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Changes Occurring in Phenolic Compounds and α-Tocopherol of Virgin Olive Oil during Storage

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Changes occurring in the concentrations of α-tocopherol, total phenols, and complex phenols linked to 3,4-dihydroxyphenylethanol (fractions FII and FIV) and *p*-hydroxyphenylethanol (FIII) during storage of virgin olive oil under environmental conditions were studied. Under diffused light, α-tocopherol was decomposed by 79% in 4 months, whereas <45% of the phenols were lost during the same period. Among the phenols, FII showed the least stability, and decreased by 72% in 6 months. Total phenols, FIII, and FIV recorded reductions in the range of 57−63% in 6 months. When the oil was stored in the dark, α-tocohperol, total phenols, FIII, and FIV exhibited similar profiles of degradation, reducing by 39−45% in the first 6 months and 50−62% in 12 months. FII was the least stable compound in the dark and recorded a loss of 64% in 6 months and 79% in 12 months. The levels of the above antioxidants were further related to peroxide formation. Remaining levels of these compounds at PV = 20 meq/kg ranged between 50 and 73% under diffused light and between 40 and 62% in the dark.

KEYWORDS: Virgin olive oil; α-tocopherol; phenolic compounds; changes during storage; peroxide formation

INTRODUCTION

Olive oil is considered to be resistant to oxidation because of its low content of polyunsaturated fatty acids and the presence of natural antioxidants such as α-tocopherol and phenolic compounds. However, like other vegetable oils, it is susceptible to oxidation. Oxidation can take place either in the presence of light (photooxidation) (*1*) or in the dark (autoxidation) (*2*). The role of antioxidants as inhibitors of oxidation can be considered well documented (*3*−*6*).

Several studies dealing with the antioxidant activity of phenolic compounds and/or tocopherols of virgin olive oil were performed under accelerated conditions involving elevated temperatures, after the addition of known concentrations of antioxidants to an oil (*5*, *7*, *8*) or a model substrate (*9*, *10*). The addition method allows the estimation of the antioxidant effectiveness of individual components; however, a model substrate does not take into consideration the possible interactions between components that may exist in a real lipid system. Furthermore, converting accelerated method values (such as the rancimat induction time values) directly into real shelf-life terms may be misleading, as elevated temperatures may involve some loss of volatile phenolic compounds.

The antioxidant activity of phenols and tocopherols present in virgin olive oil is affected by their chemical structure. Structure−activity relationships have been used as a theoretical method for predicting antioxidant activity (*11*, *12*). The in vitro activities of antioxidants are not only dependent on their absolute chemical reactivity toward hydroperoxy and other free radicals, but also on many other factors such as concentrations, temperature, light, type of substrate, solvent, and other chemical species acting as prooxidants and synergists in the system (*13*). Thus, the mode in which the phenols and α-tocopherol react is significantly affected by the interplay of all the chemical and physical parameters of the system.

A number of complex phenolic antioxidants have been identified in the polar fraction of virgin olive oil (*6*, *14*, *15*). However, the behavior of these compounds during storage of virgin olive oil has not been elucidated.

The present study aims at monitoring the changes undergone by complex phenolic fractions and α-tocopherol in virgin olive oil during storage of the oil under environmental conditions. The changes were further related to peroxide formation in order to evaluate the effect of degree of oxidation on the remaining levels of the above compounds, during actual shelf life conditions.

MATERIALS AND METHODS

Olive Oil. Twenty olive oil samples from Lianolia variety olives grown in the region of Preveza, Greece, were used. The olives were collected at various stages of ripeness during the periods December 1997/January 1998 and December 1998/January 1999, and they were processed using a centrifugation system. Samples were supplied by various local industrial olive oil mills.

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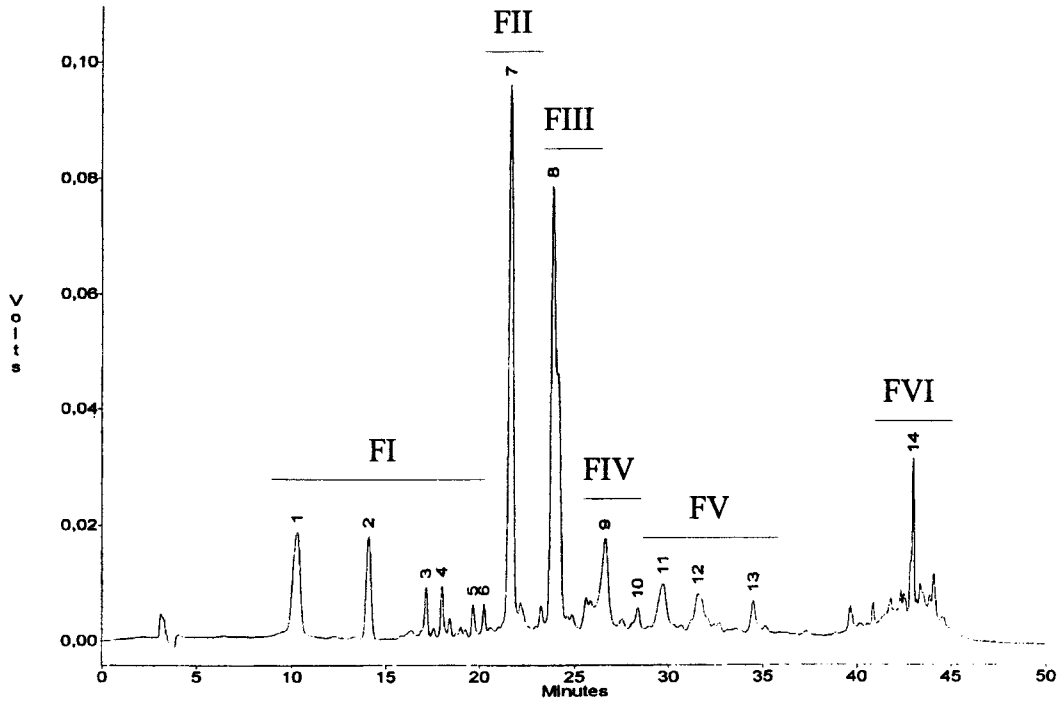

Figure 1. Separation of the phenolic extract of olive oil by reversed-phase HPLC at 280 nm. Peak numbers: (1) hydroxytyrosol; (2) tyrosol; (3) vanillic acid; (4) syringic acid; (5) *p*-coumaric acid; (6) *o*-coumaric acid; (7) hydroxytyrosol derivative; (8) tyrosol derivative; (9) complex phenol linked to hydroxytyrosol; (10) RT 28.37; (11) RT 29.71; (12) RT 31.56; (13) 34.48; (14) RT 43.00. RT = retention time in min (*19*).

Analytical Methods. The peroxide index, spectroscopic index (K_{232}) and free acidity analyses were carried out according to the methods described in IUPAC standard methods (*16*). Fatty acids were converted to methyl esters and analyzed by gas chromatography on a Carbowax column (30 cm × 0.25 mm i.d.) following the EC Regulations (*17*). Total phenols were evaluated colorimetrically at 725 nm with the Folin−Ciocalteau reagent (*18*). α-Tocopherol was evaluated by HPLC following the methodology described by Tasioula-Margari and Okogeri (*19*). The HPLC and GC−MS analyses of phenolic compounds extracted from the virgin olive oil samples were carried out according to Tasioula-Margari and Okogeri (*15*). The phenolic compounds, as shown in **Figure 1**, were grouped into fractions. Fraction I (FI) comprised peaks 1−6, while Fractions II, III, and IV (FII, FIII, and FIV) comprised peaks 7, 8, and 9, respectively.

Stability Study. Transparent glass bottles (100 mL) were filled with oil samples, such that the headspace in each bottle was about 3 mL, and were tightly capped. One set of samples was stored under diffused light, while another set was sealed in a carton and stored in the dark. At 2-month intervals (for the samples stored under diffused light) and at six-months intervals (for the samples stored in the dark), samples were withdrawn from storage and analyzed for α-tocopherol, total phenols, phenolic fractions, peroxide values, and conjugated dienes. Enough glass bottles for each oil sample were used so that no sample, once withdrawn from storage and analyzed, had to be reused. The average temperatures during winter and summer were 6 and 18 °C, respectively.

RESULTS AND DISCUSSION

Table 1 presents the results obtained from analyses of the initial compositions of the twenty olive oil samples used for this study. Peroxide values (PV), conjugated dienes as measured by extinction value at 232 nm (K_{232}), and free acidity (with few exceptions) fell within the limits for extra-type virgin olive oil quality established by the International Olive Oil Council (*20*). The mean values of the main saturated (palmitic), monounsaturated (oleic), and polyunsaturated (linoleic) acids were 15.2, 73.0, and 8.6%, respectively. Among the substances with antioxidant properties, the total phenols varied considerably

Table 1. Initial Composition of Olive Oil Samples (n = 20)

	mean ± SD	range
free acidity (%)	0.5 ± 0.4	0.1–1.5
peroxides (meq·kg^{-1})	7.2 ± 2.4	3.6–11.6
conjugated dienes (K_{232})	1.2 ± 0,4	0.8–1.9
α-tocopherol (mg·kg^{-1})	146.8 ± 41.6	82.6–235.2
total phenols[a]	320 ± 191	92–850
fraction I[b] (mg·kg^{-1})	4.9 ± 2.5	2.6–7.5
fraction II[b]	50.1 ± 49.6	7.4–204.0
fraction III[b]	70.8 ± 31.1	13.3–144.2
fraction IV[b]	6.7 ± 2.8	3.0–12.7
fatty acid (% peak area)		
C16:0	15.2 ± 1.2	13.1–17.4
C16:1	1.1 ± 0.2	0.9–1.5
C18:0	1.6 ± 0.2	1.2–2.0
C18:1	73.0 ± 2.4	68.3–77.3
C18:2	8.6 ± 0.8	7.2–10.4
C18:3	0.5 ± 0.1	0.4–0.8

[a] Evaluated colorimetrically and expressed as mg.kg^{-1} tyrosol equivalent. [b] Analyzed by HPLC and expressed as mg.kg^{-1} tyrosol equivalent.

(92−850 mg·kg^{-1}, expressed as tyrosol equivalent). Such variations are in conformity with other findings (*14*) and may be attributed to factors such as maturity stage of olives, harvesting technology, and oil extraction methodology. The concentration of α-tocopherol ranged between 83 and 235 mg/kg. Phenolic fractions II and III were present in greater amounts in all the samples analyzed. GC−MS analysis (*15*) showed that both fractions comprise complex compounds containing 3,4-dihydroxyphenylethanol (hydroxytyrosol) and *p*-hydroxyphenylethanol (tyrosol), respectively, and are identical with those reported by Montedoro et al. (*14*). Fraction I, comprising the simple phenolic compounds, did not exceed 7% of the total phenolic constituents (**Table 1**). The compounds more substantially represented in this fraction were hydroxytyrosol and tyrosol (**Figure 1**). Changes occurring in these compounds during storage and the antioxidant effectiveness of the compounds present in fraction I, at their various concentrations, have

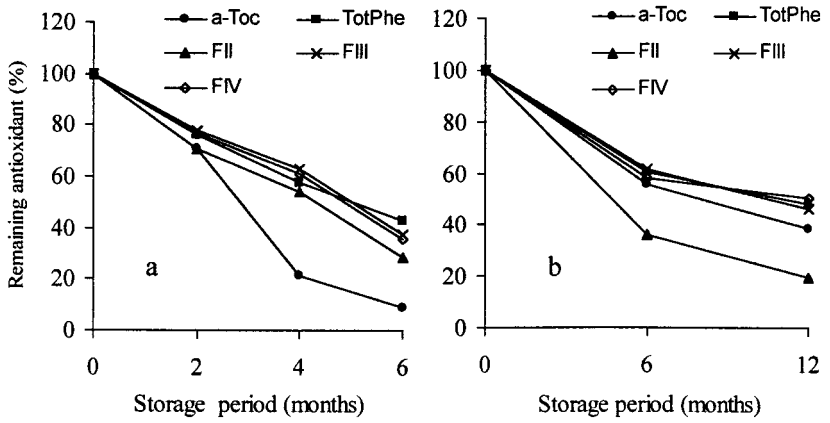

Figure 2. Changes in the concentrations of antioxidants during storage of samples under diffused light for 6 months (a) and in the dark for 12 months (b). aToc = α-tocopherol; TotPhe = total phenols; FII, FIII, FIV = fractions II, III, and IV.

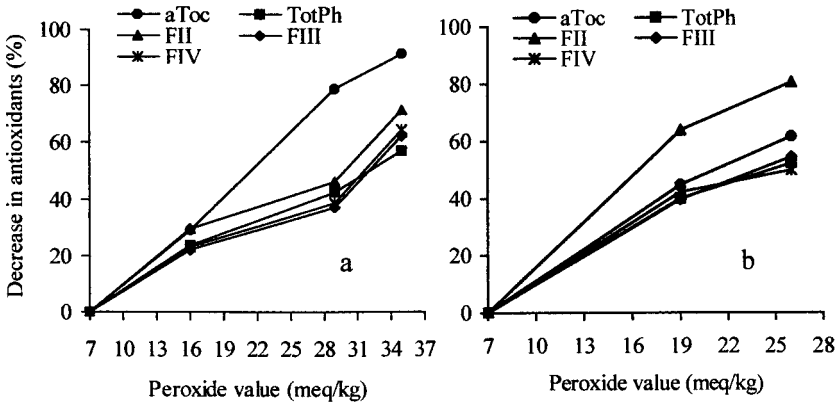

Figure 3. Changes in the levels of antioxidants at various peroxide values during storage of samples under diffused light (a) and in the dark (b). aToc= α-tocopherol; TotPhe = total phenols; FII, FIII, FIV = fractions II, III, and IV.

been reported by several authors (*4*, *6*, *21*). Therefore, interest was focused on fractions II, III, and IV (FII, FIII, and FIV), which were the major compounds (>85% of total phenolic constituents) present in all the samples analyzed.

Changes During Storage. Figure 2 depicts the relationship between remaining levels of α-tocopherol, total phenols, and phenolic fractions (expressed in percentage of initial concentrations), and storage period. During the first 2 months of storage under diffused light (**Figure 2b**), all compounds exhibited similar profiles of degradation, recording a decrease which ranged between 22% (FIII) and 30% (FII and α-tocopherol). In 4 months, the concentration of α-tocopherol decreased dramatically (79% decrease), indicating its rapid degradation. The role of α-tocopherol as a physical and chemical quencher of singlet oxygen during photooxidation (*22*) accounts for this rapid drop. However, after 4 months the drop in α-tocopherol seemed to stabilize, possibly due to its remaining low concentration. Storage under diffused light also accelerated the degradation of total phenols and the phenolic fractions, particularly FII, which decreased by 72% in 6 months. In general, the reductions observed for the phenols were remarkably less than those observed for α-tocopherol and are in conformity with the findings of Rahmani and Csallany (*23*).

An element common to all compounds during storage in the dark is the lesser rate of reduction. A similar falling trend was observed for α-tocopherol (62%), total phenols, FIII, and FIV (50−54%) during 12 months storage (**Figure 2b**). On the other hand, FII showed a degradation of 79% in 12 months, and was the least stable compound during storage in the dark. Considering that the degree of reduction is related to the degree of antioxidant activity, it is speculated that FII (a dihydroxy derivative) showed the highest antioxidant activity in the dark. The lower reduction, and therefore lower antioxidant activity, observed for FIV (also a dihydroxy derivative) may be due to its low initial concentration in the oil samples (**Table 1**). The reductions of FIII (a monohydroxy derivative) and α-tocopherol in the dark suggest a synergistic effect between these compounds and FII. It has been reported that α-tocopherol is more effective as an antioxidant when present in combination with other antioxidants or synergistic compounds (*5*, *24*).

Some authors (*21*) have reported an increase in the content of hydroxytyrosol and tyrosol during olive oil storage, and they attributed this phenomenon to the hydrolysis of complex phenols (FII and FIII in this study) which resulted in the formation of hydroxytyrosol and tyrosol. In the present study, a substantial decrease in the amounts of FII and FIII was observed both under diffused light and in the dark (**Figure 2**) however, no remarkable increase was observed in any of the compounds present in fraction I. Therefore, if there was any hydrolytic activity on FII and FIII, its degree was possibly too low to have a modification effect on hydroxytyrosol and tyrosol.

Degree of degradation during storage was determined from the formation of hydroperoxides (expressed as peroxide values) and conjugated dienes (**Table 2**). Samples exposed to light recorded an average peroxide value of 34.7 meq/kg in 6 months, whereas samples in the dark did not exceed this value in 12 months (PV = 25.8 meq/kg, **Table 2**). In the dark only conjugated dienes are formed. In the light nonconjugated dienes formed in addition to conjugated dienes resulted in higher

Table 2. Changes in Peroxide Value and Conjugated Dienes during Storage under Diffused Light and in the Dark

storage period (months)	peroxide value (meq/kg)		conjugated dienes (K232)	
	mean[a]	range	mean[a]	range
		diffused light		
2	16.1	10.8–21.4	2.1	1.7–2.5
2.6[b]	20.0	-		
4	29.1	15.1–32.2	2.2	1.8–2.6
6	34.7	18.9–50.1	2.6	2.2–5.4
		dark		
6	19.2	14.7–28.8	2.3	1.8–3.3
7.7[b]	20.0	-		
12	25.8	20.0–36.0	2.9	2.3–4.7

[a] n=20. [b] Calculated from a linear relationship between storage periods and peroxide values.

peroxide value (K232 = 2.9 at 25.8 meq/kg in the dark versus K232 = 2.6 at 34.7 meq/kg under diffused light, **Table 2**).

Levels of Antioxidants at Varying Peroxide Values. At PV of 34.7 meq/kg, which was the highest PV recorded during 6 months storage under diffused light, α-tocopherol decreased by 92%, and the phenols decreased by 57−70% (**Figure 3a**). At PV of 25.8 meq/kg, which was the maximum PV recorded during 12 months storage in the dark, FII decreased by 79%, and all the other antioxidants decreased by 52−61% (**Figure 3b**).

At PV= 20 meq/kg (which is the upper limit set by the International Olive Oil Council (*20*) for extra-type virgin olive oil) 56% of α-tocopherol and up to 73% of phenolic fractions remained under diffused light (**Figure 3a**), while in the dark and at the same peroxide value, 40% of FII and 54−62% of all the other antioxidants remained (**Figure 3b**). However, it should be noted that under diffused light, PV of 20 meq/kg was attained in 2.6 months, whereas in the dark the same value was attained in 7.7 months (**Table 2**).

The above findings indicate that significant amounts of antioxidants are retained during olive oil storage, with the degree of reductions being in the following order: α-tocopherol > FII > FIII ≅ FIV > total phenols during storage under diffused light, and FII > α-tocopherol > FIII ≅ FIV ≅ total phenols during storage in the dark.

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