6.39 ± 0.08	1.04 ± 0.03
Boiling 100 °C	120	0.70 ± 0.01	9.01 ± 0.2	2.22 ± 0.03	0.20 ± 0.02
	240	0.70 ± 0.01	10.32 ± 0.3	3.76 ± 0.03	0.34 ± 0.02

* – expressed as % of oleic acid.

implying that all the oils sampled were of extra virgin quality for both varieties. Koroneiki, which is a wide-spread variety in Greece, has a high oleic acid : linoleic acid ratio (12.6), whereas Lianolia, which is a local variety cultivated in Preveza (Epirus) and Corfu, has a low oleic acid : linoleic acid ratio (6.6).

Fig. 1 and Fig. 2 show representative HPLC chromatograms of the phenolic compounds from Koroneiki and Lianolia variety. The phenolic compounds that were quantified and identified in all samples were: tyrosol, hydroxytyrosol, vanillic acid, *p*-coumaric acid, ferullic acid, DAFOA, AFOA, DAFLA, AFLA, 1-acetoxypinoresinol, luteolin and apigenin [6, 21]. The phenolic compounds were classified as simple (vanillic acid, *p*-coumaric acid, ferullic acid), hydroxytyrosol derivatives (hydroxytyrosol, DAFOA, AFOA), tyrosol derivatives (tyrosol, AFOA, AFLA), lignans (1-acetoxypinoresinol) and flavonoids (luteolin, apigenin; Tab. 1). Similar phenolic compounds profile has been reported for the main Spanish varieties such as Picual, Picudo, Cornicabra, Arbequina, Empeltre and Hojiblanca [22]. Although the concentrations of the above phenols ranged widely between different samples, the main phenolic compounds in both varieties were tyrosol and hydroxytyrosol derivatives, similar to the results reported previously [21]. High contents of pinoresinol have been reported for Spanish and Italian cultivars [23], whereas negligible amounts were found to be present in Greek varieties studied in the present work (pinoresinol is not shown in Fig. 1 and Fig. 2, it elutes between DAFLA and 1-acetoxypinoresinol). The Koroneiki variety contained a higher amount of lignans, consisting mainly from 1-acetoxypinoresinol, compared to Lianolia, whereas both varieties contained low amounts of simple phenols (vanillic, *p*-coumaric and ferullic acid) and flavonoids (apigenin and luteolin).

Tab. 2 shows the quality characteristics during thermal treatment simulating frying conditions (180 °C) and boiling (100 °C). In general, no changes were observed in acidity values. The highest peroxide values were recorded for samples heated at 180 °C. However, values recorded at both temperatures were below 20 meq.kg^{-1}. On the contrary, the spectroscopic index K_{232} was higher than the upper limit of 2.60, at both frying and boiling temperatures, due to hydroperoxides decomposition, while conjugating dienes, measured by K_{232}, steady accumulated. Changes in the content of carbonyls, as measured by the spectroscopic index K_{270}, recorded higher values at frying than at boiling due to greater hydroperoxides degradation at the higher temperature.

Phenolic compounds changes during frying at 180 °C

The degradation profiles of phenolic compounds during frying are depicted in Fig. 3 and Fig. 4 along with the standard deviations which were lower than 10%. The phenolic content analysis of virgin olive oils from Lianolia and Koroneiki varieties, heated at 180 °C, revealed a decrease in phenolic compounds with the frying time, but this reduction was different for individual compounds. The content of hydroxytyrosol derivatives statistically significantly ($p < 0.005$) decreased, after 30 min of frying, 60% of hydroxytyrosol derivatives were lost. In contrast, contents of tyrosol derivatives and lignans seemed stable, after the same period of frying and no statistically significant ($p > 0.005$) differences were observed. After 60 min of frying, statistically significant differences were observed for both of them ($p < 0.005$). Less

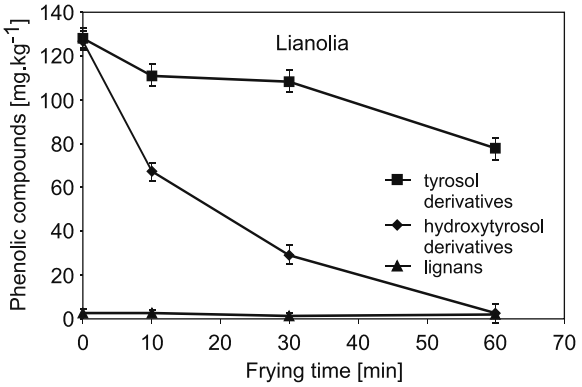

Fig. 3. Changes in phenolic compounds of virgin olive oil from Lianolia variety during frying at 180 °C.

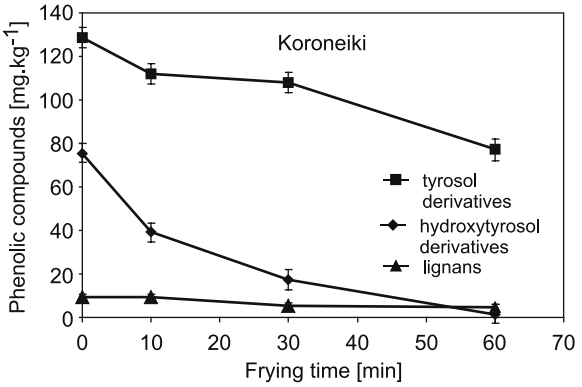

Fig. 4. Changes in phenolic compounds of virgin olive oil from Koroneiki variety during frying at 180 °C.

than 10% of hydroxytyrosol derivatives and 60% of tyrosol derivatives remained, whereas lignans remained unchanged in the oil (no statistically significant differences were observed at $p > 0.005$). The rate of hydroxytyrosol derivatives degradation was higher (higher slope) in Lianolia variety with a greater initial phenol content, than in Koroneiki. Similar trends for the reduction of the main group of phenolic compounds (hydroxytyrosol derivatives consumed after 2 h, tyrosol derivatives and lignans more stable) were reported for an Italian extra virgin olive oil (Bertolli oil) subjected to thermal treatment in an oven at 180 °C [16]. However, findings reported in the literature for Arbequina and Picual oil, which are the most popular varieties in Spain, resulted in a minor loss of polyphenols compared to our results [15]. In this case, total loss of orthodiphenols was achieved in a longer period of time (5–15 h). Although Picual oil has a higher oleic : linoleic acid ratio, (oleic : linoleic acid ratio is 24) than Koroneiki oil (oleic : linoleic acid ratio is 12), it must be noted that in this work a different quantity of olive oil (different surface : volume ratio) was subjected to treatment in a Rancimat apparatus at 180 °C. Moreover, in the above-mentioned work, the content of hydroxytyrosol and tyrosol derivatives seemed to get increased significantly in oils during the first 5 h of heating. Apparently, the oxidized products formed during heating coeluted and were determined with DAFOA and DAFLA, respectively. The evolution of oxidized phenolic compounds was observed in our work for both heating and storage conditions and is depicted in Fig. 5 and Fig. 6.

Phenolic compounds changes during heating at 100 °C

During heating at 100 °C, simulating boiling conditions, all the phenolic compounds appeared to be stable. The results are shown in Fig. 7 and Fig. 8 along with the mean standard deviations, which were lower than 10%. Even after 4 h of heating, only hydroxytyrosol derivatives decreased statistically significantly ($p < 0.005$) by 20%, while all the other phenols remained at their initial levels in both varieties (no statistically significant differences were observed at $p > 0.005$). Similar results were obtained in our previous work when the oil was heated in an oven for a period over 9 h [13]. It is reported in the literature that when boiling a mixture of virgin olive oil and water in a pressure cooker for 30 min, hydrolysis of hydroxytyrosol and tyrosol derivatives at pH lower than 6 occurs without further degradation of the hydrolysed phenols

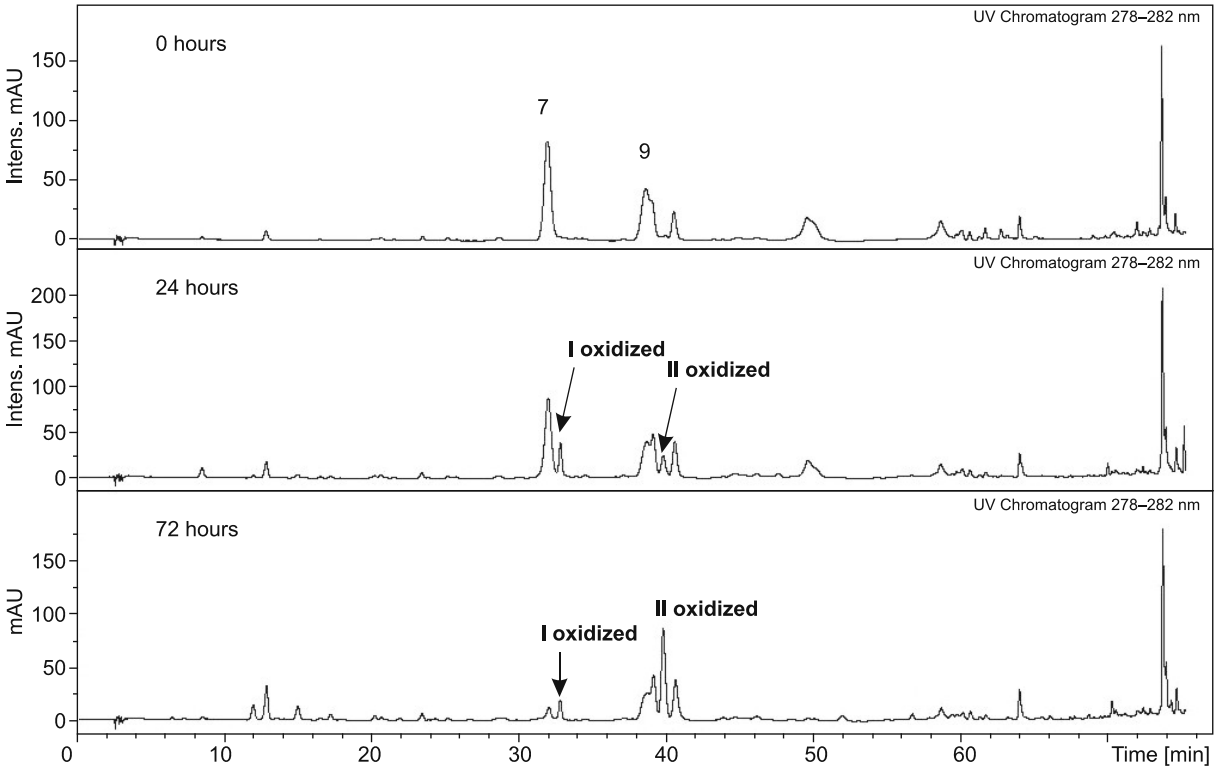

Fig. 5. Evolution of oxidized products during heating at 100 °C.

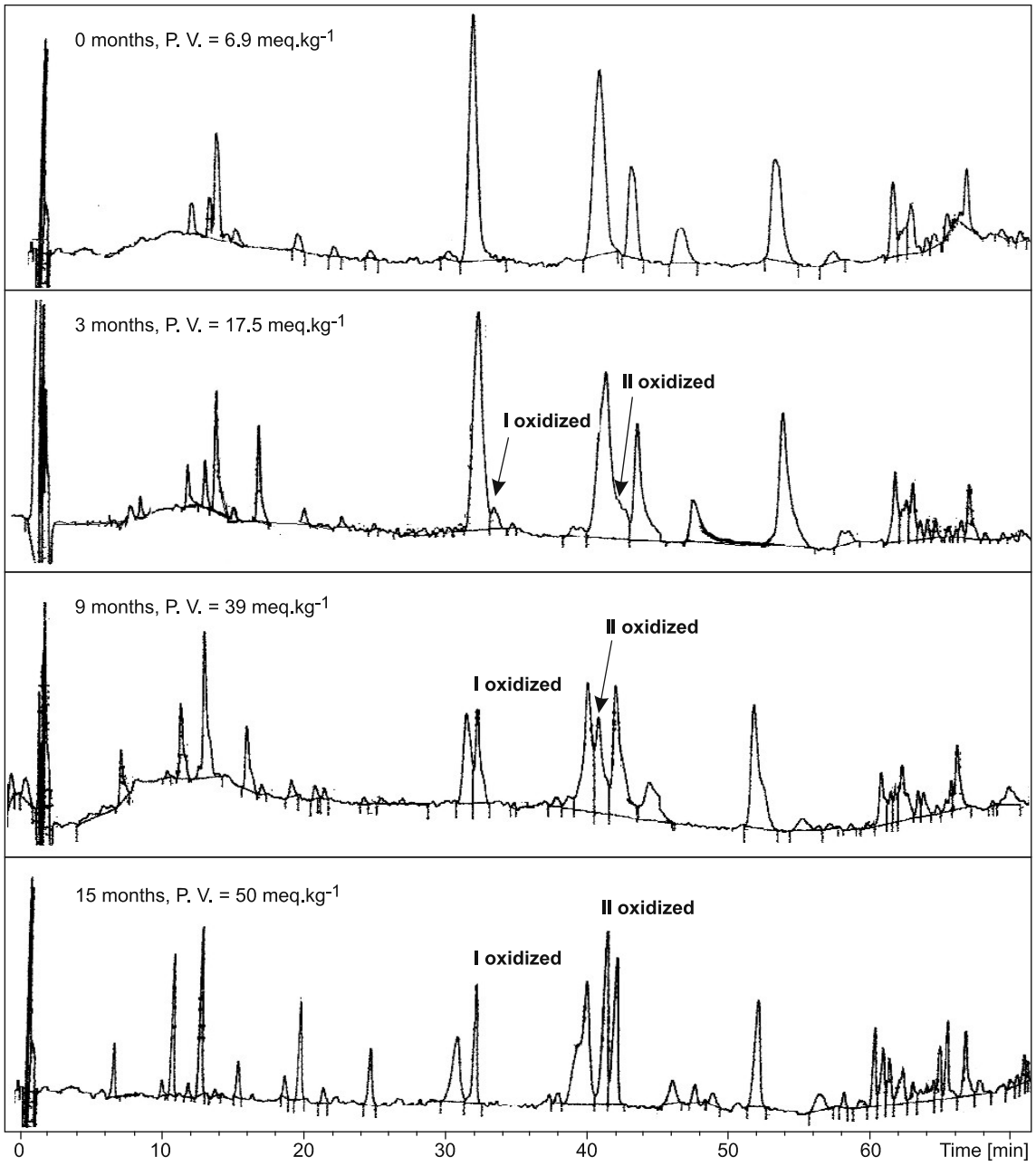

Fig. 6. Evolution of oxidized products during storage.

[15]. In conclusion, minor losses in phenolic compounds are observed during heating at boiling temperatures. This is of a great importance because in recent years, much attention has been paid to the beneficial effects of phenolic compounds [8, 24].

Phenolic compounds changes during storage

During storage for 15 months, the peroxide value did not exceed the upper limit of 20 meq.kg^{-1} (set by the European Community Regulation [20]) for the majority of samples, namely, 10 samples from Koroneiki (Group 1) and 8 samples from Lianolia variety (Group 2). Only two samples from Lianolia variety (Group 3) exceeded the upper limit of 20 meq.kg^{-1}. Lianolia is more susceptible to oxidative degradation than Koroneiki due to its higher linoleic acid content (9% versus 5%). Representative samples from each group with the maximum increase in peroxide values were selected, and their results are shown in Tab. 3. Changes occurring in phenolic compounds are dependent on the peroxide value. When the peroxide value did

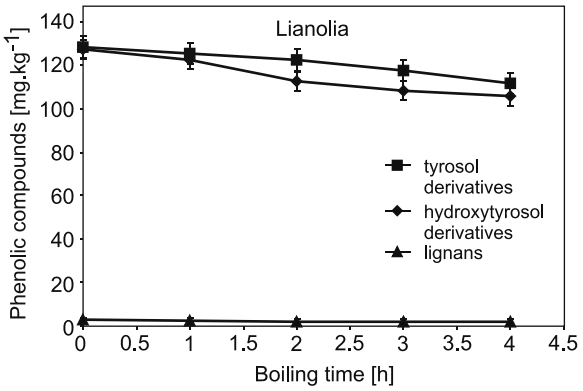

Fig. 7. Changes in phenolic compounds of virgin olive oil from Lianolia variety during heating at 100 °C.

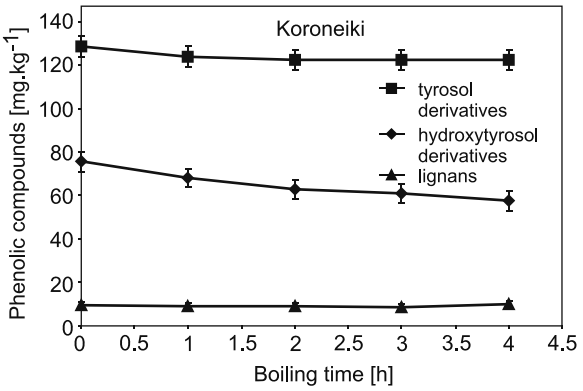

Fig. 8. Changes in phenolic compounds of virgin olive oil from Koroneiki variety during heating at 100 °C.

Tab. 3. Changes in phenolic compounds of virgin olive oils during storage.

Samples	Storage time [months]	Peroxide value [meq.kg^{-1}]	Hydroxytyrosol derivatives[a] [mg.kg^{-1}]	Oxidized product I	Tyrosol derivatives[b] [mg.kg^{-1}]	Oxidized product II	Lignans[c] [mg.kg^{-1}]	Flavonoids[d] [mg.kg^{-1}]	Simple phenols[e] [mg.kg^{-1}]
Lianolia 1	0	7.12	144.02	0.00	90.48	0.00	7.84	2.71	0.87
	15	14.01	82.17	19.81	80.59	8.21	7.79	1.06	0.29
Koroneiki 2	0	7.89	80.41	0.00	128.53	0.00	9.60	5.96	0.21
	15	16.21	64.37	5.55	120.38	2.10	8.27	4.92	0.15
Lianolia 3	0	6.92	105.14	0.00	91.32	0.00	8.76	2.41	0.30
	3	17.5	91.42	2.09	87.42	0.00	8.75	1.91	0.28
	9	39.0	52.6	10.68	72.7	8.07	8.47	1.61	0.26
	15	58.8	37.74	13.35	64.29	10.94	8.12	1.21	0.20

Concentrations of phenolic compounds were expressed as mg standard compound in kg of olive oil.
a – hydroxytyrosol, DAFOA, AFOA, *b* – tyrosol, DAFLA, AFLA, *c* – 1-acetoxypinoresinol, *d* – apigenin, luteolin, *e* – *p*-coumaric acid, ferullic acid, vanillic acid.

not exceed the upper limit of 20 meq.kg^{-1} (samples 1 and 2), approximately 60–75% of hydroxytyrosol derivatives and 80–95% tyrosol derivatives remained in the oil. Lignans remained unchanged. At a peroxide value of 58 meq.kg^{-1} (sample 3), 30% of hydroxytyrosol derivatives, 70% tyrosol derivatives and 100% lignans remained in the oil. In a previous work, samples from Lianolia variety, stored in glass bottles in the dark, exceeded the upper limit of peroxide value after 7.7 months (mean value), corresponding to a loss of 60% in hydroxytyrosol derivatives and 40% in total phenols [9]. In that case the bottles' headspace was 3% whereas in the current work, the headspace is approximately 1.5% for group 1 and 2, and 3% for group 3. When Cornicabra oil from Spain, having a ratio of oleic acid : linoleic acid of 20, was stored at 4 °C in darkness without headspace, the oils did not exceed the upper limit of 20 meq.kg^{-1} during the storage period of 21 months. The reduction of total phenolic compounds ranged in that work from 43% to 73% [11]. In another study, using olive oil from Arbequina variety with the fatty acid composition similar to Lianolia variety, a noticeable decrease was observed in hydroxytyrosol derivatives after 12 months of storage [25]. The oxidative stability of virgin olive oils correlates mainly with hydroxytyrosol derivatives and, consequently, these compounds are the first to be oxidized [2, 13]. Therefore, the oxygen availability and temperature are two important parameters for olive oil stability during storage [26]. The phenolic compounds maintenance can be achieved by oxygen exclusion by storage under nitrogen, in particular for the oils having a low oleic : linoleic acid ratio. Otherwise, more than 40% may be lost during the one year storage of olive oil.

Moreover, two oxidized phenolic compounds were detected and quantified (Tab. 3, Fig. 6). Values recorded ranged from 0 mg.kg^{-1} to 20 mg.kg^{-1} for the oxidized product I, and from 0 mg.kg^{-1} to 11 mg.kg^{-1} for the oxidized product II. Even though the highest values were recorded for the samples having the highest peroxide values,

there was no clear relationship between them and the oxidized products concentration. Decarboxymethylation of secoiridoids and further hydrolysis of phenolic esters occurred during the first six months of storage in Frantoio/Leccino and Coratina curtivars, whereas oxidation products were detected after 9 months of storage [12].

In order to confirm the structure of oxidation products (Fig. 5), HPLC-MS in negative mode was used. Phenolic compounds give peaks of a lower intensity in the negative than in the positive ion mode. Nevertheless, cleaner spectra are obtained in the negative mode. Identification of phenolic compounds was based on pseudomolecular [M-H] ions, dimers and fragments [27].

The most abundant oleuropein derivative (peak 7, Fig. 1) had a deprotonated molecule at m/z = 319, corresponding to DAFOA (Fig. 9). The ion of m/z = 639 is the dimer of the ion of m/z = 319. The ion of m/z = 195 can be explained as a hydroxytyrosol acetate fragment and the ion of m/z = 165 as the residue after the loss of hy-

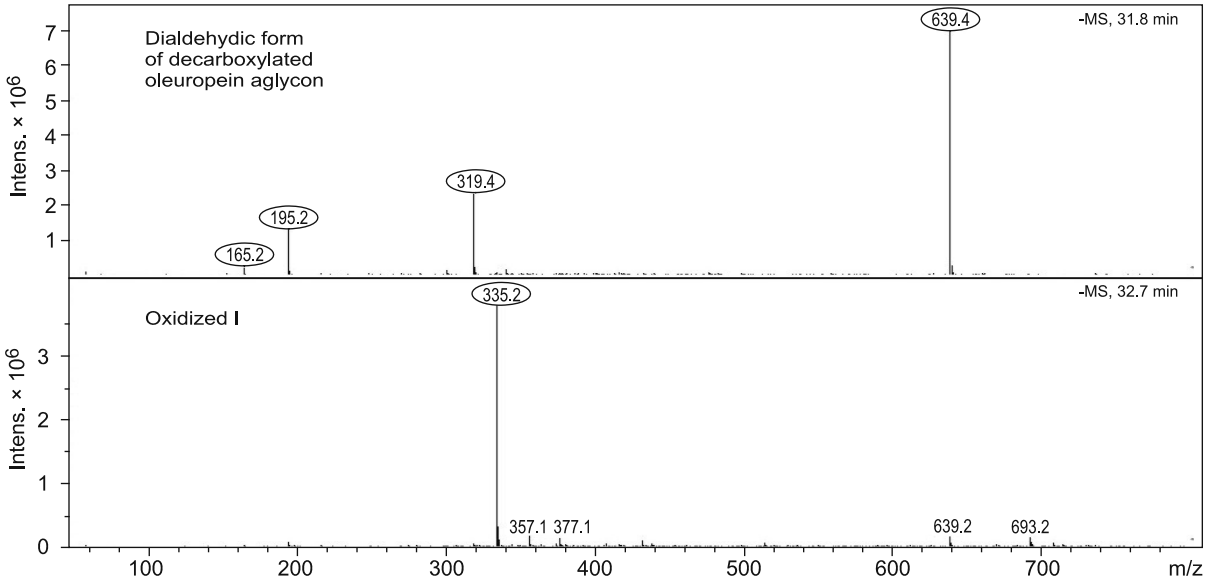

Fig. 9. Mass spectra obtained in the negative mode for dialdehydic form of oleuropein aglycon and its oxidation product.

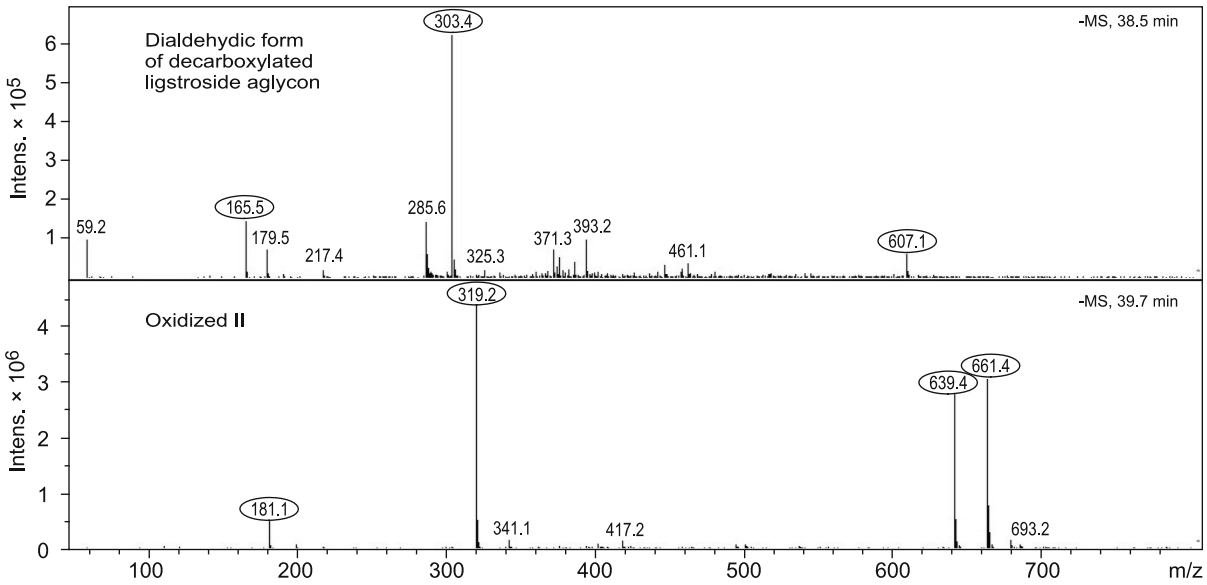

Fig. 10. Mass spectra obtained in the negative mode for dialdehydic form of ligstroside aglycon and its oxidation product.

droxytyrosol. Mass spectra of oxidation product I (Fig. 9) showed a characteristic product ion at m/z = 335, explained as the ion of m/z = 319 + oxygen, with no further fragmentation.

The most abundant ligstroside derivative (peak 9, Fig. 1) had a deprotonated molecule at m/z = 303, corresponding to DAFLA (Fig. 10). The ion of m/z = 607 is the dimer of the ion of m/z = 303, and the characteristic product ion of m/z = 165 can be explained by the loss of tyrosol. Mass spectra of oxidation product II showed a characteristic product ion at m/z = 319 (the ion of m/z = 303 + oxygen), with a further fragmentation at m/z = 181, which can be the decarboxylated elenolic acid. The dimer ion of m/z = 639 originates from the ion of m/z = 319. These findings are in accordance with mass spectra given in the literature for these oxidized products [18, 19]. It must be noted that oxidation product II has the same retention time with pinoresinol in both HPLC columns used for the phenolic compounds separation (Lichrospher and Luna). Discrepancies in the lignans concentration, observed in a previous work using NMR spectroscopy and HPLC-UV, may be due to the false estimation of oxidized product II as pinoresinol [28]. Formation of oxidized products and their estimation along with non-oxidized DAFOA may be the reason for discrepancies referred to recently in the literature. These stated that among the different classes of phenolic compounds, *o*-diphenols displayed the highest microwave heating resistance during the entire time range of application of 15 min [29]. Actually, the oil used in this study was not a virgin olive oil, as the recorded peroxide value was higher than the upper limit of 20 meq.kg^{-1}. Moreover, the wide peak of DAFOA and the low amounts of both tyrosol and hydroxytyrosol derivatives observed in this study indicated that a high degree of oxidation occured.

CONCLUSIONS

During frying (180 °C, 1 h), the content of phenolic compounds in the olive oil was remarkably reduced, especially for hydroxytyrosol derivatives, for which 90% were lost. Tyrosol derivatives and lignans appeared to be stable. During boiling (100 °C, 2 h), all phenolic compounds remained stable (only 20% were lost). When virgin olive oil is stored under environmental conditions in the dark, the reduction of phenolic compounds depends on olive oil unsaturation, headspace oxygen availability and temperature, which are factors that affect the hydroperoxides' formation. Precautions must be taken in order to avoid oxidation during storage, which mainly affect hydroxytyrosol derivatives. These findings illustrate the need to exclude oxygen from bottles' headspace during storage, especially for oils with a high linoleic acid content, in order to maintain their nutritional value.

REFERENCES

1 Angerosa, F. – Mostallino, R. – Basti, C. – Vito, R.: Virgin olive oil odour notes: their relationship with volatile compounds from lipoxygenese pathway and secoiridoid compounds. Food Chemistry, *68*, 2000, pp. 283–287.

2 Aparicio, R. – Roda, L. – Albi, M. A. – Gutierrez, F.: Effect of various compounds on virgin olive oil stability measured by rancimat. Journal of Agricultural and Food Chemistry, *47*, 1999, pp. 4150–4155.

3 Visioli, C. – Galli, C.: Biological properties of olive oil phytochemicals. Critical Reviews in Food Science and Nutrition, *42*, 2002, pp. 209–221.

4 Stark, A. H. – Madar, Z.: Olive oil as a functional food: epidemiology and nutritional approaches. Nutrition Reviews, *60*, 2002, pp. 170–176.

5 Montedoro, G. F. – Servili, M. – Baldioli, M. – Miniati, E.: Simple and hydrolyzable phenolic compounds in virgin olive oil. 2. Initial characterization of the hydrolyzable fraction. Journal of Agricultural and Food Chemistry, *40*, 1992, pp. 1577–1580.

6 Mateos, R. – Esperanto, J. L. – Trujillo, M. – Rios, J. J. – Leon-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. Journal of Agricultural and Food Chemistry, *49*, 2001, pp. 2185–2192.

7 Gimeno, E. – Castellote, A. I. – Lamuela-Raventos, R M. – Lopez-Sabater, M. C. – De la Torre, M. C.: The effect of harvest and extraction methods on the antioxidant content (phenolics, α-tocopherol and β-carotene) in virgin olive oil. Food Chemistry, *78*, 2001, pp. 207–211.

8 Servili, M. – Selvaggini, R. – Esposto, S. – Taticchi, A. – Montedoro, G. F. – Morozzi, G.: Health and sensory properties of virgin olive oil hydrophilic phenols: agronomic and technological aspects of production that affect their occurrence in the oil. Journal of Chromatography A, *1054*, 2004, pp. 113–127.

9 Okogeri, O. – Tasioula-Margari, M.: Changes occurring in phenolic compounds and tocopherols during storage. Journal of Agricultural and Food Chemistry, *50*, 2002, pp. 1077–1080.

10 Rastrelli, L. – Passi, S. – Ippolito, F. – Vacca, G. – De Simone, F.: Rate of degradation of α-tocopherol, squalene, phenolics and polyunsaturated fatty acids in olive oil during different storage conditions. Journal of Agricultural and Food Chemistry, *50*, 2002, pp. 5566–5570.

11 Gómez-Alonso, S. – Mancebo-Campos, V. – Desamparados Salvador, M. – Fregapane, G.: Evolution of

major and minor components and oxidation indices of virgin olive oil during 21 months storage at room temperature. Food Chemistry, *100*, 2007, pp. 36–42.
12 Boselli, E. – Di Lecce, G. – Strabbioli, R. – Pieralisi, G. – Frega, N. G.: Are virgin olive oils obtained below 27 °C better than those produced at higher temperatures? Lebensmittel-Wissenschaft und -Technologie - Food Science and Technology, *42*, 2009, pp. 748–757.
13 Nissiotis, M. – Tasioula-Margari, M.: Changes in antioxidant concentration of virgin olive oil during thermal oxidation. Food Chemistry, *77*, 2002, pp. 371–376.
14 Gutierrez, F. – Villafranca, M. J. – Castellano, J. M.: Changes in the main components and quality indices of virgin olive oil during oxidation. Journal of the American Oil Chemists' Society, *79*, 2002, pp. 669–676.
15 Brenes, M. – Garcia, A. – Dobarganes, M. C. – Velasco, J. – Romero, C.: Influence of thermal treatment simulating cooking processes on the polyphenol content in virgin olive oil. Journal of Agricultural and Food Chemistry, *50*, 2002, pp. 5962–5967.
16 Carrasco-Pancobro, A. – Cerretani, L. – Bendini, A. – Segura-Carretero, A. – Lercker, G. – Fernández-Gutiérrez, A.: Evaluation of the influence of thermal oxidation on the phenolic composition and on the andioxidant activity of extra-virgin olive oil. Journal of Agricultural and Food Chemistry, *55*, 2007, pp. 4771–4780.
17 Armaforte, E. – Mancebo-Campos, V. – Bendini, A. – Salvador, M. D. – Fregapane, G. – Cerretani, L.: Retention effects of oxidized polyphenols during analytical extraction of phenolic compounds of virgin olive oil. Journal of Separation Science, *30*, 2007, pp. 2401–2406.
18 Rovellini, P. – Cortesi, N.: Liquid chromatography – mass spectrometry in the study of oleuropein and ligstroside aglycons in virgin olive oil: Aldehydic forms and their oxidized products. Rivista Italiana delle Sostanze Grasse, *77*, 2002, pp 1–14.
19 Rios, J. J. – Gil, M. J. – Gutierrez-Rosales, F.: Solid phase extraction gas chromatography – ion trap – mass spectrometry qualitative method for evaluation of phenolic compounds in virgin olive oil and structural confirmation of oleuropein and ligstroside aglycons and their oxidation products. Journal of Chromatography A, *1093*, 2005, pp. 167–176.
20 Commission Regulation (EEC) No 2568/91 of 11 July 1991 on the characteristics of olive oil and olive-residue oil and on the relevant methods of analysis. Official Journal of the European Communities, *L248*, 1991, pp. 1–83.
21 Tasioula-Margari, M. – Okogeri, O.: Isolation and characterization of virgin olive oil phenolic compounds by HPLC/UV and GC-MS. Journal of Food Science, *66*, 2001, pp. 530–534.
22 Brenes, M. – Garcia, A. – Garcia, P. – Rios, J. J. – Carrido A.: Phenolic compounds in spanish olive oils. Journal of Agricultural and Food Chemistry, *47*, 1999, pp. 3535–3354.
23 Oliveras-Lopez, M. J. – Innocenti, M. – Giaccherini, C. – Ieri F. – Romani A. – Mulinacci.: Study of the phenolic composition of Spanish and Italian monocultivar extra virgin olive oils: Distribution of lignans, secoiridoids, simple phenols and flavonoids. Talanta, *73*, 2007, pp. 726–732.
24 Visioli, F. – Poli, A. – Galli C.: Antioxidant and other biological activities of phenols from olives and olive oil. Medicinal Research Reviews, *22*, 2002, pp. 65–75.
25 Morello, J.-R. – Motilva, M.-J. – Tovar, M.-J. – Romero, M.-P.: Changes in commercial virgin olive oil (cv Arbequina) during storage, with special emphasis on the phenolic fraction. Food Chemistry, *85*, 2004, pp. 357–364.
26 Kanavouras, A. – Cert, A. – Hernandez, R. J.: Oxidation of olive oil under still air. Food Science and Technology International, *11*, 2005, pp. 183–189.
27 De la Torre-Carbot. K. – Jauregui, O. – Gimeno, E. – Castellote, I. A. – Lamuela-Raventoäs, M. R. – Carmen Loäpez-Sabater. M.: Characterization and quantification of phenolic compounds in olive oils by solid-phase extraction, HPLC-DAD and HPLC-MS/MS. Journal of Agricultural and Food Chemistry, *53*, 2005, pp. 4331–4340.
28 Dais, P. – Spyros, A. – Christophoridou, S. – Hatzakis M. – Fragaki, G. – Agiomyrgianaki, A. – Salivaras, E. – Siragakis, G. – Daskalaki, D. – Tasioula-Margari, M. – Brenes, M.: Comparison of analytical methodologies based on ^{1}H and ^{31}P NMR spectroscopy with conventional methods for the determination of some olive oil constituents. Journal of Agricultural and Food Chemistry, *55*, 2007, pp. 577–584.
29. Cerretani, L. – Bendini, A. – Rodriguez-Estrada, M. T. – Vittadini, E. – Chiavaro, E.: Microwave heating of different commercial categories of olive oil: Part I. Effect on chemical oxidative stability indices and phenolic compounds. Food Chemistry, available online 27 January 2009. DOI:10.1016/j.foodchem.2009.01.060.

Received 12 December 2008; revised 24 February 2009; accepted 27 February 2009.