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Evolution of major and minor components and oxidation indices of virgin olive oil during 21 months storage at room temperature

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Abstract

This article reports the evolution of major and minor components and oxidation indices of seven samples of virgin olive oil (VOO) which differ in their initial contents of natural antioxidants, during 21 months of storage at room temperature and in darkness. As expected, statistically significant differences in the antioxidant contents were observed, with initial concentrations ranging from 0.33 to 0.55 mmol/kg for α-tocopherol and from 1.08 to 3.88 mmol/kg for total phenols. The quality indices PV, K_{232} and K_{270} increased linearly during the storage time studied (21 months), which should make it possible to predict the shelf-life of a VOO sample by extrapolation from the results obtained during a relatively short period of storage (i.e. several weeks). K_{232} was the first parameter that exceeded the established upper limit for extra VOO and therefore seems to be the most relevant index for analysis and monitoring to determine the commercial category of the olive oil. The reduction of total phenolic compounds ranged from 43% to 73%, and it was remarkable that the decrease was higher in samples whose initial phenol contents were greater. Hydroxytyrosol increased linearly in most samples, whereas its complex forms decreased considerably, with the exception of two in which the hydroxytyrosol content decreased continuously or diminished after an initial increase. This fact was probably due to the low initial concentration of hydroxytyrosol seco-iridoid forms: i.e. 0.32 mmol/kg for the sum of 3,4-DHPEA-EDA and 3,4-DHPEA-EA in one of these samples as compared to between 0.65 and 2.06 mmol/kg in the others. Finally, there was a slight and apparently linear fall in the α-tocopherol content of all samples, with a reduction ranging from 0.054 mmol/kg (12%) to 0.127 mmol/kg (23%), although there may be a short lag phase at the beginning of the assay.

Keywords: Virgin olive oil; Room temperature storage; Quality; Oxidation; Shelf-life

1. Introduction

Lipid oxidation occurs fairly slowly at room temperature. Nevertheless, it is the main cause of olive oil quality deterioration and its reaction rate determines the shelf-life of this product. The stability of virgin olive oil (VOO) usually ranges from 9 to more than 18 months and hence accelerated methods are generally employed to estimate the induction period of the oxidation reaction in a relatively short period of time (Frankel, 1998).

It is well documented in the literature that there is a good relationship between the oxidative stability of VOO, determined using accelerated oxidation tests (i.e. AOM or Rancimat), and its initial content of natural antioxidants, especially phenolic compounds (namely total polyphenols, *o*-diphenols or oleosidic forms of hydroxytyrosol) (Gómez-Alonso, Salvador, & Fregapane, 2002; Gutfinger, 1981). However, accelerated assays have a drawback in that the oxidation process takes place under drastic conditions, quite unlike those typically occurring in oil mill storage tanks or

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even during the marketing of the product. As a consequence, the methods selected to determine the end-point of the stability assays and the changes observed in the oil have no satisfactory correlation with the autoxidation process that takes place at room temperature (Frankel, 1998; Velasco & Dobarganes, 2002), or hence with VOO shelf-life.

Until recently the research community did not in depth study the evolution of the quality parameters and shelf-life of VOO, either during storage in oil mill tanks (Giovacchino di, Mucciarella, Constantini, Ferrante, & Surricchio, 2002), in bottles (Cinquanta, Esti, & Di Matteo, 2001; Gutiérrez & Fernandez, 2002; Leonardis de & Macciola, 1998; Okogeri & Tasioula-Margari, 2002) or under commercial conditions (Pagliarini, Zanoni, & Giovanelli, 2000), mainly because of the long time (12–24 months) that such studies require. Results from these studies have contributed to a better understanding of the autoxidation process; however, they are not conclusive, as yet, and the prediction of VOO shelf-life and the exact role of its natural antioxidants is still a research goal of prime interest.

This research paper examines the evolution of major and minor components and oxidation indices of seven samples of VOO which significantly differ in their initial contents of natural antioxidants, during 21 months of storage at room temperature and in darkness. It also looks at the relationship between VOO initial composition and the time required to reach the upper limits established by EU regulations for the quality parameters.

2. Materials and methods

2.1. Virgin olive oil (VOO)

Five commercial *Cornicabra* virgin olive oil samples were collected from industrial oil mills located in the area of Toledo and Ciudad Real (Castilla-La Mancha) during the crop seasons 2000/2001. Two *Cornicabra* virgin olive oils with higher concentrations of phenolic compounds were obtained separately using the Abencor system (Comercial Abengoa, SA, Sevilla, Spain). All samples were filtered with anhydrous Na_2SO_4 and stored at 4 °C in darkness using amber glass bottles without head space prior to analysis.

2.2. Purified olive oil (POO) preparation

Cornicabra virgin olive oil was stripped of pro- and antioxidants and trace metals by adsorption chromatography (Marinova & Yanishlieva, 1996). Hundred gram of virgin olive oil in 1000 ml distilled hexane were passed through a column (i.d. 2 cm) filled with 70 g alumina (type 507c, neutral, Fluka, Buchs, Switzerland) activated for 4 h at 180 °C, and collected in darkness.

2.3. Oxidation experiments

Fourteen 36.6 g (40 ml) lots of each VOO and 12 POO samples were stored in darkness at 25 °C in open 125 ml amber glass bottles (i.d.: 4.2 cm; surface area exposed to the atmosphere: 13.85 cm^2). One bottle was taken from the incubator for analysis at each scheduled time.

2.4. Analytical determinations

All reagents used were of analytical, HPLC or spectroscopic grade, and were supplied by Merck (Darmstadt, Germany).

Free acidity, given as % of oleic acid, peroxide value (PV) expressed as milliequivalents of active oxygen per kilogramme of oil (meq O_2/kg), and K_{232} and K_{270} extinction coefficients calculated from absorption at 232 and 270 nm, were measured, following the analytical methods described in European Regulation EEC 2568/91 and later amendments. *p*-Anisidine value (AnV) was determined, following the AOCS official method (Cd 18-90), using an Agilent 8453 UV–vis spectrophotometer.

2.5. Phenolic compounds

A solution of the internal standard (250 μl of a 15 mg/kg of syringic acid in methanol) was added to a sample of virgin olive oil (2.5 g) and the solvent was evaporated with a rotary evaporator at 35 °C under vacuum. The oil was then dissolved in 6 ml of hexane and a diol-bonded phase cartridge (Supelco Co., Bellefonte, USA) was used to extract the phenolic fraction. The cartridge was conditioned with methanol (6 ml) and hexane (6 ml), the oil solution was then applied, and the SPE column was washed with hexane (2 × 3 ml) and with hexane/ethyl acetate (85:15, v/v; 4 ml). Finally, the phenols were eluted with methanol (15 ml) and the solvent was removed with a rotary evaporator at 35 °C under vacuum to dryness. The phenolic residue was dissolved in methanol/water (1:1 v/v; 250 μl).

HPLC analysis was performed using an Agilent Technologies 1100 series system equipped with an automatic injector, a column oven and a diode array UV detector. A Spherisorb S3 ODS2 column (250 × 4.6 i.d. mm, 5 μm particle size) (Waters Co., Milford, Massachusetts, USA) was used, maintained at 30 °C, with an injection volume of 20 μl and a flow rate of 1.0 ml/min. Mobile phase was a mixture of water/acetic acid (95:5 v/v) (solvent A), methanol (B) and acetonitrile (C): from 95% (A)–2.5% (B)–2.5% (C) to 34% (A)–33% (B)–33% (C) in 50 min. Phenolic compounds were quantified at 280 nm using syringic acid as internal standard and the response factors determined by Mateos et al. (2001).

2.6. α-Tocopherol

Tocopherols were evaluated following AOCS Method Ce 8-89. A solution of oil in hexane was analysed on an Agilent Technologies HPLC (1100 series) on a silica gel Lichrosorb Si-60 column (particle size 5 μm, 250 mm × 4.6 mm i.d.; Sugerlabor, Madrid, Spain) which was eluted with hexane/2-propanol (98.5:1.5) at a flow rate of 1 ml/

min. A fluorescence detector (Thermo-Finnigan FL3000) was used with excitation and emissions wavelengths set at 290 and 330 nm.

2.7. Oxidative stability

This was evaluated by the Rancimat method (Gutiérrez, 1989). Stability was expressed as the induction time (hours) measured with the Rancimat 679 apparatus (Metrohm, Switzerland).

2.8. Fatty acid composition

European Regulations EEC 2568/91 and following amendments, corresponding to AOCS method Ch 2-91 were used. To determine fatty acid composition, the methyl-esters were prepared by vigorous shaking of a solution of oil in hexane (0.2 g in 3 ml) with 0.4 ml of 2 N methanolic potassium hydroxide and analysed by GC with a FID detector. A fused silica column (50 m length × 0.25 mm i.d.), coated with SGL-1000 phase (0.25 μm thickness; Sugerlabor, Spain), was used. The carrier gas was helium, at a flow through the column of 1 ml/min. The injector and detector temperatures were set at 250 °C and the oven temperature at 210 °C. The injection volume was 1 μl.

The loss in the unsaturated fatty acids due to oxidation was quantified on the basis of the ratio between each fatty acid and the palmitic acid peak areas, since saturated fatty acids are not altered by autoxidation (Dobarganes & Perez-Camino, 1988).

2.9. Chlorophyll and carotenoid compounds (mg/kg)

These were determined at 472 and 670 nm in cyclohexane, using specific extinction values, by the method of Minguez-Mosquera, Gandul-Rojas, Garrido-Fernández, and Gallardo-Guerrero (1990).

All experiments and analytical determinations were carried out at least in duplicate.

2.10. Statistical analysis

Statistical analyses were performed using SPSS 11 statistical software (SPSS Inc. Chicago, IL).

3. Results and discussion

3.1. Initial composition

Table 1 shows the values of the legal quality indices and the composition of each sample of virgin olive oil (VOO) at the beginning of the assay.

Given the object of this study, olive oil samples were selected on the basis of differences in natural antioxidant contents. As expected, there were statistically significant differences between samples in the contents of antioxidants, with initial concentrations ranging from 0.33 to 0.55 mmol/kg for α-tocopherol and from 1.08 to 3.88 mmol/kg for total phenols. Consequently, the oxidative stability, as determined by the Rancimat method, also differed considerably between samples, since samples with higher concentrations of phenolic compounds, especially *ortho*-diphenols, exhibited greater oxidative stability ($r^2 = 0.950$, $p = 0.002$), as reported elsewhere (Gómez-Alonso et al., 2002; Gutfinger, 1981).

All samples met the European Union requirements for the maximum extra virgin category as regards the values of free acidity, peroxide value, K_{232} and K_{270}, with the exception of sample VII whose free acidity value (0.84%) was slightly over the recently established limit of 0.80% (EC 1989/2003). The indices referring to primary or secondary oxidation products were well below the upper legal limit values for extra VOO (PV ⩽ 20 meq/kg; $K_{232} \leqslant 2.50$

Table 1
Initial composition and quality indices of olive oil samples

	Virgin olive oil samples							POO
	I	II	III	IV	V	VI	VII	
Free acidity (%)	0.41 ± 0.02^{d}	0.21 ± 0.01^{b}	0.14 ± 0.01^{a}	0.20 ± 0.01^{b}	0.50 ± 0.01^{e}	0.34 ± 0.01^{c}	0.84 ± 0.01^{f}	ND
PV (meq O_2/kg)	5.47 ± 0.04^{d}	5.49 ± 0.17^{d}	3.06 ± 0.07^{b}	3.68 ± 0.10^{c}	2.87 ± 0.05^{a}	6.52 ± 0.14^{f}	5.80 ± 0.08^{e}	0.00 ± 0.01
K_{232}	1.93 ± 0.05^{d}	1.87 ± 0.06^{d}	1.73 ± 0.02^{c}	$1.61 \pm 0.01^{a,b}$	1.56 ± 0.01^{a}	$1.67 \pm 0.04^{b,c}$	1.72 ± 0.01^{c}	0.91 ± 0.01
K_{270}	0.16 ± 0.01^{d}	0.16 ± 0.01^{d}	$0.13 \pm 0.01^{b,c}$	0.12 ± 0.01^{b}	0.10 ± 0.01^{a}	0.12 ± 0.01^{b}	0.14 ± 0.01^{c}	0.02 ± 0.01
AnV	25.3 ± 0.93^{f}	23.2 ± 0.96^{e}	20.6 ± 0.51^{d}	11.6 ± 0.01^{c}	$9.29 \pm 0.26^{a,b}$	9.42 ± 0.07^{b}	8.06 ± 0.12^{a}	0.09 ± 0.01
C18:1 (%)	79.0 ± 0.04^{a}	81.5 ± 0.01^{g}	81.0 ± 0.01^{f}	79.4 ± 0.03^{b}	80.9 ± 0.01^{e}	80.1 ± 0.01^{c}	80.8 ± 0.01^{d}	80.2 ± 0.02
C18:2 (%)	5.09 ± 0.01^{d}	3.61 ± 0.01^{a}	3.87 ± 0.01^{b}	5.29 ± 0.01^{e}	5.32 ± 0.01^{f}	4.94 ± 0.01^{c}	$5.31 \pm 0.01^{e,f}$	4.80 ± 0.01
C18:3 (%)	0.71 ± 0.01^{e}	0.59 ± 0.01^{c}	0.65 ± 0.01^{d}	0.58 ± 0.01^{b}	0.58 ± 0.01^{b}	0.54 ± 0.01^{a}	0.57 ± 0.01^{b}	0.50 ± 0.01
Chlorophyll[A]	11.4 ± 0.12^{e}	43.0 ± 0.12^{g}	19.6 ± 0.05^{f}	3.83 ± 0.09^{c}	2.20 ± 0.06^{a}	6.79 ± 0.20^{d}	2.58 ± 0.02^{b}	NA
Carotenoids[A]	6.32 ± 0.02^{e}	15.3 ± 0.01^{g}	12.9 ± 0.01^{f}	4.12 ± 0.01^{c}	2.71 ± 0.01^{a}	4.49 ± 0.02^{d}	3.09 ± 0.02^{b}	NA
o-Diphenols[B]	2.05 ± 0.01^{b}	2.16 ± 0.31^{b}	2.46 ± 0.13^{b}	0.70 ± 0.05^{a}	0.71 ± 0.03^{a}	0.58 ± 0.02^{a}	0.51 ± 0.01^{a}	NA
Total phenols[B]	3.70 ± 0.15^{c}	3.88 ± 0.26^{c}	3.88 ± 0.22^{c}	1.41 ± 0.07^{b}	1.38 ± 0.03^{b}	1.35 ± 0.05^{b}	1.08 ± 0.03^{a}	NA
α-Tocopherol[B]	0.55 ± 0.01^{e}	0.44 ± 0.01^{c}	0.53 ± 0.01^{e}	0.38 ± 0.01^{b}	0.45 ± 0.01^{d}	0.33 ± 0.01^{a}	0.36 ± 0.01^{b}	NA
Stability (h)	133.2 ± 2.5^{d}	158.0 ± 0.4^{e}	138.7 ± 7.2^{d}	$80.6 \pm 0.8^{b,c}$	82.2 ± 0.8^{c}	69.9 ± 2.5^{a}	75.7 ± 1.1^{b}	4.0 ± 0.1

Mean values with different letters in the same row are statistically different ($p < 0.05$). POO: purified olive oil, ND: not determined and NA: not applicable.
[A] Expressed as mg/kg.
[B] Expressed as mmol/kg.

and $K_{270} \leqslant 0.22$), indicating a low initial oxidation status, as was desired.

The fatty acid composition, with a high percentage of oleic acid and low linoleic and linolenic acid contents, was typical of the Cornicabra olive oil variety (Aranda, Gómez-Alonso, Rivera del Alamo, Salvador, & Fregapane, 2004). The contents of chlorophyll and carotenoid pigments varied over a wide range, probably because the olives were at different stages of ripeness.

3.2. Evolution of legal quality indices

The quality indices, PV, K_{232} and K_{270}, increased linearly during the storage time studied (21 months), as shown by the regression coefficients presented in Table 2. It should therefore be possible to extrapolate from this observation to predict the shelf-life of a VOO sample from results recorded over a relatively short period of storage (i.e. a matter of weeks).

In all samples, the peroxide value was lower than 6.6 meq/kg at the beginning of the assay (Table 1). In none of the oils did it exceed the upper limit (20 meq/kg) established by European Regulation for extra virgin olive oil (EVOO) during the 21-month storage period studied, and therefore the time required to reach the legal limit was extrapolated. This result showed the significant effect of minor VOO compounds on oxidative stability since, as shown in Table 2, the time required to reach the same limit in purified olive oil (POO) was less than seven weeks. However, these results contrast with the findings of Okogeri and Tasioula-Margari (2002) in that, according to their assays, VOO samples (of Lianolia variety, with low initial PV and relatively high antioxidant levels, stored in closed bottles, darkness, room temperature and only 3% of head-space) reached the upper limit for PV in less than eight months.

In order to determine whether the oxidation rate during storage was related to the initial oxidation level of the oil, the authors examined the correlation between the rate of increase of PV and its initial value in the oil samples. The results showed that there was no statistically significant correlation between these two parameters ($r^2 = 0.357$, $p = 0.156$). Moreover, the evolution of PV during storage showed no obvious correlation with the initial Rancimat oxidative stability of these oils ($p = 0.905$) or, as also reported by Cinquanta et al. (2001), with their phenolic compound ($p = 0.422$) or α-tocopherol ($p = 0.303$) contents. For example, samples III and IV, which had similar initial values for PV but very different phenolic compound contents (Table 1), exhibited very similar oxidation rates.

The behaviour observed for K_{232} index was very similar to that found for PV in that it followed a pseudo-zero-order kinetic and there was no simple correlation between the evolution of this index and the initial K_{232} values, Rancimat oxidative stability, or natural antioxidant content. As expected, the rate of increase of these two indices, K_{232} and PV, correlated directly ($r^2 = 0.764$; $p \leqslant 0.05$). However, unlike PV, K_{232} was the first index to exceed the upper limit of 2.50, established by the EU Regulations for EVOO, in all samples except for sample II; this meant that it would fail to qualify for the maximum oil category in a period of time ranging from 33 to 63 weeks. During the storage time, most oils also exceeded the upper limit for edible oil, $K_{232} \leqslant 2.60$. Consequently, K_{232} seems to be the most useful index for analysis and monitoring to determine the commercial category of olive oil. These results also show how important VOO minor compounds are in retarding the increase of K_{232} index, given that it took less than 8 weeks of storage to reach the limit value of 2.50 in POO.

With respect to the K_{270} index, a secondary oxidation product marker, samples I and III, both with high oxidative stability and phenolic compound contents, presented the highest slope over the storage time. Sample II, with a very similar initial oxidation status and phenolic content presented the lowest rate of increase for K_{270}. One possible explanation of the different behaviour observed is that the linolenic acid content of the samples was significantly higher in oils I and III (Table 1). In fact, as we know, linolenic acid is the most susceptible VOO fatty acid to autoxidation (Cosgrove, Church, & Pryor, 1987) and its hydroperoxides undergo rapid decomposition, yielding compounds some of which absorb UV radiation at 270 nm.

A multiple linear regression analysis of the data suggested that the rate of increase of K_{270} could be explained

Table 2
Slope and correlation coefficient of the linear regression of quality indices and time required to reach the upper legal limits for EVOO during storage

Sample	PV			K_{232}			K_{270}		
	Slope	r^2	Time (weeks)[a]	Slope	r^2	Time (weeks)[a]	Slope × 10^{-3}	r^2	Time (weeks)[a]
I	0.146	0.981	103[b]	0.0173	0.979	33	1.580	0.952	35
II	0.086	0.976	167[b]	0.0064	0.948	103[b]	0.702	0.940	86
III	0.156	0.982	103[b]	0.0149	0.987	54	1.330	0.978	66
IV	0.155	0.969	96[b]	0.0183	0.965	53	1.070	0.952	95[b]
V	0.133	0.976	122[b]	0.0153	0.986	63	1.210	0.985	99[b]
VI	0.123	0.965	100[b]	0.0136	0.982	56	0.864	0.958	114[b]
VII	0.094	0.951	141[b]	0.0123	0.973	62	1.000	0.951	84
POO	–	–	6.8	–	–	7.7	–	–	15.3

[a] Maximum values of quality parameter for extra virgin olive oil (EEC 2596/1991 and later amendments): PV, 20 meq/kg; K_{232}, 2.50; K_{272}, 0.22.
[b] Times calculated by extrapolation.

by taking into account initial concentrations of α-tocopherol and chlorophyll pigments in the oils ($r^2 = 0.997$; $p < 0.001$) as variables, in addition to the linolenic acid content. The positive correlation observed between α-tocopherol content and the increased rate of K_{270} could be explained by its pro-oxidant activity in the first steps of autoxidation (Blekas, Tsimidou, & Boskou, 1995), whereas the negative regression found between chlorophyll pigments in the oil and the rate of increase of K_{270} would appear to indicate that the antioxidant effect of this pigment group is slight when the autoxidation reaction occurs in darkness (Endo, Usuki, & Kaneda, 1984; Psomiadou & Tsimidou, 2002).

It is also noteworthy that K_{270} was not the first VOO quality index to exceed the legal limit during storage, contrary to what was reported by Gutiérrez and Fernandez (2002). Moreover, in the cited work, a good linear regression ($r = 0.982$) was established between the initial Rancimat oxidative stability of the oils and the time required to reach the upper limit value for K_{270}, a correlation that was not observed in the present work.

3.3. Changes in fatty acid composition

Few changes were observed in the unsaturated fatty acid composition during the 21-month storage period. This was due to the levels of antioxidant compounds and the mild storage conditions employed in this research, which reduced the oxidation in the oils.

After 93 weeks of storage, there were no detectable changes in oleic acid, the main unsaturated fatty acid of VOO. The changes observed in the polyunsaturated fatty acids (PUFA), linoleic and linolenic, in each VOO sample, are shown in Table 3. As expected, linolenic acid decreased more, the reductions ranging between 2.1% and 3.8% for linoleic acid and between 5.8% and 10.0% for linolenic acid. In all the VOO samples studied, the observed reduction was 2.5–2.8 times greater in linolenic acid than in linoleic acid. This rate is higher than was observed during autoxidation of POO (1.76; Gómez-Alonso, Salvador, & Fregapane, 2004) under the same oxidation conditions (PV between 10 and 20 meq/kg), which may suggest that natural VOO antioxidants protect linoleic more than linolenic acid. This could be explained by the fact that the activity of antioxidants differs, depending on the oxidizing substrate (Yanishlieva-Maslarova, 2001).

At the end of the assay, oxidation of total fatty acids was as high as 0.20% in all samples, except in VOO II where the loss was only 0.11% and the increases of PV, K_{232} and K_{270} were also smaller. Analysis of the correlation between the loss of PUFA and the increase of indices measuring oxidation products showed that K_{232} had the highest statistically significant regression coefficient ($r^2 = 0.924$, $p < 0.001$). In view of these results, it seems possible that the best analytical index to measure the degree to which VOO polyunsaturated fatty acids are affected by oxidation when stored in darkness and at room temperature (25 °C) is UV absorbance at 232 nm.

Finally, once again, no significant correlation was observed between changes in fatty acid composition and natural antioxidant content or Rancimat oxidative stability. This fact tends to confirm the previous results with PV, K_{232} and K_{270} indices.

3.4. Changes in phenolic compounds and other minor components

Table 4 shows VOO content in the main phenolic compounds at the beginning, in the middle and at the end of the assay. The sum of hydroxytyrosol, tyrosol and their secoiridoid derivatives during storage at 25 °C decreased considerably in all samples, but the rate of decrease was clearly different between one sample and another. The downward trend observed in the phenol content appeared roughly to follow a pseudo-first-order kinetic ($0.848 \leqslant r^2 \leqslant 0.966$). This is consistent with polyphenol contents as measured by colorimetric methods (Gutiérrez & Fernandez, 2002). The total per cent reduction ranged from 43% and 73% for samples VI and III, respectively. It should be noted that this decrease was greater in samples with higher initial phenol contents.

Hydroxytyrosol and tyrosol concentrations increased linearly in practically all samples, confirming that their secoiridoid derivatives undergo partial non-oxidative hydrolysis (Brenes, García, García, & Garrido, 2001). Only two samples differed in this respect: sample VI, whose hydroxytyrosol content decreased continuously, and sample VII, which increased initially but eventually presented a downward trend. This was probably due to the low initial concentration of hydroxytyrosol complex forms, i.e. 0.32 mmol/kg for the sum of 3,4-DHPEA-EDA and 3,4-DHPEA-EA in sample VI as compared to 0.65–2.06 in

Table 3
Decrease in fatty acid content (%) during the 21-month storage period

	Sample						
	I	II	III	IV	V	VI	VII
C18:2[a]	3.3 ± 0.4	2.1 ± 0.3	3.5 ± 0.4	3.8 ± 0.5	2.9 ± 0.3	3.3 ± 0.3	3.0 ± 0.2
C18:3[a]	8.5 ± 0.9	5.8 ± 0.4	9.6 ± 0.6	10.0 ± 1.0	8.2 ± 0.8	8.7 ± 0.9	7.7 ± 0.8
Total[b]	0.23 ± 0.03	0.11 ± 0.01	0.20 ± 0.02	0.26 ± 0.03	0.20 ± 0.02	0.21 ± 0.02	0.20 ± 0.02

[a] With respect to initial fatty acid content.
[b] With respect to initial total fatty acid content.

Table 4
Changes in VOO phenolic compound contents (mmol/kg) during storage

	Storage time (weeks)	Sample						
		I	II	III	IV	V	VI	VII
3,4-DHPEA	0	0.16 ± 0.01	0.10 ± 0.01	0.14 ± 0.02	0.03 ± 0.01	0.06 ± 0.01	0.27 ± 0.01	0.07 ± 0.01
	45	0.20 ± 0.01	0.11 ± 0.01	0.13 ± 0.01	0.05 ± 0.01	0.10 ± 0.01	0.25 ± 0.01	0.12 ± 0.01
	93	0.21 ± 0.01	0.14 ± 0.01	0.16 ± 0.01	0.07 ± 0.01	0.12 ± 0.01	0.19 ± 0.01	0.11 ± 0.01
p-HPEA	0	0.12 ± 0.01	0.10 ± 0.01	0.12 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.65 ± 0.01	0.09 ± 0.01
	45	0.16 ± 0.01	0.09 ± 0.01	0.09 ± 0.01	0.07 ± 0.01	0.08 ± 0.01	0.57 ± 0.02	0.14 ± 0.01
	93	0.19 ± 0.01	0.12 ± 0.01	0.13 ± 0.01	0.10 ± 0.01	0.12 ± 0.01	0.48 ± 0.01	0.14 ± 0.01
3,4-DHPEA-EDA	0	1.48 ± 0.07	1.60 ± 0.16	0.89 ± 0.04	0.30 ± 0.04	0.34 ± 0.01	0.05 ± 0.01	0.19 ± 0.01
	45	0.86 ± 0.07	0.90 ± 0.01	0.31 ± 0.02	0.17 ± 0.02	0.18 ± 0.02	0.02 ± 0.01	0.14 ± 0.01
	93	0.67 ± 0.06	0.63 ± 0.06	0.21 ± 0.02	0.10 ± 0.01	0.12 ± 0.01	0.01 ± 0.01	0.09 ± 0.01
p-HPEA-EDA	0	1.29 ± 0.05	1.36 ± 0.11	0.73 ± 0.03	0.47 ± 0.01	0.45 ± 0.01	0.06 ± 0.02	0.32 ± 0.01
	45	0.81 ± 0.09	0.77 ± 0.01	0.30 ± 0.04	0.36 ± 0.04	0.26 ± 0.03	0.04 ± 0.01	0.29 ± 0.03
	93	0.73 ± 0.06	0.59 ± 0.06	0.24 ± 0.03	0.24 ± 0.02	0.21 ± 0.03	0.03 ± 0.01	0.21 ± 0.02
3,4-DHPEA-EA	0	0.40 ± 0.01	0.46 ± 0.05	1.38 ± 0.06	0.53 ± 0.02	0.31 ± 0.01	0.27 ± 0.01	0.24 ± 0.01
	45	0.21 ± 0.01	0.23 ± 0.01	0.54 ± 0.03	0.23 ± 0.02	0.14 ± 0.01	0.12 ± 0.01	0.15 ± 0.01
	93	0.12 ± 0.01	0.19 ± 0.01	0.34 ± 0.02	0.13 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.08 ± 0.01
p-HPEA-EA	0	0.24 ± 0.01	0.26 ± 0.03	0.58 ± 0.05	0.33 ± 0.01	0.23 ± 0.01	0.17 ± 0.01	0.20 ± 0.01
	45	0.13 ± 0.01	0.14 ± 0.01	0.30 ± 0.03	0.23 ± 0.02	0.11 ± 0.01	0.10 ± 0.01	0.14 ± 0.01
	93	0.09 ± 0.01	0.14 ± 0.01	0.23 ± 0.03	0.15 ± 0.01	0.07 ± 0.01	0.08 ± 0.01	0.09 ± 0.01
Total phenols	0	3.70 ± 0.00	3.88 ± 0.56	3.88 ± 0.22	1.41 ± 0.07	1.38 ± 0.03	1.35 ± 0.05	1.08 ± 0.03
	45	2.36 ± 0.18	2.24 ± 0.03	1.66 ± 0.14	1.12 ± 0.11	0.88 ± 0.06	1.10 ± 0.04	0.97 ± 0.06
	93	2.01 ± 0.16	1.74 ± 0.14	1.31 ± 0.11	0.79 ± 0.05	0.70 ± 0.05	0.88 ± 0.02	0.73 ± 0.04

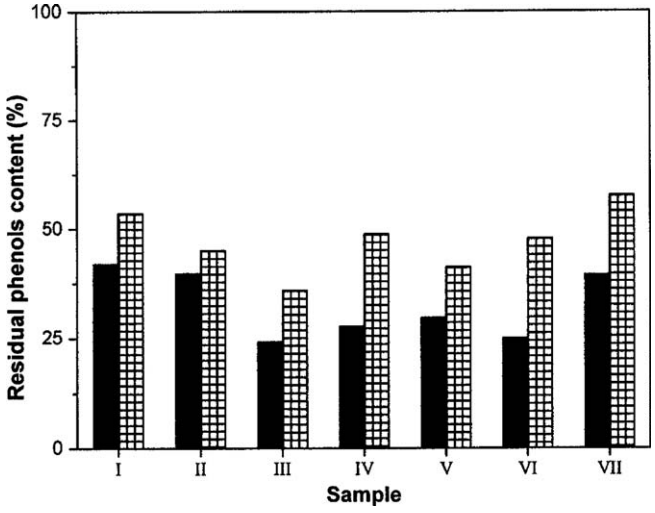

Fig. 1. Residual VOO complex phenol contents (%) after 21 months of storage at 25 °C. ■ (3,4-DHPEA-EDA + 3,4-DHPEA-EA), ▦ (*p*-HPEA-EDA + *p*-HPEA-EA).

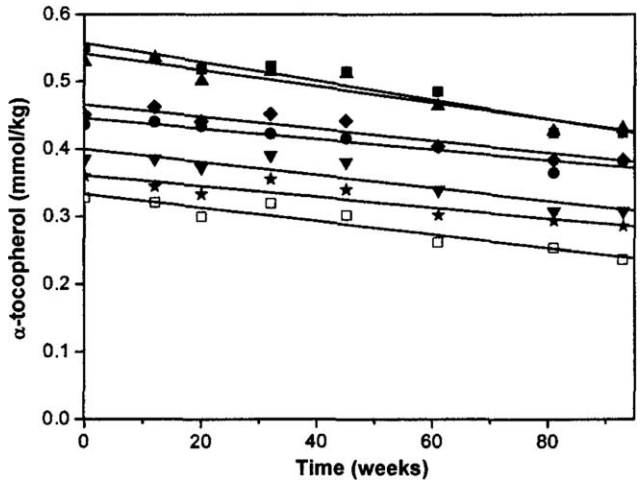

Fig. 2. Decrease of α-tocopherol content during storage. Samples: ■, I; ●, II; ▲ III; ▼, IV; ◆, V; □, VI; ★, VII.

VOO samples I–V. The evolution of tyrosol content was very similar to that of hydroxytyrosol, with the same exceptions to the overall upward trend.

Table 4 also shows that concentration of hydroxytyrosol and tyrosol complex forms in VOO decreased considerably over the storage time. As Fig. 1 shows, the decrease of hydroxytyrosol secoiridoid derivatives was greater than that of tyrosol complex forms, in all cases. However, the differences are smaller than was expected considering that tyrosol and its secoiridoid derivatives lack antioxidant activity (Baldioli, Servilli, Perretti, & Montedoro, 1996; Mateos, Domínguez, Espartero, & Cert, 2003). This fact therefore suggests that phenolic compounds are not very stable, even in mild storage conditions, which is an important aspect and merits more thorough investigation.

As depicted in Fig. 2, α-tocopherol content fell slightly and apparently linearly in all samples, although there may have been a short lag phase at the beginning of the assay. At the end of the storage period, the total reduction in the α-tocopherol content varied between 0.054 mmol/kg (12%) in sample II and 0.127 mmol/kg (23%) in sample I.

These reductions were much smaller than the 50% observed by Okogeri and Tasioula-Margari (2002) during 12 months storage of virgin olive oil in bottles with only 3% headspace at room temperature and in darkness. On the other hand, the total reduction of α-tocopherol was smaller than that of the *o*-diphenols, which, according to references in the literature (Blekas et al., 1995), may indicate that the latter had a greater antioxidant effect, especially in the early stages of oxidation.

The chlorophyll pigments of virgin olive oil have been reported to act as prooxidants under light (Rahmani & Saari-Csallany, 1998) and as antioxidants in darkness (Psomiadou & Tsimidou, 2002). With the spectrophotometric method used in the present research, only a slight decrease in the chlorophyll content could be detected, irrespective of the initial concentration in virgin olive oils. The concentrations of carotenoid pigments, to which some authors ascribe some prooxidant activity in darkness (Lee & Kim, 1992), also decreased slightly with time. The reduction was more pronounced and very similar in samples II and III, that is the ones with higher initial carotenoid contents. However, these pigments are unlikely to be of importance, given the dissimilarity in the course of the autoxidation process.

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