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Changes in commercial virgin olive oil (cv Arbequina) during storage, with special emphasis on the phenolic fraction

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Abstract

The aim of this work was to study the changes in the lipid substrate and in the minor components and specially in the phenolic fraction of commercial virgin olive oils of Arbequina cultivar after 12 months of storage. An increase of oleic acid percentage was found in the fatty acid composition. Important losses of chlorophyll, carotenoids, and total phenol content of oils occur after the storage period. Significant decreases were observed in secoiridoid derivatives and 3,4-DHPEA-AC after the storage period, while lignans were the more stable phenolic compounds. α-tocopherol disappeared after the storage period, in all oils.

Keywords: Virgin olive oil; Storage; Phenolic compounds

1. Introduction

In the Mediterranean region, the healthy properties of olive oil have been known for a long time, olive oil being the main fat source of the diet. Consumption of olive oil has also increased in non-Mediterranean areas because of the growing interest in the Mediterranean diet and the tendency of consumers to select least-processed foods. Consumers are increasingly demanding that high food quality be maintained during the period between purchase and consumption. These expectations are a consequence not only of the primary requirement that the food should remain safe but also of the need to minimise unwanted changes in sensory quality.

Virgin olive oil's characteristic aroma, taste, colour and nutritive properties distinguish it from other edible vegetable oils. It is therefore a matter of great concern for the olive industry to preserve its product without loss of these positive attributes.

Oxidative rancidity development has been recognised as the predominant cause of oil deterioration during storage. This is a reaction between unsaturated fatty acids, regardless of whether they are in their free state or esterified as a triglyceride molecule, and oxygen. It is also referred to as autoxidation because the activation energies of the first two reaction steps are very low. Therefore, autoxidation in oils can neither be prevented by maintaining cool storage conditions nor by the exclusion of light (Kristott, 2000).

The two compositional factors of oils that determine their susceptibility to oxidation are the fatty acid composition and inherent antioxidant compounds. The types of fatty acids present in an oil, and in particular their number of double bonds, determine the type and extent of chemical reactions that occur during the storage period. Abundance of oleic (C18:1), ranging from 56 to 84% of total fatty acids, is the feature that sets olive oil apart from other vegetable oils.

Virgin olive oil provides a rich source of natural antioxidants. These include carotenoids, tocopherols and phenolic compounds which may act, by different mechanisms, to confer an effective defence system against free radical attack. Some authors have estimated their contribution to oil stability, that of phenolic compounds being around 30%, fatty acids 27%, α-tocopherol 11% and carotenoids 6% (Aparicio, Roda, Albi, & Gutiérrez, 1999).

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Carotenoids, and in particular β-carotene, are efficient antioxidants owing to their ability to quench oxygen radical species, and they also can act as light filters (Van den Berg et al., 2000).

Tocopherols can act as antioxidants by two primary mechanisms, a chain-breaking electron donor mechanism, in which they donate their phenolic hydrogen atom to lipid free-radicals and a chain-breaking acceptor mechanism, which includes singlet oxygen scavenging or quenching; this inhibits the oxidations induced by electronically excited singlet oxygen (Kamal-Eldin & Appelqvist, 1996).

Phenolic compounds, which are considered to be the main antioxidant compounds in virgin olive oil, are able to donate a hydrogen atom to the lipid radical formed during the propagation phase of lipid oxidation (Shahidi & Wanasundara, 1992). The most abundant phenolic compound in virgin olive oil are mainly aglycones derived from secoiridoid compounds present in olives. These newly formed substances are polar compounds that are partitioned between the oily layer and the vegetation water, being more concentrated in the latter fraction because of their functional groups. Secoiridoid derivatives play an important role in oil stability; this has already been evaluated and shown to extend the shelf-life of olive oil (Baldioli, Servili, Perretti, & Montedoro, 1996; Gennaro, Piccioli-Bocca, Modesti, Masella, & Coni, 1998). Although the interest in phenolic compounds is related primarily to their antioxidant activity, they also show important biological activity in vivo and may be beneficial in combating diseases related to excessive oxygen radical formation exceeding the antioxidant defence capacity of the human body.

Olive oil generally has a relatively long shelf-life, during which only minor changes of sensory characteristics occur. Most producers consider 12–18 months as the maximum storage period from bottling to consumption. In any case, olive oil produced in a crop season is usually consumed before the next crop season.

The aim of this work was to study the changes in the lipid substrate (or in the fatty acid composition) and in the minor components, such as chlorophylls, carotenoids, α-tocopherol and especially in the phenolic fraction of commercial olive oils of the Arbequina cultivar after 12 months of storage.

2. Materials and methods

2.1. Materials

Twelve olive oil samples from three selected olive oil mills, defined as mill 1, mill 2 and mill 3, spread in the region of 'Les Garrigues' (Catalonia, Spain) obtained in the 2000/2001 crop season were used for the trial. Mills were selected in order to cover the wide range of variability that exists in oils from the different mills of that area, which would interfere with the study of the evolution of the compositional and quality parameters of olive oil during storage.

Oils were taken directly from the production line on the basis of a protocol established by the Regulator Organism of the Protected Designation of Origin 'Les Garrigues'. Oils collected from the first week of November to the second week of January were classified as the first harvesting period, and oils collected from the second week of January onwards were classified as the last harvesting period. In fact, from a commercial point of view, two types of oils are distinguished, depending on the time the fruit is picked. Oils from the earliest harvest are greenish in colour, fruity and have a bitter almond-like taste, while those from the latest harvest are yellow in colour, more fluid and have a sweet taste.

Oil samples were analysed as soon as they arrived to the lab (fresh oils), and after their analysis they were kept in amber glass bottles (100 ml) in the darkness at ambient temperature, for twelve months (stored), when olive oil samples were analysed once again.

2.2. Determination of pigments and chromatic ordinates

2.2.1. Pigment content

The chlorophyll fraction at 670 nm and the carotenoid fraction at 470 nm were evaluated from the absorption spectrum of each virgin olive oil sample (7.5 g) dissolved in cyclohexane (25 ml) (Mínguez-Mosquera, Rejano, Gandul, Sánchez, & Garrido, 1991). The chlorophyll and carotenoid contents are expressed as mg of pheophytin "a" and lutein per kg of oil, respectively.

2.2.2. Oil colour

A colorimeter (chromometer type Color-Eye 3000, Macbeth) was used to assess the oil colour with the Optiview 1.1 computer programme, and the CIELAB colorimetric system was applied. Oil samples were examined without dilution to avoid colour variation and the tristimulus values X, Y, and Z were calculated for illuminant C from the absorption spectrum. The oil colour is expressed as chromatic ordinates a^*, b^* and L^*.

2.3. α-Tocopherol determination

α-Tocopherol was evaluated by high-performance liquid chromatography with direct injection of an oil-in-hexane solution. Detection and quantification was carried out in a Waters 600 apparatus with a photodiode detector array (Waters 996) set at 295 nm. The 25-cm×4-mm i.d. column used was filled with Supelcosil LC-NH2, 5 μm (Supelco, Inc. Bellefonte, PA). The injection volume was 20 μl. The mobile phase consisted of hexane/ethyl acetate (70/30) at a flow rate of 1 ml/

min. α-Tocopherol was quantified by the external standard method. The linearity of the response was verified by fitting to line results of the α-tocopherol analysis of six standard solutions with known concentrations. Results are given as mg of α-tocopherol per kg of oil.

2.4. Fatty acid determination

The fatty acid composition of the oils was determined by gas chromatography (GC) as fatty acid methyl esters (FAMEs). FAMEs were prepared by saponification/methylation with sodium methylate according to the European Union Commission modified Regulation EEC 2568/91 (León-Camacho & Cert, 1994). A chromatographic analysis was performed in a Hewlett Packard 5890 Series II gas chromatograph using a capillary column (SP 2330, Supelco). The column temperature was isothermal at 190 °C and the injector and detector temperatures were 220 °C. Fatty acids were identified by comparing retention times with standard compounds. Six fatty acids were considered in this study. These were palmitic (16:0), palmitoleic (16:1), stearic (18:0), oleic (18:1), linoleic (18:2) and linolenic (18:3), acids expressed as percentages of fatty acid methyl esters.

2.5. Total phenol content

Phenolic compounds were isolated using the modified method described by (Vázquez Roncero, Janer del Valle, & Janer del Valle, 1973) with triple extraction of an oil-in-hexane solution with a 60% vol/vol water/methanol mixture. The concentration of total polyphenols was estimated with Folin-Ciocalteu reagent at 725 nm. Results were expressed as mg of caffeic acid per kg of oil.

2.6. Bitter index

The bitter index (K_{225}) was evaluated by the extraction of the bitter components of a sample of 1.0 ± 0.01 g oil dissolved in 4 ml hexane, passed over a C18 column (Waters Sep-Pack Cartridges), previously activated with methanol (6 ml) and washed with hexane (6 ml). After elution, 10 mL hexane were passed to eliminate the fat, and then the retained compounds were eluted with methanol/water (1/1) to 25 ml. The absorbance of the extract was measured at 225 nm against methanol/water (1/1) in a 1-cm cuvette (Gutiérrez, Perdiguero, Gutiérrez, & Olías, 1992).

2.7. Stability

Stability is expressed as the oxidation induction time (hours) measured with a Rancimat 679 apparatus (Metrohm Co., Switzerland), using an oil sample of 3 g warmed to 120 °C, and 20 l h^{-1} air flow. The induction time is the time needed to reach the break point of this curve. The breakpoint is designated as the intersection point of the two extrapolated straight parts of the curve. (Laübli & Bruttel, 1986).

2.8. HPLC analysis of phenolic compounds

2.8.1. Phenolic extraction

Phenols were extracted from virgin olive oil by following the procedure of Montedoro, Servili, Baldioli, and Miniati (1992). 2×20 ml of methanol/water (80:20 v/v) were added to 45 g of virgin olive oil and homogenised for 2 min with a Polytron. The two phases were separated by centrifuging at 3000 rpm for 10 min. Hydroalcholic extracts were then combined and concentrated in a vacuum at temperatures below 35 °C until a syrupy consistency was reached. 5 ml of acetronitrile were added to the extract and it was washed 3×20 ml of hexane. The apolar phases were also purified with 5 ml of acetonitrile. The resulting acetonitrile solution was evaporated under vacuum and dissolved in 5 ml of acetonitrile. Finally, an aliquot of 2 ml was evaporated under a stream of nitrogen.

2.8.2. HPLC analysis of phenolic compounds

The phenolic fraction extracted was dissolved in 1 ml of methanol and analysed by HPLC (loop 20 μl). The HPLC system consisted of a Waters 717 plus Autosampler, a Waters 600 pump, a Waters column heater module and a Waters 996 photodiode array detector managed by Millenium 2000 software (Waters Inc., Milford, MA). The column was a Inertsil ODS-3 (5 μm, 15 cm×4.6 mm i.d., GL Sciences Inc.) equipped with a Spherisorb S5 ODS-2 (5 μm, 1 cm×4.6 mm i.d., Technokroma, Barcelona, Spain) precolumn. HPLC analysis was performed by following the same procedure as Montedoro et al. (1992). The eluents were 0.2% acetic acid (pH 3.1) and methanol and the flow rate was 1.5 ml/min. The total run time was 60 min, the initial composition was 95% acetic acid, 0.2% and 5% methanol, and the gradient changed as follows: the concentration of methanol was maintained for 2 min, then it was increased to 25% in 8 min and finally, the methanol percentage was increased to 40, 50 and 100% in 10 min periods. It was maintained at 100% for 5 min. Initial conditions were reached in 15 min. Chromatograms were obtained at 280 and 339 nm.

2.8.3. Reference compounds

Tyrosol and p-coumaric acid were obtained from Extrasynthèse Co. (Genay, France). Vanillic acid, vanillin and ferulic acid were obtained from Fluka Co. (Buchs, Switzerland). Hydroxytyrosol was kindly donated by Professor Montedoro (University of Perugia, Italy). The rest of the phenolic compounds were

obtained by using a semi-preparative HPLC column Spherisorb ODS-2 (5 μm, 25 cm×10 mm i.d., Technokroma, Barcelona, Spain) and a flow rate of 4 ml/min. The mobile phases and gradient were described elsewhere (Tovar, Motilva & Romero, 2001).

Individual phenols were quantified by a four-point regression curve on the basis of the standards obtained from commercial suppliers or by preparative HPLC as described above. Quantification of the phenolic compounds was carried out at 278 nm.

2.9. Mass spectrometry

The mass spectrascopsy of selected (purified) fractions was performed on a micromass ZMD instrument (Waters Inc., Midford, MA). Operational parameters specific to the electrospray mass spectrometry included the following: capillary voltage, 2.5 kV; cone voltage 10 V; extractor voltage 5V; desolvation temperature, 400 °C; source temperature, 120 °C; ion mode, ESI^-.

3. Results and discussion

Fatty acid composition (Table 1) suffered slight changes during storage. In both harvesting periods there was an increase in the percentage of oleic acid as a consequence of the maintenance of the percentage of the saturated fatty acids and decrease of the polyunsaturated ones, linoleic and linolenic acids. The unsaturated fatty acids are very important for the stability of oils because of the chemical reactions occurring at the double bonds. The rates of those oxidation reactions depend on the number of double bonds in the carbon chain.

Chlorophyll and carotenoid contents of oils are shown in Fig. 1. At the end of the storage all samples from the first harvesting period showed >30% loss in chlorophyll content whereas, in samples from the last harvesting period, loss was generally lower (15–20%). Despite the higher initial chlorophyll content of oils from the first harvesting period, their degradation was more noticeable. Carotenoid content followed a similar trend to that of chlorophylls but the percentage loss was lower, apart from the samples processed in mill 1, showing a 40% loss, which initially had a significantly higher content of carotenoid pigments.

The values of the chromatic ordinates L^* and b^* of the oils are shown in Fig. 1. The values of the ordinate a^* are not shown because the colour of the oils from the protected Denomination of Origin 'Les Garrigues' are mainly defined by the ordinate b^* (Romero, Criado, Tovar, & Motilva, 2002). Luminosity values (L^*) increased in oils after the 12 months storage period, probably as a consequence of the reduction on the pigment content. The storage did not appear to have a significant effect on the chromatic ordinate b^*, which corresponds to the yellow zone.

Changes in the content of α-tocopherol and total phenols of oils are shown in Fig. 2. α-Tocopherol level fell 100% in all oils after the storage period. The total phenol content decreased during storage; loss was more marked in oils from the first harvesting period, which showed a higher initial content of those compounds. Reduction of the total phenol content of oils during storage is a result of the decomposition processes that occur in the more complex forms and oxidation activities.

From the results obtained in relation to α-tocopherol and total phenol contents it could be said that when oxidation takes place under non-accelerated conditions, α-tocopherol is preferentially consumed to protect oil against oxidation. However, when oxidation takes place under accelerated conditions, the best correlations have been found between total phenol content and oil stability (Baldioli et al., 1996).

The oxidative stability of oils, measured as the induction time determined using the Rancimat method, is showed in Fig. 2. It decreased during storage, especially in oils from the last harvesting period, which lost more than 50% of their initial stability at the end of the storage, maybe as a consequence of the generation of hydroperoxides during storage.

Bitter index values followed the same pattern as total phenol content (Fig. 2); in fact, it is generally accepted that the phenolic fraction of virgin olive oil is mainly responsible for the bitter attribute. At the end of the 12 month storage period, there was an important loss of

Table 1
Fatty acid composition (%) of fresh olive oil and olive oil stored for 12 months

	Harvesting period	Palmitic		Palmitoleic		Stearic		Oleic		Linoleic		Arachic		Linolenic	
		Fresh	Stored	Fresh	Stored	Fresh	Stored	Fresh	Stored	Fresh	Stored	Fresh	Stored	Fresh	Stored
Mill 1	First	13.4	13.7	1.91	1.10	2.39	2.31	70.1	72.9	10.9	9.33	0.57	0.40	0.76	0.22
	Last	12.3	12.2	1.40	0.71	2.23	2.14	74.0	75.7	9.1	8.67	0.49	0.37	0.55	0.16
Mill 2	First	13.6	13.7	1.71	1.27	1.86	1.63	71.4	73.0	10.4	9.80	0.56	0.45	0.55	0.19
	Last	11.7	11.8	1.19	0.85	2.04	1.68	75.2	76.6	8.88	8.53	0.45	0.47	0.49	0.13
Mill 3	First	14.2	14.3	1.64	1.24	2.35	2.12	70.6	72.2	10.1	9.61	0.52	0.33	0.57	0.26
	Last	11.3	11.4	1.30	0.98	2.29	2.15	75.6	76.8	8.52	8.03	0.48	0.31	0.54	0.20

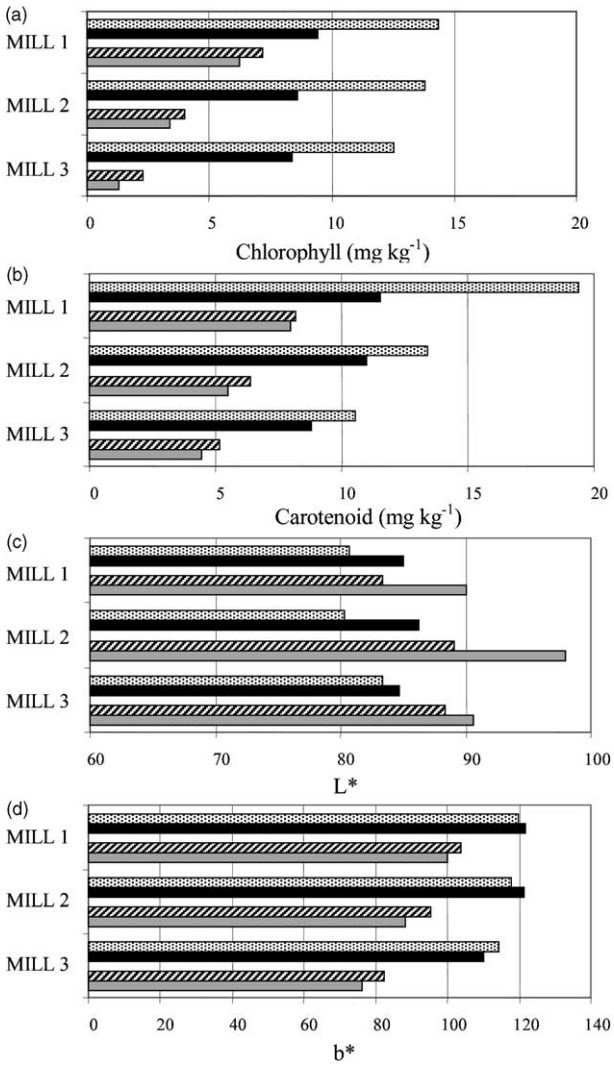

Fig. 1. Effect of storage on chlorophylls (a), carotenoids (b) and chromatic ordinates (c,d) of Arbequina olive oil from different Harvesting Periods. First Harvesting Period Fresh Oil, First Harvesting Period Stored Oil, Last Harvesting Period Fresh Oil and Last Harvesting Period Stored Oil.

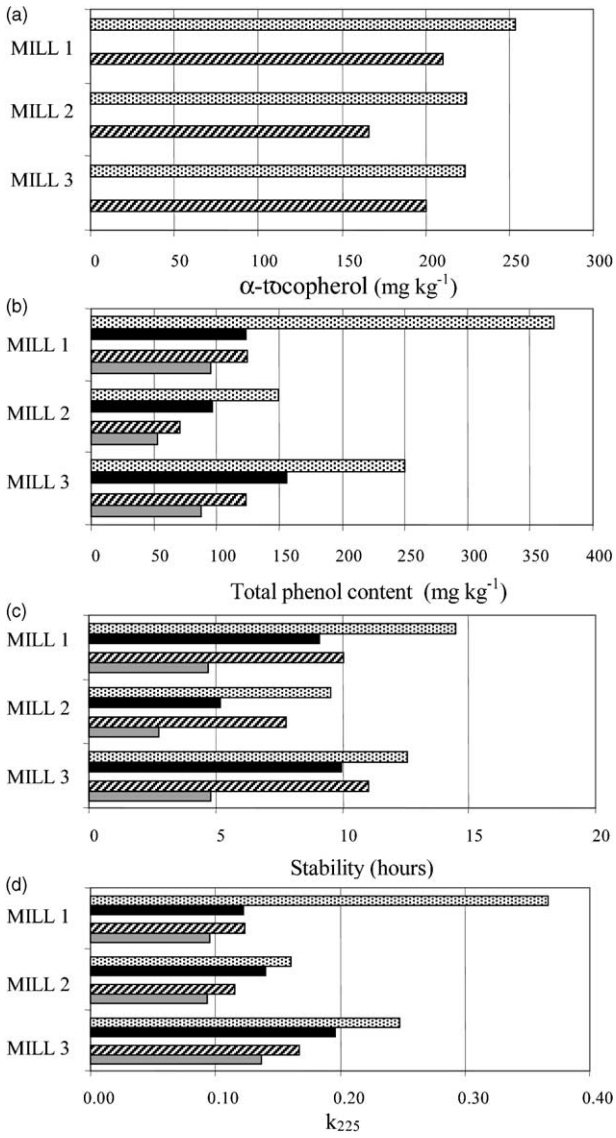

Fig. 2. Effect of storage on a-tocopherol (a), total phenol content (b), stability (c) and bitter index (d) (k_{225}) of Arbequina olive oil from different Harvesting Periods. First Harvesting Period Fresh Oil, First Harvesting Period Stored Oil, Last Harvesting Period Fresh Oil and Last Harvesting Period Stored Oil.

that positive attribute which is one of the distinctive characteristics of virgin olive oil.

As the amount of phenolic compounds is an important factor when evaluating the quality of virgin olive oil, because of their involvement in its resistance to oxidation and its sharp bitter taste, this trial also aimed at determining the changes in the phenolic fraction of oils after a 12 month storage period. Fig. 3 shows the chromatographic profile of the phenolic extracts from Arbequina oils, fresh and stored. The most noticeable is the increase of Peaks 6′ and 8′ at the end of the storage period.

The concentrations of the quantified phenolic compounds in fresh and stored oils are shown in Table 2. Among the simple phenols identified, only 3,4-DHPEA and *p*-HPEA showed a noticeable increase at the end of the storage. This could result from hydrolytic activities on the secoiridoid derivatives (Peaks 6, 7 and 9) with more complex molecular structures.

Storage did not appear to have any effect on vanillic acid or vanillin, which were present at low concentrations. However, there was a significant decrease with storage in the concentration of the rest of the quantified phenolic compounds. That reduction was more marked in the secoiridoid derivatives, 3,4-DHPEA-EDA, *p*-HPEA-EDA and 3,4-DHPEA-EA, indicating a more active participation in the oxidative processes as they were more easily oxidized. The antioxidant activity of those three phenolic compounds has already been evaluated and they have been shown to extend the shelf-

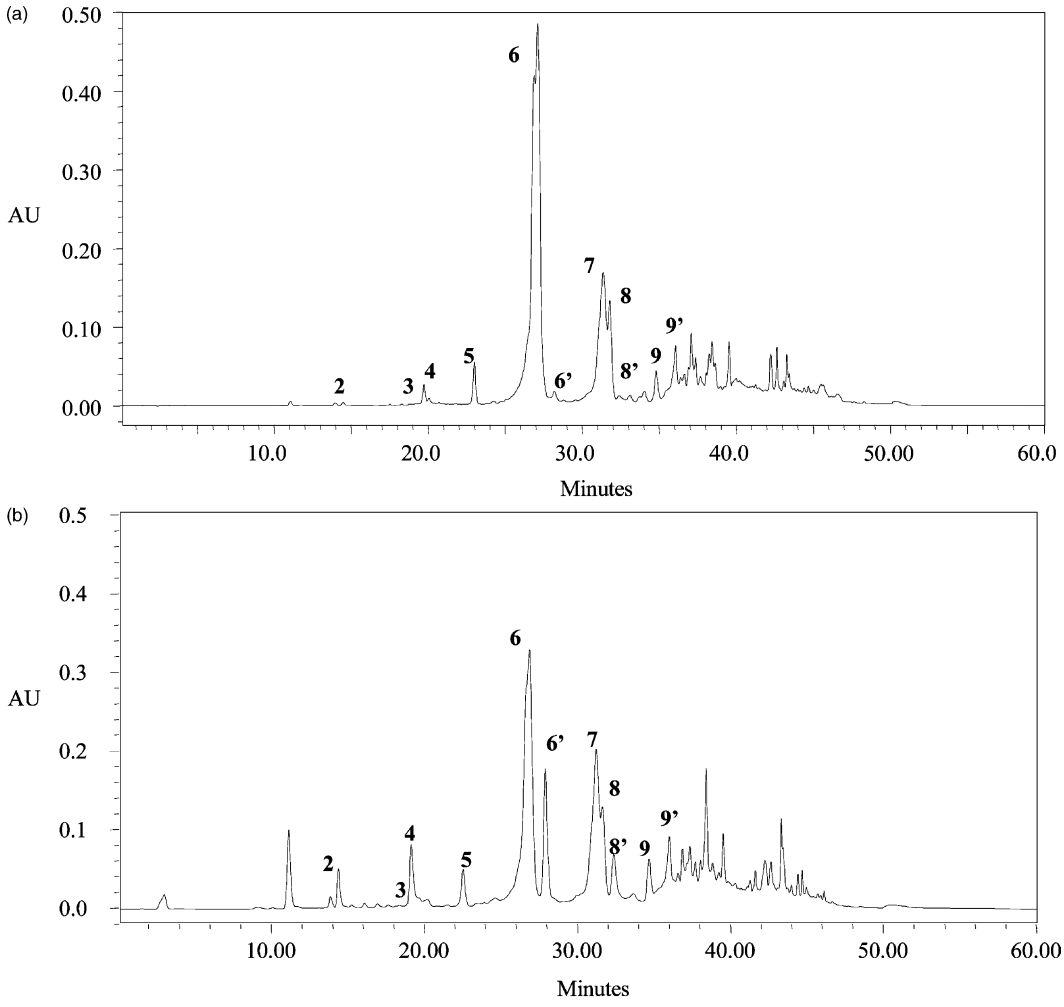

Fig. 3. HPLC chromatograms (at 278 nm) of phenolic extracts from olive oil. (a) fresh oils; (b) stored oils. See Section 2 for chromatographic conditions. See Table 1 to identify the peaks.

life of olive oil (Baldioli et al., 1996; Gennaro et al., 1998) and good correlations have been found between them and oxidative stability of oils (Tovar et al., 2001).

Among the most representative phenolic compounds in olive oil, lignans seem to be the most stable during oil storage.

The variation of Peaks 6′, 8′ and 9′ justified their purification and the determination of the mass spectra in order to elucidate the structure. Peak 6′, with RT = 28 min had the same spectrum as 3,4-DHPEA-EDA with maxima at 226.3 and 279.9 nm. The shapes of the absorbance spectra were also the same. Peak 6′ and 3,4-DHPEA-EDA were isolated and the molecular weight of both moieties was determined by MS. They both had a molecular weight of 378, suggesting that Peak 6′ could be an structural isomer of 3,4-DHPEA-EDA.

Peak 8′, which elutes at RT = 33 min after lignans (a mix of 1-acetoxypinoresinol and pinoresinol) had a slightly different UV spectrum from that of lignans, with maxima at 225 and 275 nm that are the same maxima as *p*-HPEA-EDA. The mass spectrum of this compound displayed major signals at *m*/*z* (relative intensity) 291 and 362 in the negative ESI, and 416 in the positive ESI, corresponding to the molecular weight of 1-acetoxypinoresinol and 291, which could correspond to 1-acetoxypinoresinol moieties that have lost a phenyl ring. However, the main signal is the *m*/*z* 362 that corresponds to the molecular weight of *p*-HPEA-EDA, but Peak 8′ elutes 5 min later. Therefore, we could affirm that the compound present in the Peak 8′ is an structural isomer of *p*-HPEA-EDA, Peak 8′ having a lower polarity than *p*-HPEA-EDA, so that Peak 8′ may correspond to an aldehydic form (closed ring structure) of tyrosol linked to elenolic acid (*p*-HPEA-EA) (Lennen et al., 2002).

Peak 9′, which elutes at RT = 36 min had the same UV spectrum and mass spectrum (*m*/*z* 378) as 3,4-DHPEA-EA; however, Peak 9′ elutes 2 min later. Both peaks may be chemically related, and are probably structural isomers. However, their differences cannot be

Table 2
Phenolic compounds (mg kg^{-1}) of fresh olive oil and olive oil stored for 12 months

Peak	Phenolic compounds[a]	Harvesting period	Mill 1		Mill 2		Mill 3	
			Fresh	Stored	Fresh	Stored	Fresh	Stored
1	3,4-DHPEA	First	0.13	1.63	0.48	1.35	0.33	4.77
		Last	0.24	2.08	0.10	0.21	0.14	0.94
2	*p*-HPEA	First	0.11	0.60	1.77	2.89	0.27	2.83
		Last	0.27	1.15	0.80	0.85	0.22	1.27
3	Vanillic acid	First	ND	ND	0.42	0.33	ND	ND
		Last	0.02	ND	0.11	ND	0.01	ND
4	Vanillina	First	0.25	0.20	0.35	0.24	0.34	0.82
		Last	0.17	0.18	0.23	0.11	0.18	0.15
5	3,4-DHPEA-AC	First	30.1	26.1	48.4	30.1	27.3	5.9
		Last	27.2	26.0	11.0	6.9	19.6	4.1
6	3,4-DHPEA-EDA	First	358	185	127	64.6	353	182
		Last	171	112	18.7	4.4	80.9	33.9
6′	Peak 6′	First	6.57	13.9	2.75	6.92	6.18	18.6
		Last	1.41	7.33	0.00	0.00	8.94	3.32
7	*p*-HPEA-EDA	First	69.2	33.5	47.2	24.3	64.3	42.4
		Last	51.9	29.5	42.9	10.2	65.8	26.6
8	Lignans	First	43.8	34.8	119.7	116.4	62.4	38.3
		Last	79.6	72.1	68.6	40.5	34.5	30.2
8′	Peak 8′	First	1.93	2.93	2.91	3.46	2.76	4.87
		Last	1.48	2.50	1.43	1.09	3.39	3.50
9	3,4-DHPEA-EA	First	102	45.0	64.4	42.7	127	76.7
		Last	78.1	54.1	46.9	11.9	86.9	52.2
9′	Peak 9′	First	68.8	13.6	28.3	21.4	73.7	32.8
		Last	45.8	19.4	31.4	7.51	48.4	22.2

ND: not detected.

[a] 3,4-DHPEA, hydroxytyrosol; p-HPEA, tyrosol; 3,4-DHPEA-AC, 4-(acetoxyethyl)-1,2-dihydroxybenzene; 3,4-DHPEA-EDA, dialdehydic form of elenolic acid linked to hydroxytyrosol; *p*-HPEA-EDA, dialdehydic form of elenolic acid linked to tyrosol; 3,4-DHPEA-EA, oleuropein aglycone. Each extract was analyzed in duplicate and gave reproducible results.

due to the ring of elenolic acid since the open ring structure (3,4-DHPEA-EDA) elutes at RT = 27 min. Peak 9′ structure could differ in the position of some substituent which is reflected in the polarity of the moiety.

The identified oleuropein and ligstroside-aglycone derivatives differ in that the elenolic acid ring structure is either open or closed, and also in the number of aldehydes, and the presence or absence of a carboxy-group.

Elucidations of the structures of Peaks 6′, 8′ and 9′ are in progress and should be confirmed by NMR.

4. Conclusions

An increase of oleic acid percentage has been found in the fatty acid composition as a result of the degradation of polyunsaturated acids (linoleic acid and linolenic acid) in both harvesting periods. Important losses in chlorophyll and carotenoid content of oils have been reported after storage, which has been reflected in higher values of luminosity (L^*) in oils stored for 12 months. α-Tocopherol disappeared completely after storage, while total phenol content decreased significantly, that decrease being higher in those oils collected in the first harvest period. This fact suggests that α-tocopherol plays an important role as an antioxidant in the induction period of oxidation.

In the phenolic fraction, a noticeable decrease was observed in secoiridoid derivatives and 3,4-DHPEA-AC, in both harvesting periods after 12 months' storage, while lignans were the more stable phenolic compounds. Stored oils showed a marked increase of the structural isomers (Peaks 6′ and 8′) of secoiridoid derivatives.

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