J Am Oil Chem Soc (2010) 87:369–376
DOI 10.1007/s11746-009-1517-x

ORIGINAL PAPER

Composition and Antioxidant Activity of Olive Leaf Extracts from Greek Olive Cultivars

Kostas Kiritsakis · M. G. Kontominas · C. Kontogiorgis · D. Hadjipavlou-Litina · A. Moustakas · A. Kiritsakis

Received: 14 July 2009 / Revised: 30 October 2009 / Accepted: 10 November 2009 / Published online: 10 December 2009

Abstract The olive leaf phenolic composition of the Greek cultivars *koroneiki*, *megaritiki* and *kalamon* was determined using LC/MS. Furthermore, the antioxidant activity of olive leaf extracts from the above three cultivars, using solvents of increasing polarity (petroleum ether, dichloromethane, methanol and methanol/water: 60/40) was evaluated using the stable free radical diphenylpicrylhydrazyl (DPPH) test. Furthermore the oxidative stability index (OSI) was compared to that of the synthetic antioxidant TBHQ and commercial oleoresin (rosemary extract). The ability of phenolic compounds to inhibit the lipoxygenase (LOX) activity was also investigated. The ten main components determined in the olive tree leaf extracts for the cultivars *koroneiki* and *kalamon* were: secologanoside, dimethyloleuropein, oleuropein diglucoside, luteolin-7-*O*-glucoside, rutin, oleuropein, oleuroside, quercetin, ligstroside and verbascoside. Respective compounds for the cultivar *megaritiki* were: secologanoside, dimethyloleuropein, oleuropein diglucoside, luteolin7-*O*-glucoside, oleuropein, oleuroside, quercetin and ligstroside. In all three cultivars, oleuropein represented the main phenolic component. The solvent polarity influenced the total amount of the phenolic compounds determined. When methanol/water (60/40) was used, as solvent, more phenolic compounds were determined. The total amounts of phenols determined in the extracts, obtained by successive extractions using the above solvents, were 6,094, 5,579 and 6,196 mg/kg (mg gallic acid/kg dried olive leaves) for the cultivars *megaritiki*, *kalamon* and *koroneiki*, respectively. Among all extracts, methanol/water extracts exhibited the highest antioxidant activity as shown through the application of the DPPH and OSI methods. The OSI antioxidant activity followed the sequence: synthetic antioxidant TBHQ > commercial oleoresin > olive tree leaf extracts > control. Likewise, methanol/water olive leaf extracts significantly inhibited soybean lipoxygenase, although some small differences in the activity among the olive leaf extracts of the different cultivars were observed. The solvent polarity as well as the amount of the extract influenced the inhibitory activity. A positive correlation was shown between the antioxidant activity of leaf extracts and the total phenol content.

Keywords Olive leaves · Antioxidant activity · Phenolic components · Lipoxygenase activity · DPPH · Free radical scavenging

K. Kiritsakis · A. Kiritsakis (✉)
Alexander Technological Educational Institute of Thessaloniki, Sindos, Thessaloniki, Greece
e-mail: kiritsak@food.teithe.gr

K. Kiritsakis
e-mail: kkyritsakis@yahoo.com

M. G. Kontominas
Department of Chemistry, University of Ioannina, Ioannina, Greece

C. Kontogiorgis · D. Hadjipavlou-Litina
Department of Pharmaceutical Chemistry, School of Pharmacy, Aristotle University of Thessaloniki, Thessaloniki, Greece
e-mail: kontog@pharm.auth.gr

A. Moustakas
Technical University of Crete, Chanea, Greece

Introduction

Lipid oxidation has been one of the main interests of the scientific community for centuries. Researchers are continuously seeking those natural antioxidants that will

sufficiently protect fats and oils from oxidation. Synthetic antioxidants are very effective, inexpensive and stable under usual processing and storage conditions of oils [1]. However, they have certain disadvantages, including possible toxicological effects [2]. Although there are no conclusive results on the safety of these substances, worldwide interest has arisen for the recovery and utilization of antioxidants from natural sources [3]. Recent research has focused on antioxidant compounds derived from leaves and fruit of olive trees, numerous fruits and vegetables, as well as aromatic plants and spices [4–7]. Salta et al. [8] enriched commercially available oils (olive oil, sunflower oil, palm oil and a vegetable shortening) with polyphenols by adding olive leaf extract to the product. Results showed that both antioxidant capacity and oxidative stability were substantially improved for all oils studied. A concentration of 400 ppm of free phenolics, extracted from olive leaves exhibited high antioxidant activity, superior to that of butylated hydroxy toluene (BHT) in retarding sunflower oil oxidative rancidity [9].

Aliquots of concentrated crude olive leaf juice were added to sunflower oil and heated to 180 °C. The samples exhibited remarkable antioxidant activity and at a concentration of 800 ppm were superior to that of BHT (200 ppm) in increasing sunflower oil stability [10]. A phenol extract of high hydroxytyrosol content obtained from olive leaves (*Olea europaea* L.) increased the oxidative stability of different food lipids (butter, lard and cod liver oil) [11]. Bouaziz et al. [12] showed that enrichment of refined olive and husk oils with olive leaf extract and its hydrolysate extract resulted in increased resistance to oxidative deterioration due to the extract's antioxidant content. The authors suggested that both hydrolysate and leaf extract are excellent antioxidants and may serve as substitutes for synthetic antioxidants.

Due to the increasing interest in the use of natural antioxidants, the present study was carried out in order to evaluate the phenolic composition of olive leaves of three Greek olive cultivars and to determine the antioxidant activity of olive leaf extracts obtained by different solvents of increasing polarity.

Materials and Methods

Materials

Olive leaves were collected from an olive orchard located in the area Trilofos of Thessaloniki in the fall of 2007. Four trees of each of the cultivar *koroneiki*, *megaritiki* and *kalamon* were selected. Samples were taken from each tree including different parts of the tree and were mixed. Leaves were left to dry at room temperature for 1 week before use.

1,1-Diphenyl 2-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent, lipoxygenase (1.13.11.12) type I-B (soybean) and linoleic acid (sodium salt), 99% purity, were purchased from Sigma (St Louis, MO, USA). TBHQ was obtained from Eastman Co (NY, USA), while the commercial oleoresin was purchased from Kalsec. Co., (MI, USA). All chemical reagents were of analytical grade.

Extra virgin olive oil from the island of Crete, with an acidity of 0.5% and a peroxide value of 7 mequiv O_2/kg oil, was used.

Phenol Extraction from the Olive Leaves

Phenols were extracted from the olive leaves by successive extractions, using solvents of increasing polarity (petroleum ether, dichloromethane, methanol and methanol/water: 60/40). 10 g of dried leaves from each cultivar were mechanically milled and placed in Erlenmeyer flasks. A 200-mL quantity of petroleum ether were added to each flask and left for 24 h at room temperature followed by filtration using Whatman 47 mm × 0.45 μ filters. The filtrates were evaporated in a rotary evaporator. The dry residues of the olive leaves were returned to the flasks, where 200 mL dichloromethane was added and the contents were kept for another 24 h. The procedure was repeated as shown in Fig. 1, with methanol and methanol/water: 60/40. All extracts were evaporated at mild temperatures (40–45 °C), in order to avoid the decomposition of the phenolic compounds.

Dry residues obtained from each cultivar and solvent treatment were analyzed for their phenol content.

Determination of Total Phenol Content

The total phenol content of the obtained fractions was determined using the method of Zheng and Wang [13] with

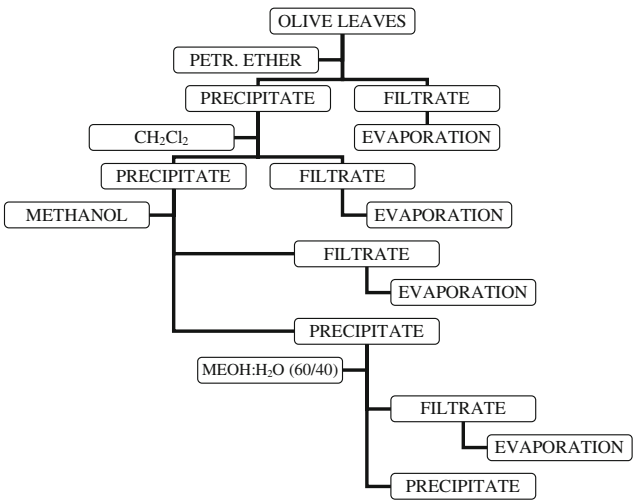

Fig. 1 Flow diagram for the preparation of olive leaf extracts with successive extractions

a few modifications: 5 mg of dry residue from each solvent extraction were dissolved in 1 mL DMSO. 100 μL of this solution were transferred to a volumetric flask to which 500 μL of Folin-Ciocalteu phenol reagent and 400 μL of 7.5% sodium carbonate solution were added. The mixture was shaken thoroughly and kept for 1.5 h at 30 °C, in the absence of light. The absorbance of the blue color formed was measured at 765 nm. The concentration of total phenol compounds for each extract was calculated on the basis of a standard curve obtained using gallic acid as the standard (twelve serial-2 fold dilutions to give a range of 0.01–0.001 mg/mL in triplicate). Results were expressed as mg of gallic acid per 100 g of dry weight.

Evaluation of the Antioxidant Activity with the OSI Apparatus

Given amounts of the dry residues from each cultivar, the synthetic antioxidant TBHQ and the commercial oleoresin (rosemary extract) were dissolved in DMSO. The stock samples were of such concentration that by adding 1 mL of each solution to 4 g of olive oil, mixtures of oil containing 100 ppm phenols were prepared. A control sample, containing 4 g of olive oil and 1 mL DMSO, was also prepared. The flow rate of air in the OSI apparatus was set at 16 L/h and the temperature at 110 °C. The OSI values, which correspond to the beginning of the propagation period or the end of the initiation period of oxidation, were automatically recorded.

Interaction with DPPH Stable Free Radical

The DPPH method [14] was used to determine antioxidant activity of olive leaf extracts. A 20-μL sample from the stock solution of the sample (approximately 2.5 mg in 1 mL DMSO) were dissolved in absolute ethanol to a final volume of 1 mL and then added to 1 mL DPPH (0.1 mM, in absolute ethanol). The reaction mixture was kept at room temperature. The optical density (OD) of the solution was measured at 517 nm, after 20 and 60 min. The optical densities of the samples in the absence of DPPH were subtracted from the corresponding OD with DPPH. The % reduction values were determined and compared to appropriate standards.

$$\%\ \text{Reduction} = \frac{\text{control OD (mean)} - \text{sample OD (mean)}}{\text{control OD (mean)}} \times 100$$

Soybean Lipoxygenase Inhibition

All extract samples were initially dissolved in DMSO (approximately 2.5 mg in 1 mL DMSO). Either 10 μL or 1 μL of the solution was mixed with 100 μL of sodium linoleate (0.1 mM) and 0.2 mL of the enzyme solution ($1/9 \times 10^{-2}$% w/v salt solution pH = 9). Samples were incubated at room temperature for 3 min. The conversion of sodium linoleate to 13-hydroperoxylinoleic acid was recorded at 234 nm and compared to an appropriate standard inhibitor (caffeic acid IC_{50} = 600 μM) [13, 14].

Analysis of the Phenolic Compounds in Olive Leaf Extracts

Phenols from 1.5 g of the leaves from each cultivar were extracted with 20 mL methanol/water 80% for 30 min. The solutions formed were filtered through GF/F filter paper. The extracts were further extracted with petroleum ether to remove chlorophyll, following filtration and centrifugation. 0.3 mL from the extracts were kept in vials prior to HPLC.

LC–MS analysis was performed using a Finningan LCQ Deca ion-trap mass spectrometer (Finningan MAT, San Jose, CA, USA) coupled with a Thermo Separation series liquid chromatographic system (Thermo products, San Jose, CA, USA) consisting of UV3000, AS3000, P4000, SCM1000, a membrane degasser and an injection valve (100 μL loop), a XTerraR RP 18, 3.5 μm, 4.6 × 150 mm column (Waters, Ireland), with a XTerraR RP 18, 3.5 μm, 4.6 × 10 mm guard column. Elution was performed at a flow rate of 1.0 mL/min, using a mixture of water/acetic acid (99.9:0.1 v/v) (solvent A) and acetonitrile/acetic acid (99.9:0.1 v/v) (solvent B) as the mobile phase. Both solvents were of HPLC grade and filtered through a 0.20-μm filter disk (for solvent A) and a 0.45-μm filter disk (for solvent B). The injection volume was 20 μL. The solvent gradient was changed according to the following conditions: from 96% (A)–4% (B) to 76% (A)–24% (B) in 35 min, 53% (A)–47% (B) in 15 min, 100% (B) in 10 min and 96% (A)–4%(B) in 10 min followed by 10 min of conditioning the column under the initial conditions. Chromatograms were acquired at 232, 280 and 370 nm. The HPLC data were collected and processed on a Chromeleon data system.

The MS analyses were carried out using an electrospray (ESI) interface operating in both positive and negative mode using the following conditions: Helium gas was used as a stealth gas at a flow rate of 28 arb. The electrospray voltage was 3.70 kV and the heated capillary temperature and voltage were maintained at 270 °C and −37.00 V, respectively, for negative mode. The tube lens offset voltage was set at −55 V. For positive mode scanning, the electrospray voltage was 4.70 kV, the heated capillary temperature and voltage were maintained at 270 °C and 15.00 V, respectively, while the tube lens offset was set to 45 V. The molecular ions were scanned from 100.0 to 2,000.0 (m/z) in such a scanning order that the full-scan mass spectrum was followed by a tandem mass spectrum (MS/MS). The MS data were collected and processed on a

Xcalibur data system. The system was optimized for oleuropein on the *m/z* ratios of 539 and 541 corresponding to the negative and positive ion of oleuropein, respectively.

Results and Discussion

Analysis of Phenolic Compounds

Figure 2 shows the phenolic compounds isolated from the olive cultivars *megaritiki*, *koroneiki* and *kalamon*. Nine compounds namely: demethyloleuropein, oleuropein diglucoside, luteolin-7-*O*-glucoside, rutin, oleuropein, oleuroside, quercetin, ligstroside, verbascoside were identified in the cultivar *megaritiki*, while ten compounds (secologanoside, demethyloleuropein, oleuropein diglucoside, luteolin-7-*O*-glucoside, rutin, oleuropein, oleuroside, quercetin, ligstroside, verbascoside) were identified in the *kalamon* cultivar and the same compounds apart from secologanoside and ligstroside were identified in the *koroneiki* cultivar. Oleuropein was by far the main constituent in all three cultivars. The compounds identified were in accordance with the related literature [15].

Two oleuropein derivatives (Fig. 3) were identified during MS analysis. The two peaks represent oleuropein (Fig. 4, retention time 31.96 min) and oleuroside (Fig. 4, retention time 34.01 min) which have the same mass. The only difference in structure between the two compounds is the position of the olefinic double bond in the elenolic acid moiety thus identification based on MS was not possible.

Determination of Total Phenols

Table 1 shows the concentration of the main phenolic compounds found in the olive leaf extract of the three tested cultivars, using methanol/water 80%. Table 2 on the other hand, shows the amount of the dry residues collected from the three cultivars and the four kinds of solvents used to obtain the phenolic extracts. The highest amount was isolated when methanol/water was used. Among the cultivars, *koroneiki* gave the highest amount of extract followed by *megaritiki* and *kalamon*. The extract weight was 1.48, 1.44 and 1.43 g, respectively, for the cultivars *koroneiki*, *megaritiki* and *kalamon*. When petroleum ether was used as the solvent, the lowest amount of phenols was obtained from all three cultivars.

Combining the results of Tables 3, 4, 5 and 6, the total mg of phenols in each cultivar were calculated:

Megaritiki 0.227 + 0.305 + 25.925 + 34.469 = 60.93 mg

Kalamon 0.238 + 0.137 + 21.052 + 34.358 = 55.79 mg

Koroneiki 0.137 + 0.267 + 27.533 + 34.024 = 61.96 mg

The amount of olive leaves used was 10 g. Thus, the phenol content in mg/kg was 6,093, 5,579 and 6,196, respectively, for the cultivars, *megaritiki*, *kalamon* and *koroneiki*. Amounts of phenols determined in the present work are substantially lower than those reported by Skerget et al. [16]. Differences observed may be related to the use of different cultivars and different procedures of extraction.

Evaluation of the Antioxidant Activity with OSI Apparatus

As Table 7 shows, all olive leaf phenolic extracts extended the initiation period of oxidation compared to the control. They enhanced the oxidative stability by 5.5–6.5 h or 30%

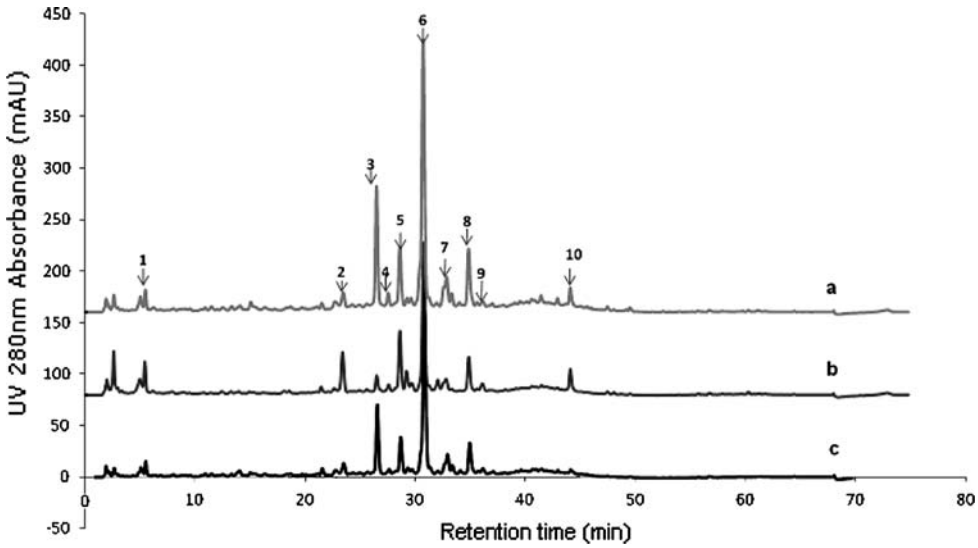

Fig. 2 RP-HPLC chromatograms of the three cultivars' extracts at 280 nm (*1* secologanoside, *2* demethyloleuropein, *3* oleuropein diglucoside, *4* luteolin-7-*O*-glucoside, *5* rutin, *6* oleuropein, *7* oleuroside, *8* Quercetin, *9* ligstroside, *10* verbascoside). (**a** *kalamon*, **b** *koroneiki*, **c** *megaritiki*)

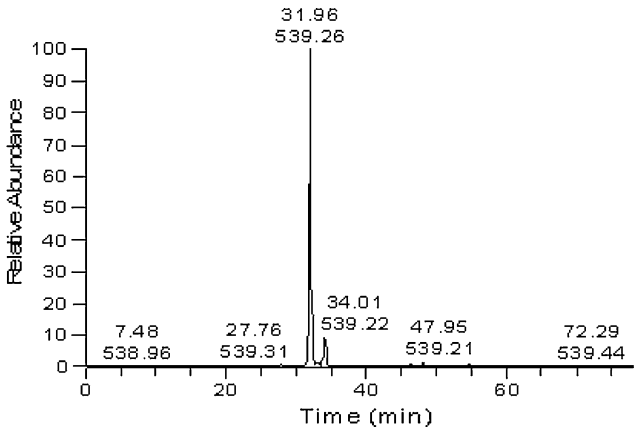

Fig. 3 Oleuropein derivatives identified during MS ion scanning (oleuropein, retention time 31.96 min and oleuroside (retention time 34.01 min)

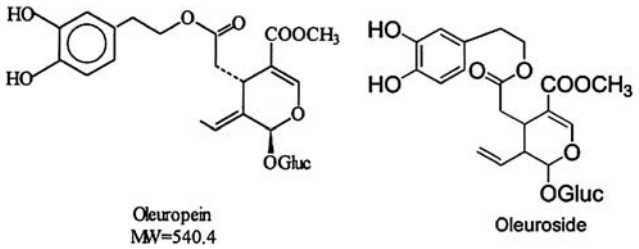

Fig. 4 Oleuropein (retention time 31.96 min) and oleuroside (retention time 34.01 min)

Table 1 Concentration of the main phenolic compounds determined in the olive leaf extract of the three cultivars, using methanol/water 80% (mg of phenols in 100 g olive leaves)

Compound	*Kalamon*	*Koroneiki*	*Megaritiki*
Oleuropein diglucoside	4.13 ± 0.19	1.44 ± 0.06	0.96 ± 0.04
Rutin	1.59 ± 0.07	1.66 ± 0.71	1.11 ± 0.05
Oleuropein	8.48 ± 0.51	4.89 ± 0.26	3.26 ± 0.18
Quercetin	1.74 ± 0.08	1.07 ± 0.06	0.71 ± 0.04

Table 2 Weights (g) of dry residues of extracts

Solvent	Cultivar		
	Kalamon	*Koroneiki*	*Megaritiki*
Petroleum ether	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.00
Dichloromethane	0.29 ± 0.01	0.30 ± 0.02	0.35 ± 0.02
Methanol	0.95 ± 0.04	1.20 ± 0.05	1.15 ± 0.05
Methanol/water (60/40)	1.43 ± 0.08	1.48 ± 0.07	1.44 ± 0.08

compared to the control. TBHQ showed the strongest effect, increasing the oxidative stability by 10 h or 47%. Commercial oleoresin also increased antioxidant activity, extending the initiation period by 9.2 h or 42%. TBHQ showed to be the strongest antioxidant in other studies as well [17].

Table 3 Phenol concentration with petroleum ether being used as solvent

Cultivar	g gallic acid/100 g sample	mg phenols in dry residues
Megaritiki	0.47 ± 0.02	0.23 ± 0.01
Kalamon	0.44 ± 0.02	0.24 ± 0.02
Koroneiki	0.28 ± 0.01	0.14 ± 0.01

Table 4 Phenol concentration with dichloromethane being used as solvent

Cultivar	mg gallic acid/100 g sample	mg phenols in dry residues
Megaritiki	86.04 ± 4.12	0.31 ± 0.02
Kalamon	46.98 ± 2.11	0.14 ± 0.01
Koroneiki	88.00 ± 4.81	0.27 ± 0.02

Table 5 Phenol concentration with methanol being used as solvent

Cultivar	g gallic acid/100 g sample	mg phenols in dry residues
Megaritiki	2.25 ± 0.10	25.92 ± 1.31
Kalamon	2.22 ± 0.12	21.05 ± 1.40
Koroneiki	2.30 ± 0.13	27.53 ± 1.55

Table 6 Phenol concentration with methanol/water (60/40) being used as solvent

Cultivar	g gallic acid/100 g sample	mg phenols in dry residues
Megaritiki	2.40 ± 0.11	34.47 ± 1.84
Kalamon	2.40 ± 0.13	34.36 ± 1.92
Koroneiki	2.30 ± 0.16	34.02 ± 2.96

Interaction of Olive Leaf Extracts with the Stable Free Radical of DPPH (1,1-diphenyl-2-picrylhydrazyl)

Figure 5 shows the results of the interaction of the olive leaf extracts from three different cultivars with the stable free radical of DPPH. The % interaction was determined after 20 and 60 min. Almost all petroleum ether and dichloromethane extracts were found to be less active against DPPH. Between the two, dichloromethane extracts presented higher reducing activity than petroleum ether extracts, except in the case of cultivar *koroneiki* after 60 min. Such a pattern may be related to the fact that the

Table 7 OSI values of the phenolic extracts of the three olive cultivars, control, TBHQ and commercial oleoresin (all samples were 100 ppm)

Cultivar	OSI Value (h)	
	Methanol extract	Methanol/water (60/40) extract
Megaritiki	27.24 ± 1.51	27.50 ± 1.44
Kalamon	28.12 ± 1.63	27.91 ± 1.28
Koroneiki	27.83 ± 1.32	28.42 ± 1.33
Control	22.00 ± 1.05	
TBHQ	32.33 ± 1.47	
Commercial oleoresin	31.21 ± 1.61	

more polar solvents dissolve more polar phenolic antioxidants from olive tree leaves which comprise the majority of phenolics in this substrate. The interaction with DPPH did not increase with time. Only in the case of *koroneiki* cultivar/petroleum ether, there was a significant increase in reducing activity with time. On the contrary, the reducing activity of the *kalamon* cultivar/petroleum ether showed a decrease with time, whereas for *megaritiki* it remained constant. Almost all methanol and methanol/water (60:40) extracts presented the same antioxidant activity. Their activity did not seem to be time dependent. Only in the *kalamon* cultivar/methanol–water extract, had the reducing activity decreased after 60 min. Such a complex behavior may be rationalized as follows:

Usually DPPH radicals react completely and rapidly with the substrate's antioxidants (within a few seconds to a few minutes). Such is the case of the more polar (methanol and methanol/water) and intermediately polar (dichloromethane) solvents in which no time dependency was observed. However in the presence of very weak antioxidants (with a small number of OH groups) or polymerized ones exhibiting stearic hindrance effects (preventing close contact between the DPPH radical and OH groups) a slow reaction occurs [18]. This is most probably the case of the *kalamon*/(non-polar) petroleum ether interaction, the scavenging activity of which decreased with time. A similar time-dependent scavenging activity was observed for various plant extracts and for varying molecular weight chitooligosaccharides [18]. The reason for the increase in scavenging activity of the *koroneiki* extract/petroleum ether system after 60 min is unclear and requires further investigation.

All of the olive leaf extracts presented antioxidant activity. However there was no statistically significant difference ($p > 0.05$) among the antioxidant activities of the cultivars. The petroleum ether extracts of *koroneiki* and *kalamon* cultivars were the least potent, due to their low polyphenol content.

Determination of Soybean Lipoxygenase Inhibitory Activity Induced by the Olive Leaf Extracts of Three Greek Cultivars

The soybean lipoxygenase assay was used as an indication of the antioxidant activity of the extracts with the higher DPPH interaction values. Linoleic acid was used as

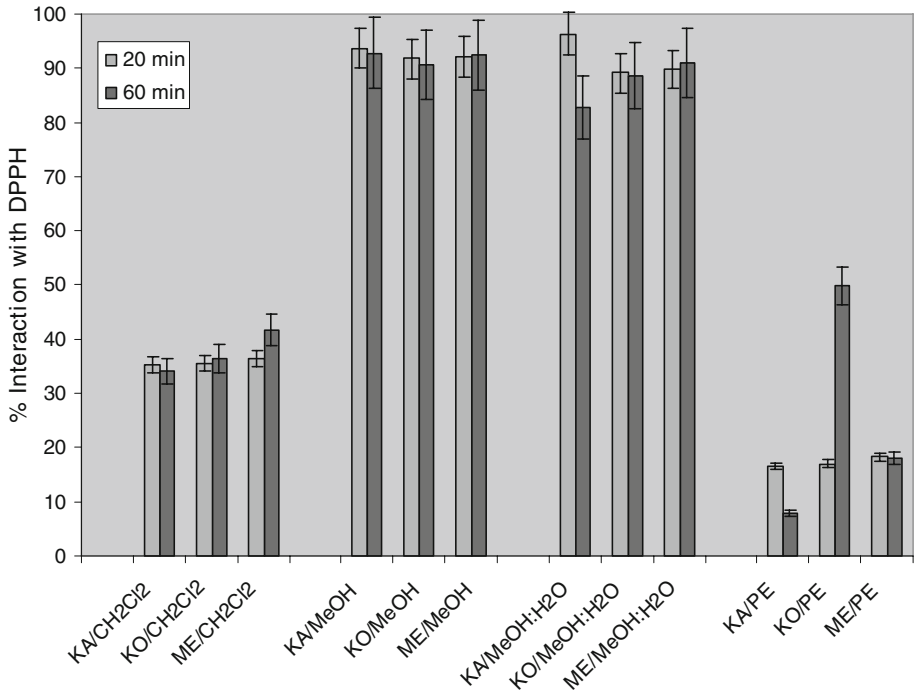

Fig. 5 Interaction of olive leaf extracts with DPPH for 20 and 60 min (Cultivars: *ME*, *megaritiki*; *KO*, *koroneiki*; *KA*, *kalamon*; CH_2Cl_2, dichloromethane; *MeOH* methanol, MeOH:H_2O (60/40); *PE*, petroleum ether)

substrate for soybean lipoxygenase. Linoleic acid is enzymatically converted to a conjugated diene, which results in a continuous increase in absorbance at 234 nm. A mixture of DMSO and buffer served as control (no enzyme inhibition), while the reported value for caffeic acid was used as a positive control.

The inhibitory activity of olive tree leaf extracts from the three different Greek cultivars was tested at two different concentrations (0.1 and 1 mg/ml). Almost all the extracts presented the same inhibitory activity (around 100%) at the higher concentration. Significant differences ($p < 0.05$) were observed at the lower concentration (0.1 mg/ml) of the extracts, which are presented at Fig. 6. The inhibitory activity was concentration dependent.

There was a statistically significant difference ($p < 0.05$) among the extracts and the cultivars, respectively. In most cases the dichloromethane, methanol and methanol/water (60:40) extracts were the most potent against lipoxygenase. The dichloromethane extracts of *kalamon* and *megaritiki* cultivars presented a higher inhibitory activity against LOX than the *koroneiki* cultivar. Most of the LOX inhibitors are antioxidants or free radical scavengers [16], since lipoxygenation occurs via a carbon-centered radical. Based on present results, it is suggested that the % DPPH scavenging activity does not always positively correlate to the LOX % inhibition as is the case of *kalamon* and *megaritiki* varieties (Fig. 6). Certain studies [19] suggest a relationship between LOX inhibition and the ability of the inhibitors to reduce Fe^{+3} at the active site to the catalytically inactive Fe^{+2}. Many flavonoids and other phenolic derivatives inhibit soybean lipoxygenase through the mechanism proposed above [19]. Thus the presence of compounds with a free –OH group could account for the inhibition effect shown in tested samples. It has been suggested that olive leaves are a source of antioxidants acting as radical scavengers [20].

Conclusion

The present study showed that the great diversity and complexity of the natural mixtures of phenolic compounds in the olive leaf extracts of various cultivars render comparison of their antioxidant activities difficult. There was also some antioxidant activity in olive leaves that may be attributed to other unidentified compounds or to synergistic interactions.

The concentration of phenols obtained from the three Greek cultivars (*koroneiki*, *megaritiki* and *kalamon*) varied with type and polarity of solvent used for the extraction. The highest amount of phenols was extracted when methanol/water (60:40) was used. Among the cultivars, *koroneiki* showed itself to have the highest concentration of phenols. Many different phenolic compounds were determined in the leaf extracts of the three olive cultivars. Of all the compounds determined, oleuropein was found in the highest concentration in all three cultivars.

When the OSI method was applied, using extra virgin olive oil as the substrate, the antioxidant activity of additives followed the order: synthetic antioxidant TBHQ > commercial oleoresin > olive tree leaf extracts > control.

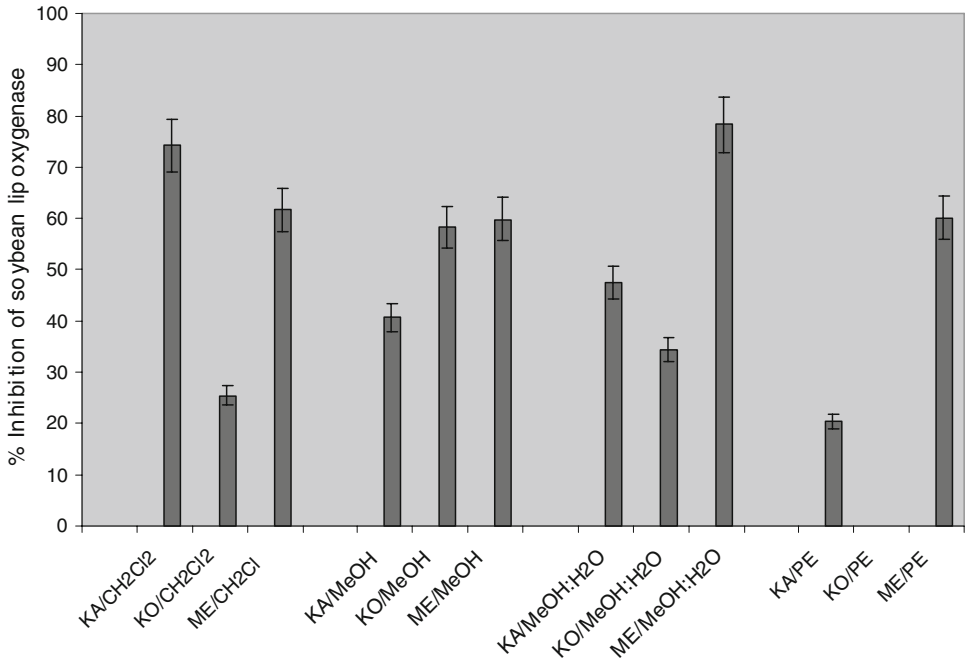

Fig. 6 Soybean lipoxygenase inhibition at concentration 0.1 mg/ml of olive leaf extracts (Cultivars: *ME*, *megaritiki*; *KO*, *koroneiki*; *KA*, *kalamon;* CH_2Cl_2, dichloromethane; *MeOH* methanol, MeOH:H_2O (60/40); *PE*, petroleum ether)

The LOX inhibitory activity was affected by the solvent polarity used. Results obtained for this enzyme, using petroleum ether as the solvent, confirmed the results of the DPPH method and gave an indication for the higher antioxidant activity of the phenolic compounds present in the cultivar *koroneiki*.

The olive leaf extracts present significant antioxidant activity which renders such products useful for the enhancement of the oxidative stability of edible oils.

Acknowledgments This project was funded by the Research Committee of the Alexander Technological Educational Institute of Thessaloniki, Thessaloniki, Greece and the Greek Ministry of Education through the ''Food Science and Nutrition'' Graduate program.

References

1. Kiritsakis A, Stine CM, Dugan LR (1983) Effect of selected antioxidants on the stability of virgin olive oil. J Am Oil Chem Soc 60:1286
2. Gharavi N, Haggarty S, El-Kadi A (2007) Chemoprotective and carcinogenic effects of *tert*-butylhydroquinone and its metabolites. Curr Drug Metab 8:1
3. Loliger J, Lambelet P, Aeschbach R, Prior EM (1996) Natural antioxidants: from radical mechanisms to food stabilization. In: McDonald RE, Min DB (eds) Food lipids and health. Marcel Dekker Inc, New York, pp 68–77
4. Gamel TH, Kiritsakis A (1999) Effect of methanol extracts of rosemary and olive vegetable water on the stability of olive oil and sunflower oil. Grasas y Aceites 50:345
5. Papagrigoriou Ch, Kiritsakis A, Botsoglou N, Kiritsakis K, Polymenopoulos Z (2007) Evaluation of the antioxidant activity of methanol plant extracts. In: Proceedings, vol. 2, Published by Hellenic Association of Food Technologists and Alexander Technological Education Institute of Thessaloniki, pp 595–599
6. Gkanatsiou K, Kiritsakis A, Kiritsakis K (2007) The effect of saffron extracts (*Crocus Sativus* L.) In: Preventing the oxidation of oils, vol. 2. Hellenic Association of Food Technologists and Alexander Technological Educational Institute of Thessaloniki, Greece, pp 595–604
7. Kähkönen M, Hopia A, Vuorela H, Rauha JP, Pihlaja K, Kujala T, Heinonen M (2000) Antioxidant activity of plant extracts containing phenolic compounds, Abstracts, 91st AOCS Annual Meeting and Expo, S121
8. Salta FN, Mylona A, Chiou A, Boskou G, Andrikopoulos NK (2007) Oxidative stability of edible vegetable oils enriched in polyphenols with olive leaf extract. Food Sci Tech Int 13:413
9. Farag RS, El-Baroty GS, Basuny AM (2003) The influence of phenolic extracts obtained from the olive plant (cvs. Picual and Kronakii), on the stability of sunflower oil. Int J Food Sci Technol 38:81
10. Farag RS, Mahmoud EA, Basuny AM (2007) Use crude olive leaf juice as a natural antioxidant for the stability of sunflower oil during heating. Int J Food Sci Technol 42:107
11. De Leonardis A, Aretini A, Alfano G, Macciola V, Ranalli G (2008) Isolation of a hydroxytyrosol-rich extract from olive leaves (*Olea europaea* L.) and evaluation of its antioxidant properties and bioactivity. Eur Food Res Technol 226(4):653
12. Bouaziz M, Fki I, Jemai H, Ayadi M, Sayadi S (2008) Effect of storage on refined and husk olive oils composition: stabilization by addition of natural antioxidants from chemlali olive leaves. Food Chem 108:253
13. Zheng W, Wang SY (2001) Antioxidant activity and phenolic compounds in selected herbs. J Agric Food Chem 49:5165
14. Kontogiorgis C, Hadjipavlou-Litina D (2005) Synthesis and antiinflammatory activity of coumarin derivatives. J Med Chem 48:6400
15. Savourin C, Baghdikian B, Elias R, Dargouth-Kesraoui F, Boukef K, Balansard G (2001) Rapid high performance liquid chromatography analysis for the quantitative determination of oleuropein in *Olea europaea* Leaves. J Agric Food Chem 49:618
16. Skerget M, Kotnik P, Hadolin M, Rizner Hras A, Simonic M, Knez Z (2004) Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities. Food Chem 89:191
17. Wanasundara UN, Shahidi F (1994) Canola extract as an alternative natural antioxidant for canola oil. J Am Oil Chem Soc 71:517
18. Wolniak M, Tomczykowa M, Tomczyk M, Gudej J, Wawer I (2007) Antioxidant activity of extracts and flavonoids from Bidens tripartita. Acta Pol Pharm 64:441
19. Muller K (1994) 5-Lipoxygenase and 12-lipoxygenase: attractive targets for the development of novel antipsoriatic drugs. Arch Pharm 327:3
20. Papoti V, Tsimidou M (2009) Impact of sampling parameters on the radical scavenging potential of olive leaves. J Agric Food Chem 57:3470