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Loss of stability of "veiled" (cloudy) virgin olive oils in storage

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Abstract

Filtration of veiled virgin olive oil samples indicated a loss in stability that was assigned to a loss in total polyphenol content. Oil samples, after filtration, had quite similar peroxide values and absorbance in the UV region to the respective veiled ones. Nine months storage of veiled and filtered oils at ambient temperature, in the dark, revealed that gradual loss in stability, expressed as OSI values, was greater in the latter. This finding was reflected by the greater increase in peroxide values and also faster decrease in total polar phenol content in filtered samples. The RP-HPLC analysis of the phenolic fractions, at various stages of storage, indicated interesting differences in the evolution of individual phenols between the two types of samples. It is suggested that, not only the higher content, but also the forms of individual phenols liberated in veiled oils, due to hydrolytic processes, may be responsible for the unexpected high oxidative stability of this promising commercial category of virgin olive oil.

Keywords: Veiled olive oil; Filtration; Phenols; Stability

1. Introduction

Veiled (cloudy) virgin olive oil (VVOO) is the fresh olive fruit juice prior to final filtration and bottling. Apart from being an intermediate product in olive oil technology, VVOO can be bottled and sold separately, as some consumers prefer its flavour and consider it to be of higher nutritional value. Experimental results concerning the stability of veiled oil are rather conflicting and the pertinent literature is rather poor. Lercker, Frega, Bocci, and Servidio (1994) and also Frega, Mozzon, and Lercker (1999) claimed that the water and the small particles dispersed in the oil have some antioxidant effect. According to these authors, filtering causes a decrease in the stability of the oil. Ambrosone, Angelico, Cinelli, Di Lorenzo, and Ceglie (2002) monitored the oxidation of model emulsified olive oils and found that the dispersed water reduces the rate of oxidation. Suggestions have also been made that traces of peptides may contribute to the stability of non-filtered oils (Hidalgo, Alaiz, & Zamora, 2001, 2002) although the level of such compounds is very low and a significant effect can hardly be expected in the presence of strong antioxidants such as polar phenols and α-tocopherol. Brenes, Garcia, Garcia, and Garrido (2001), reported that storing of olive oil without filtration gave higher values of parameters indicating oxidation, such as peroxide value and K_{232}. Thus, whether veiled oil is more stable or not remains ambiguous although the reported presence of oxidative enzymes (Georgalaki, Sotiroudis, & Xenakis, 1998; Vaglimigli, Sanjust, Curreli, Rinaldi, & Rescigno, 2001) in addition to esterases and glycosidases (Brenes et al., 2001), suggests a more rapid oxidation in the unfiltered oil.

An evaluation of the oxidative status in the suspension is necessary to better understand some important reactions, which affect the quality of the bottled VVOO and the technology of storing before decanting and bottling. The present work aims at measuring the oxidative

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stability and shelflife of bottled cloudy olive oils, as well as of unfiltered oils, obtained soon after pressing. The oils were examined before storing and after 1, 2, 4, 6 and 9 months on the shelf. Each sample was examined for P.V., K_{232}, K_{270}, total phenol content and HPLC analysis of the phenolic fraction. Stability was measured periodically using a Rancimat apparatus at 120 °C (20 l/h). Other determinations (e.g., tocopherol content and pigments) were carried out where necessary.

2. Materials and Methods

2.1. Materials

Bottled VVOO samples were donated from a packer in Crete (samples 1 and 2). Unfiltered oil, just after pressing, was obtained from an olive mill in Kalamata, Greece (sample 3), from Crete (sample 4), and from a plant located in the area of Athens (samples 5 and 6). All the samples fell within the category of ''extra virgin olive oil''. Part of the oil samples was filtered in the laboratory using a common filter paper. The filtration of the samples was performed in the dark.

All chemicals were of analytical grade. *n*-Hexane, acetic acid (glacial), trichloromethane and isooctane (spectra grade) were purchased from Riedel-de-Haën (Seelze, Germany). Folin–Ciocalteau reagent and potassium iodate were obtained from Panreac (Barcelona, Spain), sodium carbonate anhydrous and sodium thiosulfate were from Riedel de Haën (Seelze, Germany). Soluble starch was purchased from Merck Co. (Darmstadt, Germany) and caffeic acid standard (98%, purity) was from Fluka (Germany). The HPLC grade solvent acetonitrile was obtained from (Baker Inc, Deventer, Holland), and methanol and formic acid were purchased from Riedel de-Haën (Seelze, Germany). *n*-Hexane was Baker Analyzed HPLC 95% Reagent (Deventer, Holland), 2-propanol acetone and acetonitrile (Chromasolv) and methanol (pro-analysis), were from Riedel de Haën (Seelze, Germany) and the diethylether was Labscan, Analytical Sci. (Dublin, Ireland). DL-α-Tocopherol (99% for biochemistry) (α-T) and β-carotene (for biochemistry) were purchased from Merck (Darmstadt, Germany). Chlorophylls *a* and *b* (Chl) *a* and *b* were from Sigma Chemical Co. (St. Louis, MO, USA). Pheophytins *a* and *b* (Pheo *a* and *b*) were prepared from the respective chlorophylls by acidification (HCl, 1 N).

2.2. Methods

2.2.1. Free acidity, peroxide value, and specific ultraviolet absorbance K_{232}, K_{270}

The determinations were made in triplicate following the analytical methods described in the EC Regulation No. 2568/1.7.91.

2.2.2. Total phenol content determination

The total phenol content of the oils was determined colorimetrically using the Folin–Ciocalteau reagent (Gutfinger, 1981). The instrument used was a U-2000 Hitachi spectrophotometer (Tokyo, Japan). Results were expressed as caffeic acid equivalents (mg CA/kg of oil).

2.2.3. Rancimat assay

Stability indices were determined at 120 °C and a flow rate of 20 l/h using a Rancimat 679 Metrohm (Metrohm Ltd., Switzerland). Oxidative stability index (OSI) values were expressed in hours.

2.2.4. Shelflife tests

Two of the VVOO samples (5 and 6), and also the respective filtered ones in a series of sealed glass bottles filled with oil (headspace 4%), were kept in a carton box at room temperature. Samples were periodically removed and stored at −23 °C before analysis. HPLC analysis for all samples was carried out after completion of the experimentation.

2.3. High-pressure liquid chromatography of polar fractions

2.3.1. Sample preparation

A 2.5 g oil sample was weighed in a centrifugal tube, dissolved in 5 ml hexane and 5 ml of a methanol:water 60:40, v/v, mixture were added. The tube was centrifuged for 10 min at 3500 rpm. After the oil layer removal, the methanol–water extract was condensed in a rotary evaporator under vacuum at 40 °C. The dry residue was first flushed with nitrogen and then diluted in 1.5 ml of methanol.

2.3.2. Chromatogaphic separation

Phenol separation and characterization were achieved using a P400 Finnigan MAT (Thermoseparation Products Inc., San Jose, USA) liquid chromatograph equipped with a 505 LC oven (Rigas Labs, Thessaloniki, Greece), and a UV 6000LP diode array detection system. Data were processed using ChromQuest software (Thermoseparation Products Inc., USA). A Spherisorb ODS-2, 5 μm, 250 mm × 3.0 mm I.D. (Rigas Labs, Thessaloniki, Greece) column was used. Solvent A was formic acid:water (99:1), v/v and solvent B was methanol:acetonitrile 50:50, v/v, at a flow rate of 0.9 ml/min. The elution gradient was: t = 0 min: 4% B, t = 1 min: 4% B, t = 26 min: 30% B, t = 66 min: 98% B, t = 70 min: 4% B, t = 80 min: 4% B. The injected volume was 20 μl.

2.3.3. HPLC analysis of tocopherols and pigments of VVOO

The solvent delivery system consisted of two Marathon IV Series HPLC pumps (Rigas Labs, Thessaloniki,

Greece) and a Rheodyne injection valve (Model 7125) with a 20 μl fixed loop (Rheodyne, Cotati, CA, USA). The liquid chromatograph was equipped with a UV–Vis spectrophotometric detector SPD-10AV (Dual Wavelength) Shimadzu (Kyoto, Japan) connected in series with a Diode Array Linear UVIS-206 Multiple Wavelength system (Linear Instr. Fermont, CA, USA). Computer software, Linear UV–Vis-206 (Linear Instr.), was used to obtain and store absorption spectral chromatographic data. The data from the UV–Vis SPD-10AV detector were stored and processed with the chromatographic software EZChrom (Sci. Software, Inc., San Ramon, CA, USA). A gradient elution was used with *n*-hexane/2-propanol (99:1, v/v) (A) and 2-propanol (B) as eluents. The gradient was: 0% (B) for 10 min; 0–5% (B) in 4 min; 5% (B) for 6 min; 5–0% (B) in 4 min; 0% (B) for 6 min. Separation was achieved on a 250 × 4 mm i.d., LiChrospher-Si, 5 μm, column (MZ Analysentechnik, Mainz, Germany) at 1.2 ml/min flow rate. The injection volume was 20 μl. Detection of tocopherols was achieved using a UV detector at 294 nm while pigments (β-carotene; lutein; pheophytin α) were monitored using a diode array detector. Detailed information for the identification and quantification of individual compounds is given in a previous paper by Psomiadou and Tsimidou (1998).

3. Results and discussion

Values indicating the stability, other quality characteristics and total polyphenol content of all the virgin olive oils (VOO) samples are presented in Table 1. As shown in the Table, the stability (OSI value) of the unfiltered samples is in all cases significantly higher than that of the corresponding filtered oils. This coincided with a higher total phenolic content in the unfiltered oils. Filtration did not affect the level of peroxide values or K_{232} and K_{270} in most of the samples. To understand the phenomena a shelflife test was carried out. Samples 5 and 6 were examined for loss in stability during storage. The samples were stored for 9 months at 20 °C in the dark, though no bottled virgin olive oil is expected to remain for more than a 3–4 month period on the shelf. Monitoring of the oxidation was carried out by peroxide value, OSI value and total polar phenol determination (Table 2).

Table 2
Quality characteristics and oxidative stability of virgin olive oil sample pairs 5/5* and 6/6* in relation to storing (20 °C, dark, closed bottles with headspace 4%)

Samples[a]	P.V. (meq O_2/kg oil)	Rancimat stability (h, 120 °C)	Polyphenol content (mg CA/kg oil)
Storage time: t = 1 months			
5	14.4	11.8	308
5*	16.2	9.0	215
6	14.6	12.1	333
6*	19.0	7.7	194
Storage time: t = 2 months			
5	14.0	11.7	341
5*	16.2	8.4	243
6	14.4	11.0	338
6*	18.7	7.2	227
Storage time: t = 4 months			
5	15.1	10.5	248
5*	19.3	7.3	125
6	15.4	10.6	225
6*	20.0	7.1	97
Storage time: t = 6 months			
5	16.4	10.4	280
5*	26.4	5.5	89
6	16.2	10.0	261
6*	25.4	5.8	102
Storage time: t = 9 months			
5	26.0	10.4	231
5*	43.3	4.9	80
6	24.7	10.7	175
6*	38.7	5.9	49

[a] For the sample numbers see Section 2 part. The asterisk denotes the same oil after filtration.

Table 1
Quality characteristics and oxidative stability of olive oil samples before storing ($t = 0$)

Samples[a]	P.V. (meq O_2 kg oil)	Rancimat stability (h, 120 °C)	K_{232}	K_{270}	Polyphenol content (mg CA/kg oil)
1	8.8	N.d.[b]	1.70	0.15	178
1*	8.8	N.d[b]	1.70	0.15	148
2	10.3	9.0	1.60	0.17	190
2*	10.3	8.0	1.60	0.17	171
3	10.9	9.3	1.85	0.26	166
3*	10.9	8.8	1.85	0.26	167
4	7.3	12.9	1.49	0.18	245
4*	7.3	12.1	1.46	0.18	199
5	13.8	12.5	2.15	0.14	418
5*	13.9	6.9	2.15	0.14	328
6	14.1	12.1	2.15	0.15	449
6*	18.1	8.0	2.15	0.15	210

[a] For the sample numbers see Section 2 part. The asterisk denotes the same oil after filtration.
[b] Not determined.

Table 3
HPLC quantitative results for tyrosol (TY) and hydroxytyrosol (HTY) of samples 5 and 6 in relation to storing time (*t*: 0, 6 and 9 months)

Months	Samples	Tyrosol[a] (mg/kg oil)	Hydroxytyrosol[a] (mg/kg oil)
$t = 0$	5	42.5 ± 0.5	32.9 ± 0.1
	5*	22.1 ± 0.9	29.6 ± 0.6
	6	42.5 ± 0.9	48.3 ± 2.5
	6*	13.8 ± 0.5	31.5 ± 0.6
$t = 6$	5	41.1 ± 0.4	42.2 ± 0.5
	5*	14.2 ± 0.7	21.0 ± 0.7
	6	37.8 ± 0.02	47.1 ± 0.5
	6*	12.3 ± 0.5	29.2 ± 0.6
$t = 9$	5	26.4 ± 1.1	26.9 ± 0.4
	5*	12.6 ± 0.3	19.3 ± 0.5
	6	29.0 ± 2.0	33.5 ± 1.0
	6*	14.9 ± 0.3	30.2 ± 1.0

[a] Values are the means of three injections.

Changes in quality characteristics and stability of oil samples were observed for both filtered and unfiltered oils after the second month of storage. However, the two types of oils showed different behaviour in storage, especially after the 4th month. The unfiltered oils appeared to be better protected against oxidative deterioration since the rate of oxidation in terms of peroxide value was lower. Stability of VVOO was only slightly affected during the first four (4) months. The % loss in polyphenols by the end of the experimentation was found to be lower than that observed for the filtered counterparts. Filtered oil had a greater loss of polyphenols in actual values at every step of storing, which was reflected to the corresponding PV, in particular after the 4th month.

Analysis of certain samples indicated significant changes in the profile of phenols before and after storage

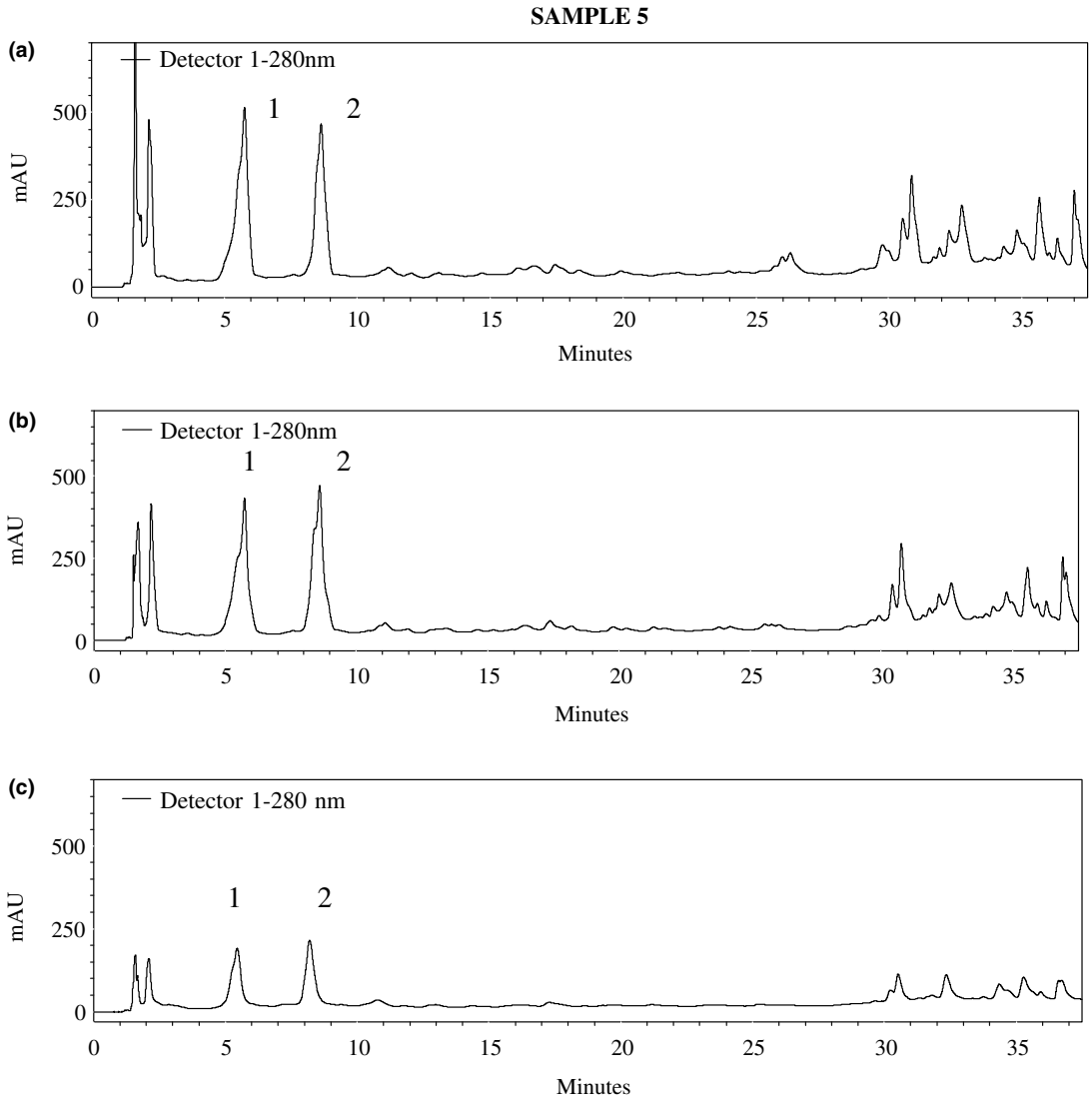

Fig. 1. Chromatographic profile of the olive oil polar fraction for samples 5 and 5* at 280 nm, (a) $t = 0$, (b) $t = 6$, (c) $t = 9$ months. Peak characterisation: HTY,1; TY, 2.

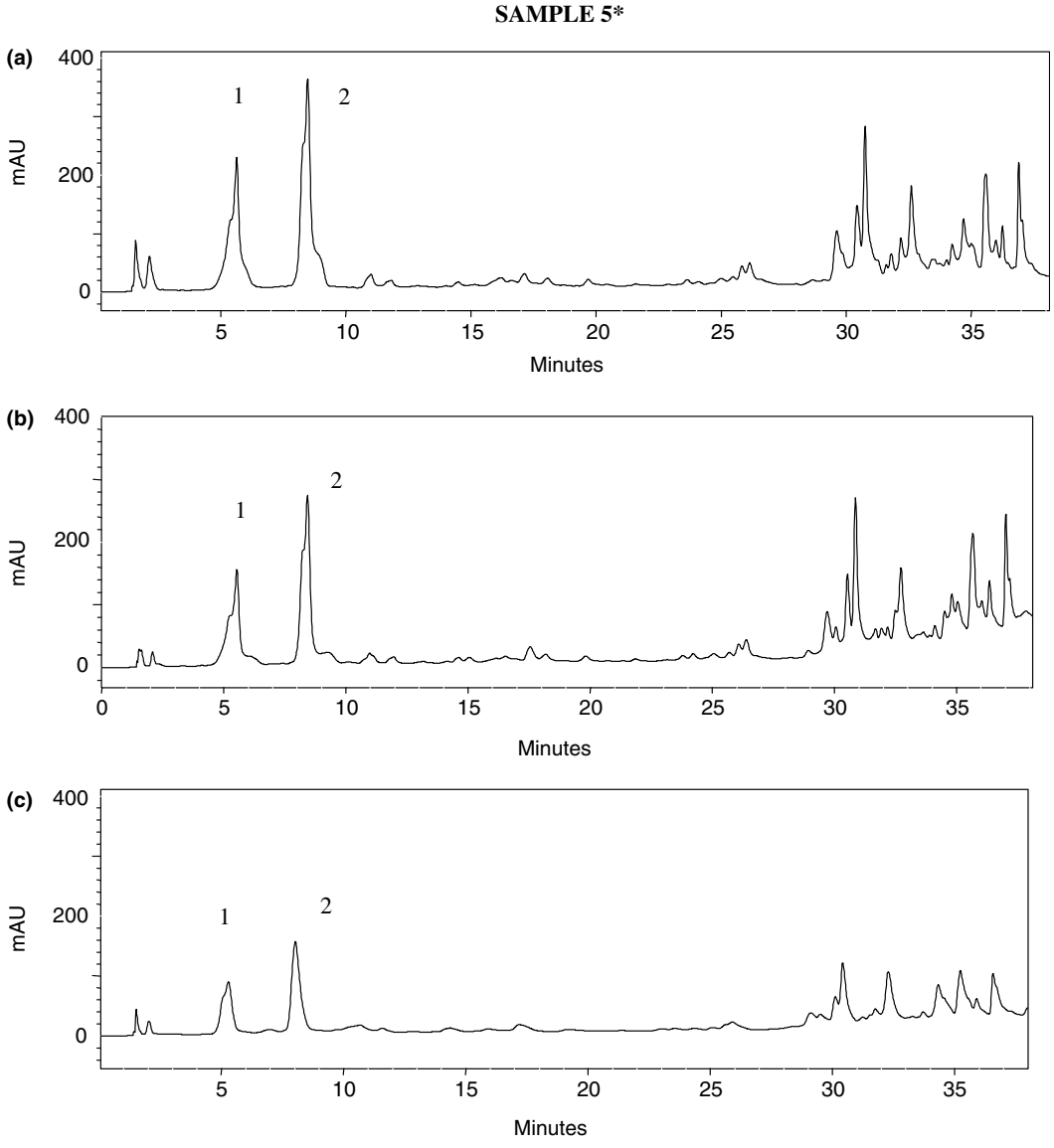

Fig. 1 (*continued*)

of the veiled and filtered oils. Qualitative and quantitative changes in the content of free hydroxytyrosol (HTY) and tyrosol (TY) during storage showed that the loss of the two phenols was continuous for nine (9) months (Table 3). The loss in free phenols was more rapid in the filtered oils. In the non-filtered oils, changes occurred after nine months of storage while, for the filtered oils, changes were observed much earlier. Filtration reduced, not only the levels of free phenols and the stability of oils, but also the levels of HTY, which has a strong antioxidant activity. The levels of TY were also significantly decreased.

The loss of complex phenols was continuous (Fig. 1). It could be that hydrolytic processes occurred in parallel with oxidation, which is in line with Montedoro et al. (1993) and also Cinquanta, Esti, and La Notte (1997) and Pagliarini, Zanoni, and Giovanelli (2000), who suggest hydrolysis of aglycone esters in long term storage. The water content and a_w levels for veiled oils support such a hypothesis, since the respective values for sample 5 were: 0.34% moisture/0.66 a_w versus 0.04(<0.01) for the 5* and 0.52% moisture/0.70 a_w versus 0.06(<0.01) for the sample pair 6/6*. Part of the total water content present in a food product is bound and part is free and available for chemical and enzymic reactions. This suggests a hydrolytic process in VVOO samples during storage. A higher water activity level is expected to favour enzymic activities, including lipase, lipoxygenase and phenoloxidase activities. Thus, a more rapid oxidation of the unfiltered oil could be expected. On the other hand, oxidation rates found in the literature indicate two maxima in the oxidation curve versus a_w, one below

$a_w < 0.1$ and the other $a_w > 0.65$. The first max corresponds to the water activity of the filtered oil and the second to the veiled one. Nevertheless, it is difficult to conclude which oil is more prone to oxidation.

Some assumptions have been made for the explanation of the higher stability of veiled olive oil. As already suggested by Lercker et al. (1994) and also by Frega et al. (1999), the suspended material contains chemical compounds acting as antioxidants and the avoidance of filtration is highly desirable to extend the shelflife of the oil. Ambrosone and his co-investigators (2002) claimed that, in the artificially prepared w/o emulsions using 3% water, the dispersed water itself exerted a positive antioxidant effect.

Undoubtedly, a loss of a significant part of polyphenols during precipitation or filtration is related to the reduction of oxidative stability. Some of the phenols present may act as inhibitors of oxidative enzymes and enhance (in an indirect way) the total antioxidant activity, although the conditions in the veiled oil (water activity ~0.7) favour enzymic reactions. It is possible that, in addition to the total content of polyphenols, the nature of individual phenolic compounds, mainly various forms of oleuropein aglycones, play a role. Moreover, for four months veiled oils showed no losses in tocopherol content, chlorophylls or carotenoids, a fact that adds to their marketing position. This trend is illustrated in Fig. 2 for the VOO samples 6/6*.

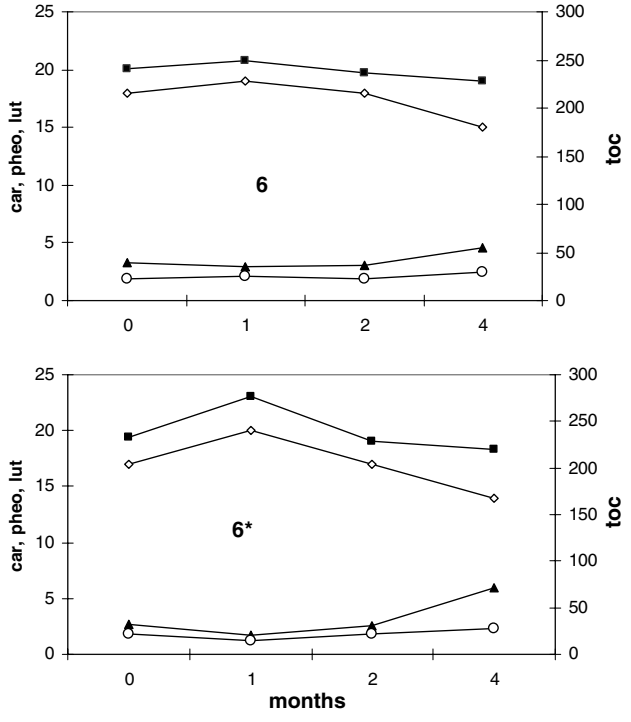

Fig. 2. Changes in α-tocopherol and pigment content during storage for VOO samples 6 and 6* (mg α-tocopherol/kg oil, ■ mg β-carotene/kg oil, lozenge; mg pheophytin α kg oil, ▲; lutein/kg oil, ○).

Although literature on the stability of olive oil in relation to concentration of phenolic compounds is relatively abundant, few researchers have focussed on the relative changes of filtered and unfiltered oil phenolic compounds during storage. It has to be assumed that the greater stability of the unfiltered oil is due to the higher total polyphenol content since the levels of other major oil antioxidants do not change after filtration. The additional polyphenols obviously protect the veiled oil, either by interfering with the free radical reaction, or by acting as antioxidants in an indirect way, i.e., by inhibiting oxidizing enzymes. This has to be further investigated. From the above it can be concluded that a more thorough investigation of chemical but also enzymic reactions taking place in the veiled oil is necessary for a better evaluation of this natural product and its commercial appreciation by interested parties.

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