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Solid-Phase Microextraction in the Analysis of Virgin Olive Oil Volatile Fraction: Modifications Induced by Oxidation and Suitable Markers of Oxidative Status

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Modifications of virgin olive oil subjected to accelerated storage were evaluated by HS-SPME analysis. To find a suitable marker of oxidative degradation, the volatile compounds showing variable concentration during the oxidative process have been identified and quantified by SPME coupled to GC-MS and GC-FID, respectively. The SPME analysis results were then compared with the parameters usually applied to assess the oxidative status of lipids, such as peroxide value, spectrophotometric absorbance, and loss of unsaturated fatty acids. Finally, the assessment of nonanal has been suggested as a marker of oxidative degradation. This rapid, inexpensive, and reliable method may allow screening of oils prior to testing by a panel of assessors.

KEYWORDS: Virgin olive oil; oxidation; volatiles; oxidative status; aldehydes; quality; solid-phase microextraction; SPME

INTRODUCTION

The main process that alters vegetable oils and fat-containing foods is rancidification. Autoxidation of unsaturated lipids modifies the organoleptic characteristics through the development of off-flavors and may also decrease nutritional quality and safety through the formation of secondary reaction products (*1*).

Compared with other vegetable oils, virgin olive oil has been shown to be more resistant to oxidation because of its low polyunsaturated fatty acid content and the presence of natural antioxidants, such as tocopherols and phenolic compounds (*2*). However, despite its stability, virgin olive oil remains susceptible to rancidification. Oxidation reactions reduce the high nutritional value of virgin olive oil, mainly due to the loss of tocopherols and phenolic compounds (*3*, *4*), and lead to the deterioration of its characteristic flavor through the development of undesirable sensory attributes from hydroperoxide decomposition products (*5*−*8*).

The characteristic virgin olive oil aroma and, in particular, its green and fruity attributes depend on many volatile compounds that derive from the degradation of polyunsaturated fatty acids through a chain of enzymatic reactions known as the lipoxygenase pathway (*9*−*13*). Variable amounts of hexanal, hexanol, and hexyl acetate derive from the degradation of linoleic acid, whereas (*Z*)-3-hexenal, (*E*)-2-hexenal, (*E*)-2-hexenol, (*Z*)-3-hexenol, and (*Z*)-3-hexenyl acetate result from the enzymatic degradation of linolenic acid (*14*−*16*). For this reason, the content of aldehydic compounds such as hexanal cannot, as is the case for other vegetable oils, be used as an index of oxidative status.

According to European Commission (EC) regulations the oxidative deterioration in olive oils is assessed by the peroxide value, spectrophotometric absorbance at 232 and 270 nm, and sensory evaluation (*17*, *18*). The peroxide value determines the amount of primary oxidation products, whereas spectrophotometric absorbance measures the formation of conjugated dienes and trienes due to the formation of primary or secondary oxidation products (*19*−*22*). Sensory evaluation is based on the detection of volatile secondary oxidation products affecting the organoleptic properties of the oil. As the majority of these compounds present a low threshold odor (*19*, *23*, *24*) the method is very sensitive, even at low concentrations, and able to detect oxidative deterioration at earlier stages than are other parameters. However, there are some disadvantages to sensory analysis, namely, the different sensitivity of each panelist and the need for a costly and time-consuming training of assessors.

Several methods have been studied to assess the oxidative status of lipidic substances, based on the detection of primary or secondary oxidation products and analysis of the oxidation substrate (*19*, *20*). Among these, some studies have examined how the volatile fraction of virgin olive oil is modified during oxidation, mainly by applying dynamic headspace (DH) tech-

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Table 1. Analytical Parameters of the Virgin Olive Oil Submitted to Accelerated Storage

analytical parameter	value
acidity	0.67%
peroxide value	8.6 mequiv of O_2/kg
K_{270}	0.13
K_{232}	2.04
fatty acids	
C16:0	13.2%
C16:1	1.3%
C17:0	0.0%
C17:1	0.1%
C18:0	2.5%
C18:1	76.2%
C18:2	5.1%
C20:0	0.4%
C18:3	0.6%
C20:1	0.3%
C22:0	0.1%
total phenols	193 μg/g as tyrosol
total tocopherols	223 μg/g

niques (*5*−*8*). The advantage of volatile analysis is that it has low detection limits for volatile secondary oxidation products related with the generation of off-flavors detected by sensory evaluation.

Recently, headspace solid-phase microextraction (HS-SPME) has been introduced as an alternative to the dynamic headspace technique and as a sample preconcentration method prior to chromatographic analysis. SPME is a rapid, sensitive, and solvent-free sampling technique developed by Arthur and Pawliszyn (*25*) for the analysis of pollutants in water, and it has since been applied to food flavor analysis. The suitability of HS-SPME for qualitative and quantitative analysis of virgin olive oil volatile fraction has recently been evaluated (*26*), and qualitative data obtained by applying this technique to virgin olive oil were also reported by other authors (*27*−*31*). HS-SPME has also been used to characterize the volatile compounds in other vegetable oils. In the case of refined vegetable oils, volatile compounds formed during oxidation have been isolated by SPME and characterized by GC-MS (*24*, *32*). In the analysis of lipid oxidation products in milk HS-SPME demonstrated better precision, accuracy, repeatability, and linearity of response than did the DH technique (*33*).

In the present paper, HS-SPME was applied to evaluate the modifications occurring in the volatile composition of virgin olive oil during oxidation in order to designate a valid indicator of oxidative degradation at early stages. The method was then compared with other validated techniques usually applied to assess the oxidative status of olive oil.

MATERIALS AND METHODS

Accelerated Storage Test. A virgin olive oil from Bianchera variety olives produced in northern Italy was used. The oil was recently produced at industrial scale (4 months) and was properly conserved in the dark and at low temperature until the beginning of the study. It corresponded to the extra virgin olive oil commercial class according to EC regulations (*17*, *18*) and was characterized by high quality and antioxidant content (**Table 1**).

Thirty-two glass flasks (50 mL) were filled with the oil so that the headspace was ∼1% of the volume. Thirty of the capped flasks were placed in a thermostat at 60 °C, whereas the fresh oil of 2 flasks was immediately analyzed. At 1 week intervals, from 0 to 16 weeks, samples were withdrawn in duplicate from the thermostat to be analyzed.

The accelerated storage test was carried out in the dark and in the presence of a low percentage of headspace volume in order to reproduce, as far as possible, the conditions normally applied in virgin olive oil storage. For the same reason the oxidation process was accelerated by using a moderate temperature (60 °C), thus enabling the progressive appearance of oxidative degradation products to be detected (*19*) and avoiding secondary reactions of hydroperoxide decomposition, which could take place under extreme conditions but are not relevant during real oil storage (*34*).

Analytical Methods. Peroxide value, free acidity, fatty acid composition, and spectrophotometric absorbance at 232 and 270 nm were determined according to EC regulations (*17*). Total phenolic content was determined by HPLC as described by Cortesi et al. (*35*), and tocopherol content was assessed as described in ref *36*. The appearance of rancid odor in the olive oil samples was evaluated by means of the judgment of 19 assessors.

HS-SPME Sampling of Volatile Compounds. As previously reported (*26*), 1.5 g of sample spiked with 4-methyl-2-pentanol (Sigma-Aldrich, St. Louis, MO) to a concentration of 1.5 μg/g was placed in a 10 mL vial fitted with a silicone septum. The SPME sampling was performed by exposing the divinylbenzene/Carboxen/polydimethylsiloxane fiber (50/30 μm, 2 cm long from Supelco Ltd., Bellefonte, PA) for 30 min in the headspace of the sample maintained in a water bath at 40 °C under magnetic stirring.

The fiber was then desorbed for 1 min at 260 °C in the gas chromatograph injection port.

GC-FID and GC-MS Analysis. After SPME sampling, GC analyses were performed using two Hewlett-Packard 5890 series II gas chromatographs, one equipped with an FID detector and one coupled to a Hewlett-Packard 5971A quadrupole mass selective spectrometer. Both were provided with a split−splitless injection port. Helium was the gas carrier, at linear velocities of 23 and 17 cm^3/s for GC-FID and GC-MS, respectively.

Changes in the headspace oil composition during the accelerated storage test were quantitatively evaluated by the GC-FID analysis as this method is more sensitive than mass spectrometry, applied to identify the chromatographic peaks of interest. Separation of compounds was performed on two columns of different polarities: Supelcowax-10 and SPB-1 (both 30 m × 0.25 mm i.d., 0.25 μm film thickness), both purchased from Supelco Ltd. Column temperature was held at 40 °C for 10 min and increased to 200 °C at 3 °C/min. The FID temperature was set at 280 °C, and the temperatures of the ion source and the transfer line were 175 and 280 °C, respectively. Electron impact mass spectra were recorded at 70 eV ionization energy in the 15−250 amu mass range, 2 scan/s.

Quantitative analysis of each of the two replicates at the different oxidation times was carried out in duplicate by HS-SPME/GC-FID. Relative amounts of volatile compounds were calculated by the internal standard method, considering the relative response factor to be 1. Concentrations were expressed as micrograms per gram equivalents of 4-methyl-2-pentanol. Finally, concentrations of nonanal were calculated by using the experimental response factor (*26*) and expressed as micrograms per gram.

Statistical Analysis. Data were analyzed using the Statistica 5.0 package (StatSoft '97 edition). Pearson linear correlation was applied to relate the concentration of volatile compounds to the time of oxidation.

RESULTS AND DISCUSSION

Qualitative Analysis. Figure 1 shows the chromatographic profiles relative to the nonoxidized and oxidized virgin olive oils, obtained by separation on both of the capillary columns used.

To investigate the oxidative changes in the olive oil headspace we focused on the main secondary oxidation products derived from oleic, linoleic, and linolenic acid, together with some other compounds showing variable concentration during the oxidative process. These compounds were identified, as shown in **Table 2**, either by comparing their mass spectra and retention times with those of standard compounds or by comparing the mass spectra with those of the Wiley 6.0 mass spectrum library. Kovats retention indices were also determined on two chro-

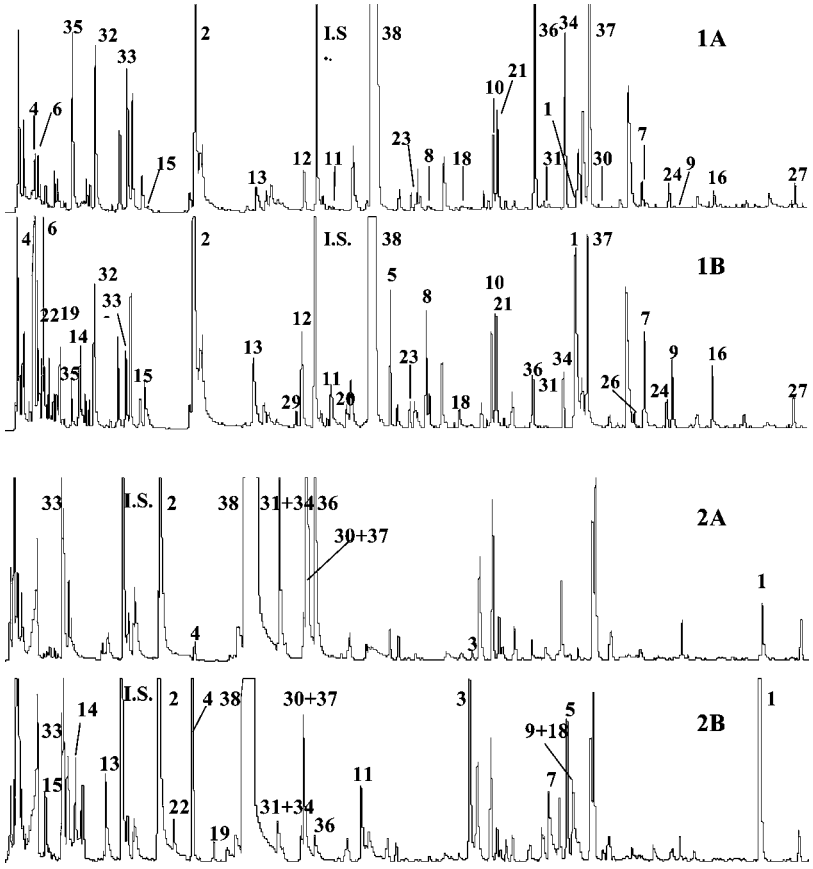

Figure 1. Chromatographic profile of the nonoxidized (A) and oxidized (B) virgin olive oil, obtained by separation on Supelcowax-10 (1) and SPB-1 (2) capillary columns, respectively. The identification numbers correspond to the compounds listed in **Table 2**.

matographic capillary columns of different polarities and compared with retention indices of compounds available in the literature. A number of compounds were tentatively identified on the basis of their mass spectra alone. This was the case for 2,3-dihydro-4-methylfuran and 2,3-dihydro-5-methylfuran (*m*/*z* 41, 55, 69, and 84 and *m*/*z* 43, 72, and 84, respectively).

Evolution of Volatile Compounds during the Oxidation Process. To select a number of compounds as possible markers of the oxidation process, the amount of each compound was monitored during the test and correlated with the time of oxidation. **Table 2** shows the slope of the linear regression, the *r* value, and the significance of the correlation between the concentration of each compound and the time of oxidation. It should be pointed out that the response factor was considered to be 1 for all of the compounds studied; thus, it was not their real concentrations that were evaluated, but rather the ability of the technique to detect modifications of the volatile fraction related to oxidative reactions. In terms of the slope values of the regression straight line, the peaks corresponding to nonanal and hexanal are those that most rapidly increase during oxidation, their rates being quite similar. Among volatile aldehydes the presence of other saturated species, such as pentanal, heptanal, and octanal, was detected. The behavior of pentanal, formed from 13-LOOH, could not be considered because it coeluted with 3-pentanone on both of the columns used, as previously reported (*26*). However, the sum of these compounds was only weakly influenced by the oxidative process, in accordance with the results reported by Solinas et al. (*7*). As expected, the responses of both heptanal and octanal also proved to be related to the time of oxidation, even though the rate of increment was lower (**Table 2**). Although the presence of heptanal, octanal, and nonanal may be attributed to decomposition reactions of hydroperoxides formed by the autoxidation of oleic acid, the amount of hexanal is due to both autoxidation and the lipoxygenase cascade, through the formation of 13-LOOH (*15*, *16*, *41*).

2-Alkenals, ranging from (*E*)-2-propenal to (*E*)-2-heptenal, together with traces of (*E*)-2-decenal were also found. The trend of 2-alkenal amounts was positive except for (*E*)-2-hexenal, the presence of which was mainly attributed to enzymatic oxidation of linolenic acid rather than to autoxidation of linolenic acid (*41*), this compound being the main product of the lipoxygenase pathway (*14*). Increasing amounts of (*E*)-2-propenal, (*E*)-2-pentenal, and (*E*)-2-decenal were assumed to have originated from secondary reactions of the primary autoxidation products 16- and13-LnOOH and 9-OOOH, respectively (*41*). Using the present method, the amount of (*E*)-2-heptenal could not be directly determined because of its coelution with (*Z*)-2-pentenol on the polar column and with peak 8 on the apolar column. This unidentified compound (fragment ions *m*/*z* 41, 53, 81, 95, 109, and 124) was found to be present in increasing amounts during the storage test. As shown in **Figure 2**, peak 8 increased linearly from time 0 to time 5 weeks, approximately, at which point the slope of the curve changed, finally following a negative trend. As the behavior of peak 8 could be monitored by separation on the polar column (**Figure 2**) and the slope of the sum of peak 8 and (*E*)-2-heptenal proved to be steeper than the slope of peak 8 alone, it may be deduced that (*E*)-2-heptenal increased during oxidation. The absence of significant variations for the sum of (*E*)-2-heptenal and (*Z*)-2-pentenol may be due to a simultaneous decrease of the latter. Also, the increase of (*E*)-2-butenal was observed during accelerated storage. These compounds are mainly formed by photosensitized oxidation (*1*, *41*), but in the present experiment this cannot explain the

Table 2. Identification of Volatile Compounds and Their Correlation with Oxidation Time

		RI[k]		RI refs					
	compound[a]	SW[k]	SPB[k]	SW	SPB	ID[k]	slope[b]	*r*	*p*
1	nonanal[c]	1391	1082	1382 (*37*)	1087 (*37*)	*d,e*	9.00	0.975	*f*
2	hexanal[g]	1084	769	1084 (*37*)	780 (*37*)	*d,e*	8.27	0.970	*f*
3	(*E*)-2-heptenal[c] + peak 8[c]	1321	931	1314 (*39*)	954 (*38*)	*e*	5.01	0.962	*f*
4	octane[c]	800	800			*d,e*	4.14	0.970	*f*
5	2-pentylfuran[g]	1230	979	1220 (*39*)		*d,e*	2.99	0.966	*f*
6	(*E*)-2-propenal[g]	840	nd[k]	838 (*39*)		*e*	2.89	0.925	*f*
7	(*E,Z*)-2,4-heptadienal[g]	1460	970	1454 (*39*)	1000 (*38*)	*e*	1.99	0.888	*f*
8	peak 8[g]	1259	931				1.91	0.800	*h*
	peak 8[g,i]	1259	931				6.52[i]	0.967	*f*
9	(*E,E*)-2,4-heptadienal[g]	1487	981	1483 (*39*)		*e*	1.60	0.931	*f*
10	hexenyl acetate[g]	1315	990	1300 (*37*)	987 (*37*)	*d,e*	1.33	0.933	*f*
11	heptanal[g]	1185	879	1186 (*37*)	883 (*37*)	*d,e*	1.22	0.929	*f*
12	1-penten-3-ol[g]	1165	nd	1157 (*38*)	792 (*38*)	*e*	1.20	0.876	*f*
13	(*E*)-2-pentenal[g]	1131	718	1131 (*38*)	766 (*38*)	*e*	1.16	0.800	*h*
14	2-ethylfuran[g]	941	676	944 (*39*)		*e*	1.12	0.970	*f*
15	(*E*)-2-butenal[g]	1033	619	1035 (*39*)		*e*	0.87	0.889	*f*
16	propanoic acid[g]	1528	nd			*e*	0.86	0.780	*h*
17	hexanoic acid[g]	1836	nd	1850 (*38*)	890 (*38*)	*d,e*	0.74	0.903	*f*
18	octanal[g]	1287	981	1278 (*37*)	985 (*37*)	*d,e*	0.56	0.865	*h*
19	2-octene[c]	864	810	880 (*37*)	810 (*37*)	*e*	0.51	0.867	*h*
20	2,3-dihydro-4-methylfuran [g,j]	1196	nd			*e*	0.48	0.878	*f*
21	(*Z*)-2-pentenol[g,j] + (*E*)-2-heptenal[g]	1321	nd			*e*	0.36	0.422	
22	1-octene[c]	836	783	850 (*39*)		*e*	0.34	0.838	*h*
23	peak 23[g]	1246	1204				0.34	0.916	*f*
24	α-copaene[g]	1482	1368	1488 (*41*)	1380 (*41*)	*e*	0.32	0.885	*f*
25	(*E,E*)-2,4-decadienal[c]	nd	1287	1710 (*38*)	1283 (*38*)	*e*	0.31	0.934	*f*
26	1-octen-3-ol[g]	1449	967	1420 (*37*)	968 (*37*)	*e*	0.28	0.910	*f*
27	(*E,E*)-α-farnesene[g]	1747	1486	1751 (*41*)	1515 (*41*)	*e*	0.25	0.887	*f*
28	(*E*)-2-decenal[g]	1641	1237	1590 (*38*)	1234 (*38*)	*e*	0.18	0.941	*f*
29	2,3-dihydro-5-methylfuran[g,j]	1160	nd			*e*	0.13	0.389	
30	(*Z*)-2-hexenol[g]	1417	855			*d,e*	0.04	0.425	
31	(*E*)-3-hexen-1-ol[g]	1361	836			*d,e*	–0.13	–0.960	*f*
32	pentanal + 3-pentanone	979	669	935 (*38*)	791 (*38*)		–0.13	–0.105	
33	1-penten-3-one[g]	1013	654	973 (*38*)	680 (*38*)	*d,e*	–1.36	–0.860	*h*
34	(*Z*)-3-hexen-1-ol[g]	1380	838	1391 (*38*)	844 (*38*)	*d,e*	–2.58	–0.985	*f*
35	ethanol[g]	926	551	929 (*38*)	500 (*37*)	*d,e*	–3.42	–0.958	*f*
36	1-hexanol[g]	1353	858	1360 (*38*)	858 (*38*)	*d,e*	–4.19	–0.984	*f*
37	(*E*)-2-hexen-1-ol[g]	1403	853	1377 (*38*)	854 (*37*)	*d,e*	–6.35	–0.971	*f*
38	(*E*)-2-hexenal[g]	1218	824	1220 (*38*)	826 (*38*)	*d,e*	–15.54	–0.553	

[a] Sorted by linear regression slope relative to concentration as a function of oxidation time. [b] Slope of the linear regression (multiplied by 100) between concentration (μg/g) and oxidation time (weeks). [c] Determined on SPB-1 capillary column. [d] Identified by comparison with standard compounds. [e] Tentatively identified by Wiley 6 mass spectra library search and retention index. [f] (Significance of the correlation) $p \leq 0.000005$. [g] Determined on Supelcowax-10 capillary column. [h] $p \leq 0.0005$. [i] Calculated from time 0 to time 5 weeks. [j] Tentatively identified. [k] RI, Kovats retention index; SW, polar capillary column (Supelcowax-10); SPB-1, apolar capillary column (SPB-1); ID, identification method; nd, not determined.

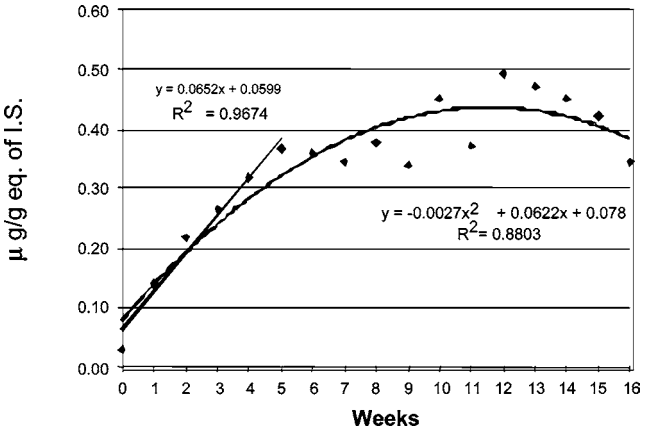

Figure 2. Amounts of compound relative to peak 8 as a function of time of oxidation; fitted line from 0 to 5 weeks and from 0 to 16 weeks.

increase in these compounds as the oxidation test was carried out in the dark. However, traces of (*E*)-2-butenal have been reported as resulting from the autoxidation of linolenate (*41*), and Keszler et al. (*42*) proposed a mechanism by which 2-heptenal is formed from 9-LOOH β-scission and successive degradation of the diene radical. At low temperatures, such as that applied in the present study, the homolytic type β-scission is favored (*43*). Moreover, there is evidence of other oxygen-activating mechanisms that do not require light, and these may be the cause of the increase in (*E*)-2-butenal and (*E*)-2-heptenal during the present experiment. The most important termination process for secondary peroxyl radicals at room temperature is the Russell mechanism, involving a tetraoxide intermediate that produces a ketone, an alcohol, and oxygen. There is evidence that the oxygen produced is activated at the singlet state (*1*). In addition, metals can initiate fatty acid oxidation by reaction with oxygen: the anion thus produced may either lose an electron to give singlet oxygen or react with a proton to form a peroxyl radical (*1*).

In terms of the hydrocarbon fraction, among the alkanes originating from autoxidation reactions only octane, known to be formed from 10-OOOH (*41*), could be determined (**Table 2**). Octane showed one of the highest increment velocities in this study. Furthermore, 1-octene and 2-octene were detected, and their response was noted to increase over time. Although no data are available on the origin of the first, the second is

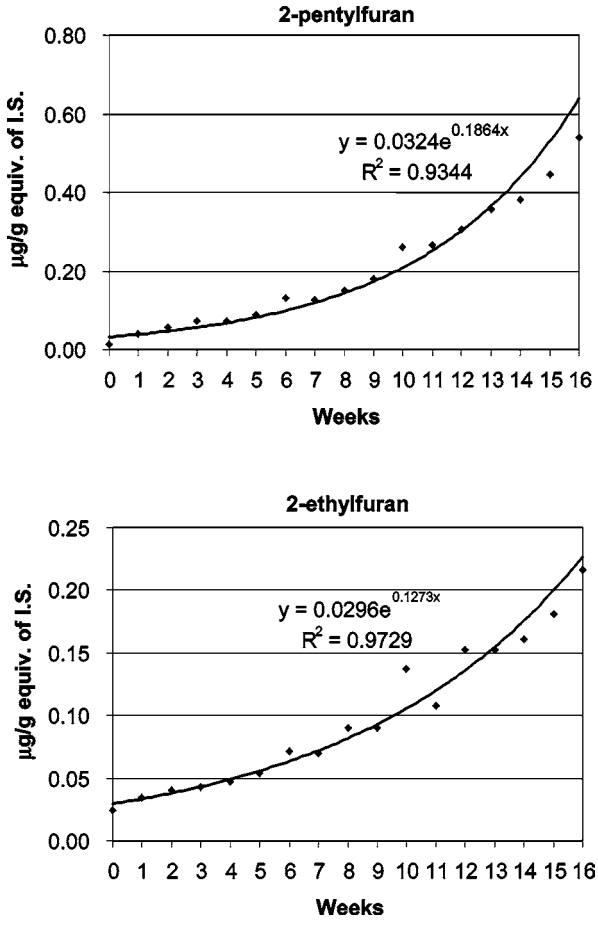

Figure 3. Amounts of 2-pentylfuran and 2-ethylfuran as a function of time of oxidation, expressed as micrograms per gram equivalents of 4-methyl-2-pentanol.

considered to be a consequence of the photo-oxidation of linolenate, followed by the decomposition of 10-LOOH (*34*).

During the oxidation process the formation of a number of substituted furans was also observed, mainly represented by 2-pentylfuran and 2-ethylfuran (**Table 2**). Their presence seems to be due to degradation reactions of linoleate and linolenate hydroperoxides, respectively, although the mechanisms of formation remain unclear (*1*, *34*, *43*). In fact, Frankel (*34*) reports that autoxidized linoleate hydroperoxides produced more 2-pentylfuran and less (*E*)-2-heptenal than photo-oxidized linoleate hydroperoxides. Moreover, two compounds tentatively identified as 2,3-dihydro-4-methylfuran and 2,3-dihydro-5-methylfuran appeared in lower amounts during oxidation. The presence of similar compounds in the headspace of oxidized virgin olive oil was reported by Morales et al. (*8*), whereas 2-ethylfuran and 2-pentylfuran were detected by the same authors in fresh virgin olive oil (*11*). Except for the compound tentatively identified as 2,3-dihydro-5-methylfuran, the rest of the detected substituted furans showed a significant correlation with the time of oxidation. 2-Ethylfuran, and, in particular, the peak corresponding to 2-pentylfuran rapidly increased from traces to considerable amounts during the time of oxidation, the exponential curve being shown in **Figure 3**.

2,4-Heptadienal isomers and traces of (*E*,*E*)-2,4-decadienal from 12-LnOOH and 9-LOOH decomposition, respectively, were detected in the samples' headspace. Whereas heptadienal isomers presented discrete responses and high correlation with the time of oxidation, the response of (*E*,*E*)-2,4-decadienal was quite low (**Table 2**).

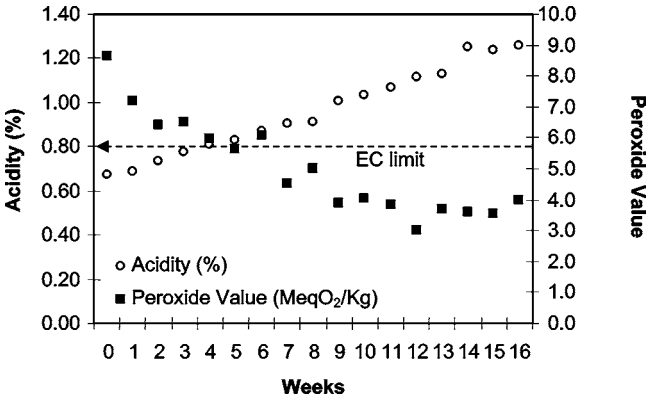

Figure 4. Variations of acidity (percent) and peroxide value during accelerated storage of the oil.

Alcoholic compounds showing variable concentration during oxidation were also monitored. Only 1-penten-3-ol and 1-octen-3-ol increased during oxidation (**Table 2**). Some authors have proposed that the 1-penten-3-ol in olive originates from pentene radicals produced by the β-scission of 13-linoleic alkoxy radicals (*45*), whereas 1-octen-3-ol derives from 10-LOOH decomposition (*46*). In contrast with the above-mentioned alcohols, ethanol and products of the lipoxygenase pathway, such as 1-hexanol, 2-hexenol, and 3-hexenol, showed a negative trend with a high degree of inverse correlation with the time of oxidation (**Table 2**). These results are not consistent with those of Angerosa et al. regarding the oxidation of virgin olive oils by light exposure (*5*). It may be that their decrease during accelerated storage is due to alcohols undergoing further oxidative reactions.

In terms of carboxylic acids, a positive trend was observed for hexanoic and propanoic acid, although responses proved to be quite low (**Table 2**). Hexanoic acid may result from the secondary decomposition of hexanal and 2,4-decadienal formed from fatty acid oxidation (*41*). In contrast, there is little information about the formation of propanoic acid observed in the present study.

Other compounds normally present in nonoxidized virgin olive oil were noted to vary their concentration during the test, such as hexenyl acetate, α-copaene, (*E*,*E*)-α-farnesene, and 1-penten-3-one. Whereas this last compound was reduced slightly, a moderate increment was observed for the other compounds.

Modification of Analytical Quality Indices. In terms of fatty acid composition (**Table 1**), no variations were detected during the oxidation test, probably because the extent of oxidation was insufficient to reveal the disappearance of those fatty acids undergoing oxidation.

In contrast, a progressive increase of free acidity was observed (**Figure 4**), reaching, after 4 weeks of storage, the maximum limit for extra virgin olive oil established by EC regulations (0.8 g of oleic acid/100 g of oil, in force from November 2003) (*47*). The peroxide value was observed to decrease during the oxidation process (**Figure 4**). Under the oxidation conditions applied, low concentrations of oxygen were available for the reaction with alkyl radicals to form peroxyl radicals and then hydroperoxides. The decreasing peroxide value across the whole experimental time may be explained by the fact that the rate of formation of new hydroperoxides was lower than the rate of decomposition. These results are consistent with those obtained by other authors for the storage or accelerated oxidation of olive oil in the presence of limited amounts of oxygen (*5*, *45*).

As shown in **Figure 5**, a correlation with the time of oxidation was found for K_{232} and, especially, K_{270} ($r = 0.855$ and $r =$

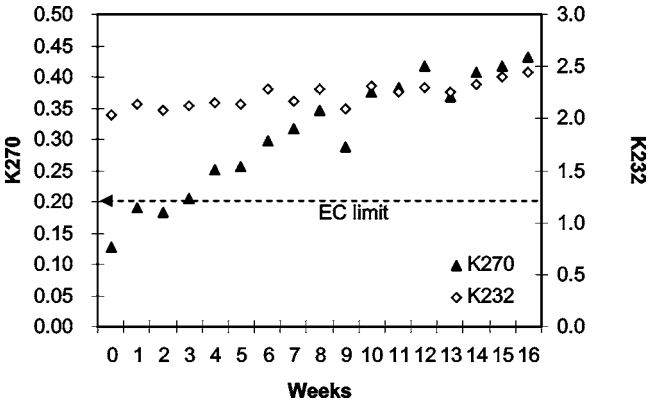

Figure 5. Variations of K_{232} and K_{270} during accelerated storage of the oil.

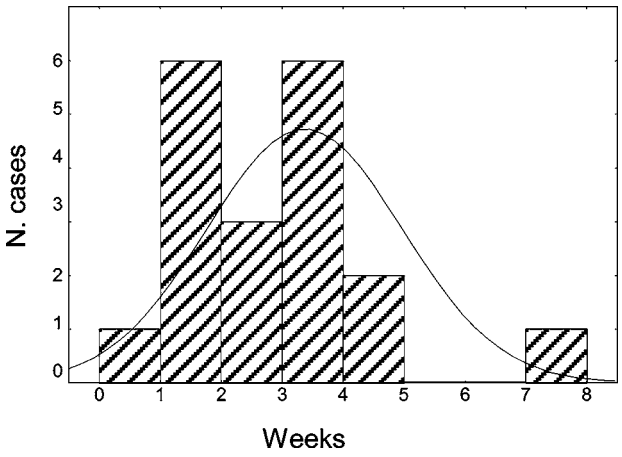

Figure 6. Frequency histogram of the minimum oxidation time required by the panelists to detect the rancid defect.

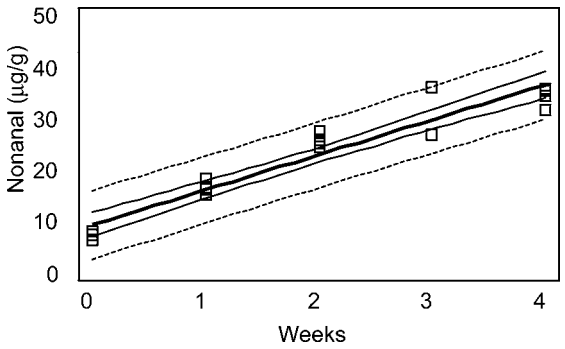

Figure 7. Fitting for nonanal concentrations within 0 and 4 weeks of accelerated storage. The 95% confidence values for the mean of observations (inner bound) and the 95% prediction values for new observations (outer bound) are shown.

0.961, respectively), reflecting the presence of secondary oxidation products (*22*, *45*). The value of K_{270} exceeded the limit of 0.2 established by EC regulations for extra virgin olive oil (*18*) in the fourth week of accelerated storage.

Markers of Oxidation Status. Headspace analysis showed a similar trend to that of K_{270}, K_{232}, and free acidity, but not to that of peroxide value. In terms of assessing an oil's oxidative status, the peroxide value is not a valid parameter because it is not always correlated with the oxidative deterioration of the oil. In fact, although the peroxide value decreased, a significant increment in volatile oxidation products was observed.

Among the volatile oxidation products analyzed by HS-SPME, the highest rates of formation were observed for nonanal, hexanal, and octane, followed by 2-pentylfuran, (*E*)-2-propenal, 2,4-heptadienal isomers, and peak 8. Except for hexanal, which derives either from autoxidation or from the lipoxygenase pathway, all of these compounds can be considered as markers of the degree of oxidation, although some of them do not have a particularly significant impact on flavor due to their high odor thresholds (*8*, *46*).

As 2-pentylfuran and 2-ethylfuran seem to increase more slowly during the earlier stages of the oxidative process (**Figure 3**), they may be useful for distinguishing oils at a high degree of oxidation but are less sensitive with oils of higher quality. In contrast, peak 8 increases rapidly during the first stages, and after 4 weeks of accelerated storage, when free acidity and K_{270} exceeded the EC regulation limits for extra virgin olive oil, its concentration is ~10-fold higher than at the initial time (**Figure 2**). Further information on the structure of this compound and its mechanism of formation is required prior to its being considered as a suitable marker of olive oil oxidation.

Evaluation of oxidation products with oleic acid as their precursor should be a more reliable way of assessing oxidative status because their presence in olive oil headspace is due exclusively to chemical oxidation. In fact, enzymatic oxidation affects only linoleic and linolenic acid, leading to the formation of C_6 volatile compounds. Either nonanal or octane derives from the autoxidation of oleic acid, and given that nonanal showed the highest rate of increment and fitting during the oxidation, it was considered to be the most suitable index of the oxidative degree.

Figure 6 displays a frequency histogram of the minimum oxidation time required by the panelists to detect the rancid defect. As in the fourth week 85% of assessors perceived the rancid odor in the oil samples, and other parameters such as free acidity and K_{270} exceeded the EC regulations limit for extra virgin olive oil, the behavior of nonanal was evaluated within the oxidation time interval from 0 to 4 weeks. **Figure 7** reports the fitting for nonanal concentrations expressed as micrograms per gram within this time range (response factor reported in ref *26*). The plot shows the 95% confidence values for the mean of observations (inner bound) and the 95% prediction values for new observations (outer bound). The upper and lower prediction limits at time 0 were 2.6 and 16.0 μg/g, respectively, and at time 4 weeks the same limits were 29.8 and 43.3 μg/g, respectively. The upper prediction limit at oxidation time 0 may represent the value to consider that a virgin olive oil is of high quality in terms of oxidative status. If nonanal concentration exceeds the lower limit calculated at 4 weeks of accelerated storage, it may indicate that the virgin olive oil is not of extra quality class. For values of nonanal between 16 and 29.8 μg/g, the oils are located in an intermediate interval, and their oxidative degradation should be stated by means of a panel test. However, the study of a large number of oil samples is necessary to propose a reliable limit to classify extra virgin olive oils in terms of oxidative status.

The results suggest that headspace analysis by SPME is a suitable method for evaluating the degree of oxidation of virgin olive oils and, in particular, that the determination of oxidation compounds such as nonanal may be considered as a marker of oxidative status. This rapid, inexpensive, and reliable method may allow screening of the oils previously to tested by a panel of assessors, being a valid support to the sensory analysis.

ABBREVIATIONS USED

LOOH, linoleic acid hydroperoxide; LnOOH, linolenic acid hydroperoxide; OOOH, oleic acid hydroperoxide.

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