α-Tocopherol Content of Greek Virgin Olive Oils

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α-Tocopherol, the main tocopherol homologue found in olive oil, was determined using normal phase HPLC. Ninety Greek virgin olive oils, selected according to a designed sampling protocol from different cultivars and regions all over Greece for three successive crop years, were analyzed. For a specific olive cultivar, which is widely used for the production of olive oil in Greece, additional measurements were made to study the effect of milling conditions on α-tocopherol concentration. Finally, a significant number of commercial olive oil samples (25) obtained from the retail market were analyzed. High concentrations of α-tocopherol were observed in most of the samples selected from various regions. Values ranging between 98 and 370 mg/kg were found (>200 mg/kg in 60% of samples). Extraction conditions were not found to influence α-tocopherol level. α-Tocopherol content of retail market samples was high, ranging from 120 to 250 mg/kg of oil (>180 mg/kg in 60% of samples). Storage of samples under domestic conditions for two years showed that good handling is quite important for retaining high α-tocopherol levels and for increasing, thus, the storage life and nutritional value of this exquisite oil.

Keywords: *α-Tocopherol content; Greek virgin olive oil; milling conditions; keepability; nutritional value*

INTRODUCTION

Tocopherols are methyl-substituted chromanols with a three-isoprene moiety side chain. The four tocopherols, α-, *β*-, *γ*, and *δ*-tocopherols, differ from one another by the number and position of methyl groups in the phenolic part of the chromane ring. The α homologue contains three methyl groups, the *β* and *γ* homologues are dimethylated positional isomers, and *δ*-tocopherol is monomethylated. The configuration at the three asymmetric centers, 2, 4′, and 8′, is *R* (Kamal-Eldin and Appelqvist, 1996). These compounds possess vitamin E activity, and the biopotency of natural *R*,*R*,*R*-α-tocopherol is the highest, 1.49 IU/mg relative to the *all-rac*-α-tocopheryl acetate, which is the reference standard with a biological activity of 1.0 IU/mg (Eitenmiller and Landen, 1999). Moreover, tocopherols effectively inhibit lipid oxidation in foods and biological systems (Kamal-Eldin and Appelqvist, 1996).

Major dietary sources of tocopherols are vegetable oils. Data from the National Health and Nutrition Examination Survey (NHAVES II) showed that fats and oils accounted for >20% of the vitamin E in the United States diet (Eitenmiller and Landen, 1999). The daily requirement increases when the diet contains a high content of unsaturated fatty acids. For example, the requirement of α-tocopherol is 0.09 mg/g of monoene fatty acids and 0.4−0.6 mg/g of dienoic acids (Belitz and Grosch, 1999). Published lists (National Research Council, 1989) for recommended daily requirements suggest 10 and 8 mg for men and women, respectively. According to prevailing nutritional views, olive oil, although rich in monounsaturated fats, also supplements the required daily essential fatty acids intake and protects from vitamin E deficiency (Kafatos and Comas, 1990; Lenart et al., 1998). Olive oil is now recognized worldwide as a healthful oil, and this commodity is now gaining popularity in other countries. Significant quantities are exported from Italy, Spain, and Greece to the United States, Australia, and Japan.

Extensive research work concerning the occurrence and levels of α-tocopherol has been carried out mainly for Italian (Conte et al., 1993; Fedeli and Cortesi, 1993; Esti et al., 1996; Ranalli and Angerosa, 1996; Manzi et al., 1998) olive oils and, to a lesser extent, for olive oils from other producing countries (Cert et al., 1996; Salvador et al., 1998). Such a systematic work is lacking for Greek olive oil. The present work aims at obtaining compositional data by analyzing a large number of Greek virgin olive oils selected from different cultivars and regions all over Greece during a period of three production years according to a designed sampling protocol. In addition, for the Koroneiki cultivar, which is widely used for the production of olive oil in Greece, the effect of the conditions of milling on tocopherol content was studied. Finally, a significant number of commercial olive oil samples obtained from the retail market were analyzed, and keepability of samples preserved under domestic conditions was examined.

MATERIALS AND METHODS

Samples. Ninety virgin olive oil samples from various olive oil mills all over Greece for three successive crops were obtained directly from the production line with the aid of Elais, SA (Piraeus, Greece). The oils were collected on the basis of a certain protocol related to Good Manufacturing Practice (Petrakis, 1994) and were stored in a freezer at −18 °C until analysis.

For the study of the effect of milling conditions on α-tocopherol content, 34 samples were produced at a pilot scale in

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10.1021/jf990993o CCC: $19.00
Published on Web 04/21/2000

Table 1. α-Tocopherol (α-T) Concentration[a] of Greek Virgin Olive Oils from Various Geographic Areas and Different Cultivars

1994−1995			1995−1996			1996−1997		
geographic region	cultivar	α-T, mg/kg	geographic region	cultivar	α-T, mg/kg	geographic region	cultivar	α-T, mg/kg
Chania, Crete	Koroneiki	309	Chania, Crete	Koroneiki	199	Chania, Crete	Koroneiki	212
Chania, Crete	Koroneiki	251	Chania, Crete	Koroneiki	239	Chania, Crete	Koroneiki	247
Chania, Crete	Koroneiki	286	Chania, Crete	Koroneiki	189	Rethimno, Crete	Koroneiki	274
Chania, Crete	Koroneiki	332	Rethimno, Crete	Koroneiki	207	Iraklio, Crete	Koroneiki	210
Rethimno, Crete	Koroneiki	254	Iraklio, Crete	Koroneiki	192	Iraklio, Crete	Koroneiki	252
Iraklio, Crete	Koroneiki	236	Iraklio, Crete	Koroneiki	225	Lasithi, Crete	Koroneiki	240
Iraklio, Crete	Koroneiki	225	Lasithi, Crete	Koroneiki	193	Lasithi, Crete	Koroneiki	212
Lasithi, Crete	Koroneiki	257	Lasithi, Crete	Koroneiki	221	Korinthia, Peloponnese	Manaki	197
Lasithi, Crete	Koroneiki	263	Achaia, Peloponnese	Koroneiki	133	Argolida, Peloponnese	Manaki	213
Lasithi, Crete	Koroneiki	220	Korinthia, Peloponnese	Chondrolia + Manaki + Megaritikes	166	Argolida, Peloponnese	Manaki	210
Lasithi, Crete	Koroneiki	209	Argolida, Peloponnese	Manaki + Chondrolia	279	Arcadia, Peloponnese	Manaki	171
Lasithi, Crete	Koroneiki	193	Arcadia, Peloponnese	Manaki	219	Lakonia, Peloponnese	20% Koroneiki + 80% Athinolia	298
Argolida, Peloponnese	Tsounati	247	Lakonia, Peloponnese	20% Koroneiki + 80% Athinolia	179	Lakonia, Peloponnese	Koutsourelia	125
Arcadia, Peloponnese	Chondrolia	288	Lakonia, Peloponnese	50% Koroneiki + 50% Asprolia	291	Lakonia, Peloponnese	Athinolia	349
Achaia, Peloponnese	Patrini	127	Lakonia, Peloponnese	95% Koroneiki + 5% Athinolia	309	Lakonia, Peloponnese	Koroneiki	181
Korinthia, Peloponnese	Tsounati	233	Messinia, Peloponnese	Koroneiki	228	Lakonia, Peloponnese	90% Athinolia + 10% Mourtolia	243
Korinthia, Peloponnese	Tsounati	194	Messinia, Peloponnese	Koroneiki	200	Lakonia, Peloponnese	Athinolia	241
Lakonia, Peloponnese	60% Koroneiki + 40% Asprolia	345	Ilia, Peloponnese	Koroneiki	315	Messinia, Peloponnese	Koroneiki	100
Lakonia, Peloponnese	Athinolia	232	Ilia, Peloponnese	Koroneiki	158	Messinia, Peloponnese	Koroneiki	131
Lakonia, Peloponnese	90% Koroneiki + 10% Athinolia	271	Ilia, Peloponnese	Koroneiki	147	Messinia, Peloponnese	Koroneiki	134
Ilia, Peloponnese	Koroneiki	309	Etoloakarnania	Koroneiki + Chondrolia	143	Messinia, Peloponnese	Koroneiki	199
Ilia, Peloponnese	Koroneiki	260	Evia	Kalamon + Amfissis	154	Ilia, Peloponnese	Koroneiki	324
Messinia, Peloponnese	Koroneiki	287	Fthiotida	Amfissis	220	Ilia, Peloponnese	Koroneiki	153
Messinia, Peloponnese	Koroneiki	242	Magnisia	Amfissis	196	Ilia, Peloponnese	Koroneiki	146
Messinia, Peloponnese	Koroneiki	251	Leukada	Lianolia	333	Etoloakarnania	Koroneiki	246
Messinia, Peloponnese	Koroneiki	195	Zakinthos	Koroneiki	266	Zakinthos	Koroneiki	365
Evia	Koroneiki	155	Lesvos	Kolovi	215	Lesvos	Adramatiani + Kolovi	140
Preveza	Koroneiki + Chondrolia	370	Chalkidiki	Chondrolia	171	Lesvos	Kolovi	163
Zakinthos	Koroneiki	364	Chalkidiki	Chondrolia	98	Chalkidiki	Chondrolia	134
Dodekanissa	Chondrolia	162	Samos	Koroneiki + Chondrolia + Throumbolia	247	Samos	Throumbolia	204

[a] Mean values of at least two replications. Repeatability data are given under Materials and Methods.

the Institute of Subtropical Plants and Olive Tree (Chania, Crete, Greece). These samples were obtained from Koroneiki cultivar fruits, harvested at three different periods for two successive crop years. The olive fruits were processed just after harvest in the Institute using three different extraction systems: a "classical press" a "three-phase centrifugal system", and a "two-phase decanter". The olive paste was subjected to malaxation at two different temperatures, 30 and 45 °C. The temperature of 30 °C is the condition used for the extraction of the so-called "cold pressed oils". The temperature of 45 °C was chosen as a condition that supports higher oil yields. Twenty-five commercial extra virgin olive oils were purchased from local supermarkets.

Solvents and Standards. The solvents, HPLC grade, were used without further purification. *n*-Hexane was Baker, HPLC 95% (Deventer, Holland), and 2-propanol (Chromasolv) was from Riedel de-Häen (Seelze, Germany). DL-α-Tocopherol (99% for biochemistry) and a set of α-, β-, γ−, and δ-tocopherols (for biochemistry) were purchased from Merck (Darmstadt, Germany).

Apparatus. The solvent delivery system consisted of two Marathon IV series HPLC pumps (Rigas Laboratories, Thessaloniki, Greece) and a Rheodyne injection valve (model 7125) with a 20 μL fixed loop (Rheodyne, Cotati, CA). The liquid chromatograph was equipped with a UV−vis spectrophotometric detector SPD-10AV (dual wavelength) Shimadzu (Kyoto, Japan). Fluorescence detection was accomplished by an SSI 502 programmable fluorescence detector (Scientific Systems Inc., State College, PA) set at 294 nm (excitation) and 330 nm (emission). The data from the UV−vis SPD-10AV detector were stored and processed with the chromatographic software EZChrom (Scientific Software, Inc., San Ramon, CA). Absorbance measurements were taken by a Hitachi U-2000 spectrophotometer (Hitachi Ltd., Tokyo, Japan) in 1 cm quartz cells.

HPLC Separation, Identification, and Quantification of Tocopherols of Virgin Olive Oil. The elution system was *n*-hexane/2-propanol (99:1, v/v). Separation was achieved on a 250 × 4 mm i.d. LiChrospher-Si 60, 5 μm, column (Analyzentechnik, Mainz, Germany) at 1.2 mL/min flow rate. The injection volume was 20 μL. α-Tocopherol standards and samples (8% m/v) were prepared for analysis by dilution into the elution solvent. Oil sample solutions were filtered through a 0.45 μm membrane filter (Schleicher & Schuell, Dassel, Germany) just before HPLC analysis. Care was taken to exclude sunlight exposure of samples and standard solutions throughout the analytical procedure. Tocopherols were detected at 294 nm by the UV−vis detector. Identification of β-, γ-, and δ-tocopherols was based on standards. Their relative retention times were also verified using (a) a fluorescence detector and (b) peak spectra using a diode array detector (linear UVIS-206 multiple wavelength system, Linear Instruments, Fremont, CA). Standard curves (concentration of α-tocopherol versus peak area) were constructed by linear regression analysis of data. The repeatability (CV%) of injection volume within the same day was 2.3% ($n = 6$). To determine measurement precision, an oil sample was injected six times. The calculated coefficient of variation was 5.0%.

Figure 1. Main olive oil producing regions in Greece: 1, Chalkidiki; 2, Magnisia; 3, Evia; 4, Fthiotida; 5, Etoloakarnania; 6, Preveza; 7, Lefkada; 8, Zakinthos; 9, Achaia (Peloponnese); 10, Ilia (Peloponnese); 11, Arcadia (Peloponnese); 12, Messinia (Peloponnese); 13, Lakonia (Peloponnese); 14, Argolida (Peloponnese); 15, Korinthia (Peloponnese); 16, Chania (Crete); 17, Rethimno (Crete); 18, Iraklio (Crete); 19, Lasithi (Crete); 20, Dodekanissa; 21, Samos; 22, Lesvos.

Injections in duplicate were made at each concentration for both standards and samples. Concentration of α-tocopherol standard solutions was calculated from absorbance values at 292 nm divided by a correction factor of 0.0076 as suggested by Pocklington and Dieffenbacher (1988). Calibration curves were tested daily, and injections of standard solutions were made after every two samples.

Keepability Tests. Five samples were selected from the set of the 90 virgin olive oils on the basis of some quality parameters such as fatty acid composition, acidity, peroxide value, absorbance at 232 nm (EC, 1991), total phenol content (Gutfinger, 1981), and induction periods (Rancimat at 120 °C; Servili et al., 1996). Virgin olive oil samples were stored in the dark at 22 ± 6 °C. Transparent glass bottles were completely filled with oil and sealed. No headspace was left in the bottles, which were covered with aluminum foil and kept in a carton box. Peroxide values and absorbance at 232 nm were periodically measured (EC, 1991).

Statistical Analysis. A computer package, Statgraphics version 5 (Statistical Graphics Corp.) was used for univariate and multiple regression analysis between α-tocopherol content and acidity and between α-tocopherol content and fatty acid composition (16:0, 18:0, 18:1, 18:2, 18:3).

RESULTS AND DISCUSSION

Table 1 presents the results obtained from the analysis of 90 samples from the main olive oil producing regions in Greece (Figure 1) during three crop years (1994−1995, 1995−1996, 1996−1997). It is clear from the data of this table that ranges for samples from each year of harvest are wide (127−370, 98−333, and 100−365 mg/kg). For the overall range of α-tocopherol (98−370 mg/kg) frequency distribution analysis indicated that the majority of samples (>60%) contained >200 mg/kg. These values appear to be much higher compared to those found for a limited number of virgin olive oil samples analyzed by Tsimidou (1985) and Andrikopoulos et al. (1989), who reported ranges of 59−186 mg/kg ($n = 14$) and 81−142 mg/kg ($n = 5$), respectively. Such differences should be related to the improvement of milling technology and overall quality control programs and the European Union regulations (EC, 1991), which impose strict limits for all edible types of olive oil. Compared to Italian and Spanish virgin olive oils, Greek oils have α-tocopherol levels that are among the highest reported [Conte et al., 1993 (55−264 mg/kg, $n = 18$); Fedeli and Cortesi, 1993 (97−315 mg/kg, $n = 52$); Cert et al., 1996 (103−283 mg/kg, $n = 14$); Esti et al., 1996 (100−320 mg/kg, $n = 23$); Ranalli and Angerosa, 1996 (147−187 mg/kg, $n = 6$); Manzi et al., 1998 (160−253 mg/kg, $n = 15$); Salvador et al., 1998 (55−234 mg/kg, $n = 65$)].

Homologues β- and γ-tocopherols were found to range from trace to 9 mg/kg and from trace to 40 mg/kg, respectively, whereas the mean value for δ-tocopherol was 4 mg/kg. It has been reported (Kamal-Eldin and Andersson, 1997) that factors such as the degree of unsaturation and the presence of anti- and/or prooxidants may influence the forms and levels of tocopherols naturally encountered in an oil. Virgin olive oil contains chlorophyll pigments, and this may partially explain the high levels of α-tocopherol, which is the most active homologue in deactivating singlet oxygen (Grams and Eskins, 1972). The reported loose relationship between tocopherol level and acidity (Tsimidou, 1985) was not observed in this work using univariate or multivariate regression analysis ($r = 0.18$, $p < 0.05$). This is probably due to the mode of sample selection. A strict protocol was applied, and the samples had very small differences in acidity (<0.9% as oleic acid) and other quality parameters. A low correlation was found between α-tocopherol content and the sum of oleic and linoleic content (0.49, $p < 0.05$). No correlation was found with linoleic acid content.

The samples used in this study were obtained from the main oil-producing cultivars grown in various regions in Greece. Greek cultivars can be divided into two major categories based on the distribution of oleic and linoleic acids (Synouri et al., 1995). The first one includes cultivars such as Koroneiki, Tsounati, and Athinolia, whereas the second covers cultivars such as Manaki, Adramytiani, Kolovi, Throumba, and Hondrolia. Oils from the former group are obtained from cultivars bearing small sized fruit; those in the latter group are produced from cultivars with medium sized fruit. Frequency histograms for oil samples belonging to the two groups revealed some differences (Figure 2), which may be due to varietal characteristics. In a total of 64 samples 40% contained >250 mg/kg α-tocopherol, whereas only 11.5% of the 26 samples belonging to the second group appeared to contain such levels of α-tocopherol. Moreover, 20% of the samples of the small-sized fruit category presented levels of tocopherols >300 mg/kg. Although no marked differences were observed for oils from Koroneiki fruits in relation to geographical origin, it could be said that oils from Crete had somewhat higher contents of α-tocopherol (mean value = 239 mg/kg, $n = 25$) in comparison to oils from Peloponnese (mean value = 198 mg/kg, $n = 18$).

To gain insight into the effect of extraction technology on the concentration of α-tocopherol in olive oil, a separate set of samples was analyzed. The latter were obtained from Koroneiki fruits at a pilot scale using

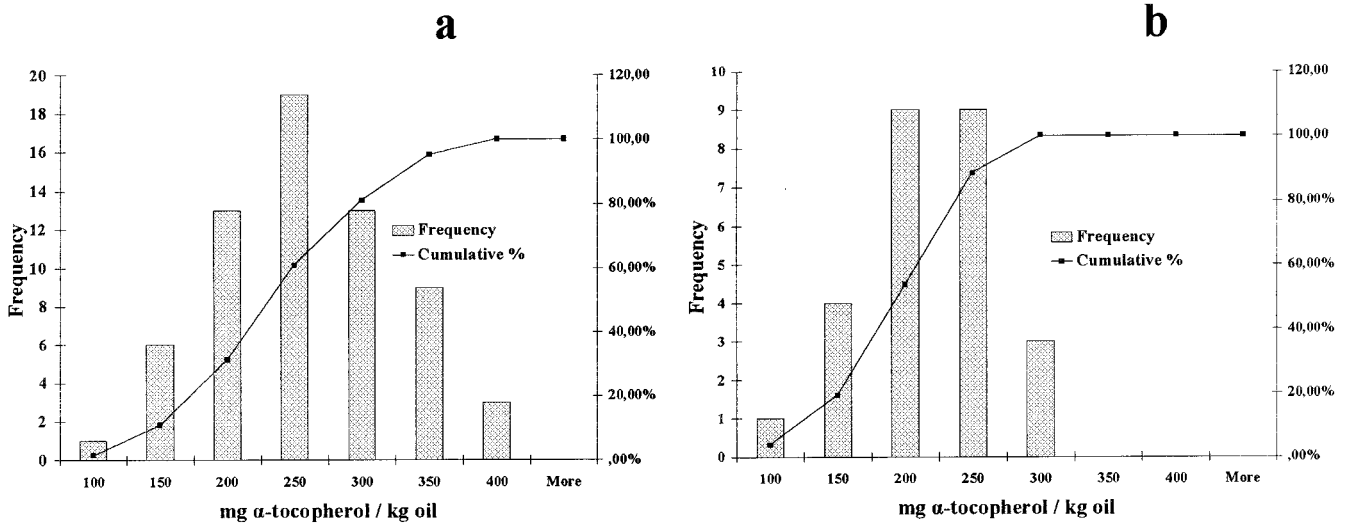

Figure 2. Frequency histograms of α-tocopherol content of virgin olive oils from different cultivars: (a) small sized olives; (b) medium sized olives.

Table 2. α-Tocopherol Content of Monovariety Virgin Olive Oils (Koroneiki) from Different Extraction Systems

1995−1996		1996−1997	
sample (harvest period/extraction system)	α-tocopherol, mg/kg	sample (harvest period/extraction system)	α-tocopherol, mg/kg
November/classical, 30 °C	271	November/classical, 30 °C	nm[a]
November/classical, 45 °C	277	November/classical, 45 °C	nm
November/two-phase, 30 °C	288	November/two-phase, 30 °C	321
November/two-phase, 45 °C	295	November/two-phase, 45 °C	315
November/three-phase, 30 °C	277	November/three-phase, 30 °C	304
November/three-phase, 45 °C	287	November/three-phase, 45 °C	313
December/classical, 30 °C	270	December/classical, 30 °C	249
December/classical, 45 °C	275	December/classical, 45 °C	258
December/two-phase, 30 °C	283	December/two-phase, 30 °C	250
December/two-phase, 45 °C	286	December/two-phase, 45 °C	260
December/three-phase, 30 °C	269	December/three-phase, 30 °C	250
December/three-phase, 45 °C	285	December/three-phase, 45 °C	269
January/classical, 30 °C	274	January/classical, 30 °C	281
January/classical, 45 °C	298	January/classical, 45 °C	302
January/two-phase, 30 °C	290	January/two-phase, 30 °C	278
January/two-phase, 45 °C	325	January/two-phase, 45 °C	302
January/three-phase, 30 °C	294	January/three-phase, 30 °C	253
January/three-phase, 45 °C	305	January/three-phase, 45 °C	278

[a] nm, the values are not given because the trial was unsuccessful due to technical problems.

three different extraction systems operated under the rules of Good Manufacturing Practice. Table 2 shows the α-tocopherol content of 34 samples produced in two successive crop seasons using two different temperatures for malaxation of olive paste. The use of classical systems in Greece is now rare because in the past decade they were gradually replaced by centrifugals (three-phase). A novelty in this area is the "ecological" or "two-phase decanters". In these systems no water is added during the milling process, and in this way polar phenolic antioxidants are retained (Di Giovaccino, 1996). High levels of α-tocopherol were found in all of the samples (range = 249−325 mg/kg), but no observable differences were found in relation to the system of extraction used. This was verified by three different tests with samples obtained from olives harvested at three different dates. The results are in agreement with those reported earlier by Ranalli and Angerosa (1996) for Italian oils. Obviously, milling conditions are important for the level and nature of other quality characteristics (aroma, total phenol content), but the impact to the tocopherol content seems to be negligible when Good Manufacturing Practice is applied.

Table 3. α-Tocopherol Content of Commercial Greek Extra Virgin Olive Oil Samples

brand[a]	sample[b]	α-tocopherol,[c] mg/kg	brand	sample	α-tocopherol, mg/kg
A	i	184	G	i	177
	ii	122		ii	150
B	i	200	H	i	194
	ii	168		ii	215
C	i	219	I	i	158
	ii	250		ii	180
D	i	129	J	i	206
	ii	163		ii	178
E	i	188	K	i	191
	ii	222		ii	204
F	i	227	L	i	197
	ii	198	M	i	136
			N	i	244

[a] A−N, various brands. [b] i, ii, samples analyzed within a certain brand. [c] Mean values of at least two replications

Table 3 presents the results from the analysis of 25 samples of extra virgin olive oil obtained from local retail stores. Bearing in mind the biological value of olive oil, it is important to monitor α-tocopherol levels in bottled olive oil, which is actually consumed. From

Table 4. Quality Characteristics of Virgin Olive Oil Samples Used in the Keepability Tests

sample	acidity, % oleic acid	C18:2, %	peroxide value, mequiv of O_2/kg	K_{232}[a]	total phenol content, mg/kg of caffeic acid	OSI induction period, h
1	0.91	8.36	16.3	2.48	238	10.1
2	0.24	7.14	12.1	1.94	96	8.3
3	0.39	9.20	6.4	1.56	91	6.7
4	0.74	13.54	11.9	2.46	47	4.8
5	0.46	4.58	7.0	1.45	76	12.0

[a] K_{232} = absorbance at 232 nm/$C_{g/100mL}$.

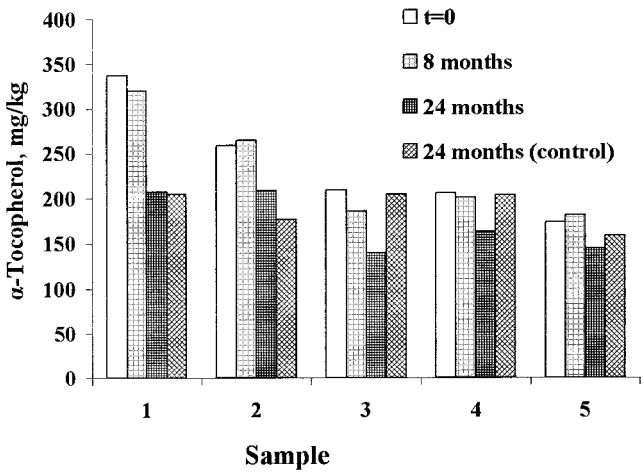

Figure 3. Changes in α-tocopherol content of samples stored in the dark at 22 ± 6 °C for 24 months.

the values of Table 3 it is clear that the high levels of α-tocopherol found in the selected samples are also met in commercial oils that were randomly picked up from the market (range = 120−250 mg/kg of oil, >180 mg/kg in 60% of the samples). Taking into account that the per capita consumption of olive oil in Greece is ~20 kg (Boskou, 1996) and that most of this quantity is virgin olive oil, the intake of α-tocopherol from this source covers >50% of the daily requirement.

In a previous paper (Psomiadou and Tsimidou, 1998) it was shown that α-tocopherol losses may occur during storage under the conditions prevailing in store shelves (relatively high temperatures, alternate exposure to dark and diffused light). Lower values observed in some commercial samples can be explained by such losses because ideal conditions of storage are not always easy to obtain in the chain of product distribution. Further losses may be expected during domestic use. It is therefore important to educate people to handle the oil properly so that its nutritional value is preserved until its final use. To demonstrate how important handling is for the preservation of olive oil quality and nutritional value, keepability tests were additionally carried out. Five samples selected on the basis of the quality characteristics shown in Table 4 were stored in the dark. Changes in α-tocopherol content were measured periodically in one series of bottles imitating domestic use. A second series of bottles was stored under the same conditions and opened only after 24 months, at a date beyond the legal "expiration date" of samples. The results are shown in Figure 3. Considerable tocopherol losses were observed in samples opened periodically within the period of two years due to the renewal of oxygen supply. Tocopherol losses in samples kept sealed (coded as control samples) for two years were insignificant with the exception of oils 1 and 2. This is probably due to the overall oxidative status of these oils as indicated by the high acidity and initial peroxide values (Table 4). It can be suggested that although virgin olive oil is a very stable lipid material, it should be consumed within a short period after bottling, if nutritional value is also considered and not only oxidative stability. There is no need to store large quantities of virgin olive oil in the house as is the practice in rural areas of the producing countries. Long storage periods result in reduced levels of tocopherols.

The results of the above study show that specific precautions should be taken during handling of oil to maintain its nutritional value. It has to be also stressed that tables for tocopherol contents of vegetable oils (Gunstone et al., 1994; Belitz and Grosch, 1999) give values (~100 mg/kg) which are much lower than those found in the literature for virgin olive oil. This is due to the fact that in these tables the values refer to refined olive oil. However, pure refined olive oil is not among the permitted edible forms according to EC regulation (EC, 1991) which aims at supporting the natural product. Therefore, these tables should be revised.

ACKNOWLEDGMENT

We express our sincere thanks to Elais SA (Piraeus, Greece) for providing the 90 samples and data for their fatty acid compositions and to the Subtropics and Olive Tree Institute (Chania, Greece) for the samples from pilot plant tests.

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Received for review September 7, 1999. Revised manuscript received February 25, 2000. Accepted March 2, 2000. This work has been partially financed by the General Secretariat for Research and Technology, Greek Ministry of Development (Program EPET II-691, Greek Olive Oil: Extraction Technology, Origin and Quality). E.P. thanks the Foundation of State Scholarships (I.K.Y., Athens, Greece) for a Ph.D. grant.

JF990993O