Changes in the Volatile Composition of Virgin Olive Oil during Oxidation: Flavors and Off-Flavors

M. T. Morales, J. J. Rios, and R. Aparicio*

Instituto de la Grasa (CSIC), Avenida Padre García Tejero 4, 41012 Seville, Spain

A thermoxidation process has been applied to extra-virgin olive oil to develop new knowledge on the evolution of the volatile compounds responsible for virgin olive oil flavor during oxidative deterioration. The initial volatiles (a total of 60), many of them responsible for the pleasant sensory characteristics of the oil and produced mainly through biochemical pathways, disappeared in the first hours, and the formation of off-flavors, produced through oxidative pathways, gradually increased. The main volatile compounds possibly responsible for off-flavors (51) were identified, and their evolution during the oxidative process was studied. The fatty acids content was determined during the process. Unsaturated fatty acids were found to be the main precursors of the volatile compounds found in oxidized samples. The early measurement of nonanal (which was not detected at all, or only at trace levels, in extra-virgin olive oil samples) could be an appropriate method to detect the beginning of the oxidation. The ratio hexanal/nonanal was used to differentiate between oxidized and good-quality virgin olive oil samples. Sensory evaluation of the samples and peroxide value agreed on the evolution of the oxidation.

Keywords: *Oxidation; volatiles; flavor; off-flavor; virgin olive oil*

INTRODUCTION

Olive oil is the oil obtained from the fruit of the olive tree (*Olea europaea* L.). When the oil is properly processed from fresh and mature fruit of good quality, an oil with abundant flavor is obtained, the so-called "virgin olive oil", which is greatly liked by native consumers and internationally appreciated by gourmets (Kiritsakis and Min, 1989).

Normal processing of oils and fats removes almost all undesirable minor components such as colored compounds, free fatty acids, metals, and volatiles, but retains the major neutral lipids and most of the natural antioxidant tocopherols present in the oil. On the contrary, virgin olive oil is unique among the vegetable oils in that it can be consumed crude, thus conserving flavor compounds, vitamins, and other important natural compounds.

The complex flavor of virgin olive oil is mainly produced by volatile and phenol compounds (Flath et al., 1973; Morales et al., 1994), most of which have been identified and quantified in virgin olive oils obtained from different olive varieties at different stages of ripeness (Morales et al., 1996a). The formation of volatiles is related to olive fruit cell destruction. The total amount of volatiles changes during the processing steps (milling, malaxation, centrifugation, etc.) of olive fruit in the olive mill (Morales et al., 1996b). Many authors (Flath et al., 1973; Guth and Grosch, 1993; Morales et al., 1995; Aparicio et al., 1996) have clearly demonstrated that volatile compounds are responsible for flavor, and it has been shown that the main components contributing to the fragrant global flavor of the virgin olive oil are aldehydes, alcohols, esters, and ketones. Most of these volatiles are produced through the lipoxygenase pathway (Olías et al., 1993)—in olives this biochemical pathway promotes the formation of C_6 volatile compounds, from linoleic and linolenic hydroperoxides, rather than the C_9 compounds.

* Author to whom correspondence should be addressed (fax +34-5-4616790; e-mail aparicio@cica.es).

The sensory characteristics of these volatile compounds have been studied previously, most of them being responsible for pleasant attributes (Morales et al., 1995; Aparicio et al., 1996). Lipolysis and oxidation are the processes leading to the most serious deterioration of olive oil. Lipolysis usually starts while the oil is still in the fruit, while oxidation begins after the oil is obtained from the fruit and proceeds mainly during storage. Due to these processes, the pleasant sensory characteristics of the oil change to unpleasant ones.

Though virgin olive oil is considered to be a stable oil due to the presence of α-tocopherol and phenolic compounds such as hydroxytyrosol, tyrosol, caffeic acid, and others (Tsimidou et al., 1992), it is susceptible to oxidation, like other vegetable oils, and some off-flavors due to volatile compound deterioration can be detected when oxidation processes start. Consequently, the initially pleasant sensory characteristics of the oil eventually give way to unpleasant sensory attributes.

The advance of oxidation processes in refined vegetable oils is indicated by the increase of total volatiles and the concentration of some specific volatile compounds such as hexanal (Snyder et al., 1988; Warner et al., 1988). However, as virgin olive oil initially has a great amount of volatile compounds and some volatiles, e.g. hexanal, are also present in the original flavor, it was necessary to look for some other way to ascertain when the oxidation process starts.

These changes, and the volatile compounds responsible for them, were evaluated during the accelerated oxidation process.

The present paper studies the changes produced in the initial content of virgin olive oil volatile compounds by applying an accelerated thermoxidation process. The progress of the oxidation is monitored by determining different parameters related to oxidation. The ratio hexanal/nonanal is discussed as an appropriate way to detect the beginning of oxidation and its evolution. The paper also studies the changes produced in the sensory characteristics and fatty acid content of the virgin olive oil during the oxidative process.

S0021-8561(96)00585-7 CCC: $14.00

Table 1. Volatile Compounds and HRGC/Olfactometry of Virgin Olive Oil Sample

scan no.[a]	volatile compound	sniffing	scan no.[a]	volatile compound	sniffing
67	hexane		424	2-methylbutan-1-ol	fish oil
90	methyl acetate		429	3-methylbutanol	
100	octene	solvent-like	438	3-methyl-2-butenyl acetate	putty-like, unpleasant
114	ethyl acetate	sweet, aromatic	446	dodecene	
119	butan-2-one	fragrant, pleasant	449	ethenylbenzene	
125	3-methylbutanal	sweet, fruity	463	pentan-1-ol	pungent
139	1,3-hexadien-5-yne		467	1,2,4-trimethylbenzene	
148	ethylfuran	sweet	484	hexyl acetate	sweet, fruity
155	ethyl propanoate	sweet, strawberry, apple	488	C_8 ketone	fruity, mushroom-like
162	pentan-3-one	sweet	496	octan-2-one	
173	4-methylpentan-2-one	sweet	499	3-(4-methyl-3-pentenyl)furan	moldy
187	pent-1-en-3-one	sweet, strawberry	510	(*E*)-2-penten-1-ol	
194	2-methylbut-2-enal	solvent-like	513	3-hexenyl acetate	green-banana, fruity
206	methylbenzene	glue, solvent-like	519	(*Z*)-2-penten-1-ol	banana
209	2-methylbut-3-enol		532	6-methyl-5-hepten-2-one	fruity
245	butyl acetate	green, pungent, sweet	536	nonan-2-one	fruity
249	hexanal	green, apple	566	hexan-1-ol	fruity, aromatic
273	2-methylbutyl propanoate	aromatic, ketone	570	4-methyl-1-penten-3-ol	
276	2-methyl-propan-1-ol	ethyl acetate-like	575	(*E*)-3-hexen-1-ol	
282	(*E*)-2-pentenal	green, apple	582	alcohol C_6 branched	sweety
294	(*Z*)-2-pentenal	green, pleasant	594	tridecene	
306	ethylbenzene	strong	599	(*Z*)-3-hexen-1-ol	banana
309	(*E*)-3-hexenal	artichoke, green, flowers	609	2,4-hexadienal	
312	(*Z*)-3-hexenal	green leaves, grassy	625	(*E*)-2-hexen-1-ol	green, grassy
330	2-methylpent-4-enal		630	(*Z*)-2-hexen-1-ol	green fruit
337	1-penten-3-ol	wet earth	640	2-octenal	fruity, soap
355	3-methylbutyl acetate	banana	664	acetic acid	pungent
373	heptan-2-one	fruity	710	methyl nonanoate	fruity
378	aldehyde C_6 branched	fruity	747	methyl decanoate	fresh
396	(*E*)-2-hexenal	bitter, almonds, green	781	propanoic acid	aromatic, pungent

[a] Scan numbers correspond to Figure 1.

MATERIALS AND METHODS

Extra-virgin olive oil from the cultivar Spanish Arbequina (Seville, Spain) was properly obtained from fresh, mature fruits of good quality. The cultivar was selected as representing a substantial proportion of the bottled olive oil trade in Europe. Samples were obtained under the best conditions (20 °C), using the centrifugation system, from an olive oil mill, and freeze-stored, in glass bottles, until the moment of analysis, which was carried out in duplicate.

Dynamic Headspace Volatile Concentration. Flavor and off-flavor components were analyzed using dynamic headspace techniques employing Tenax TA (Chrompack) as the adsorbent. Different conditions were used for each:

Flavor Components. A 25 g sample of virgin olive oil was stirred and heated at 40 °C; flavor components were swept from the surface by a nitrogen stream of 12 L/h flow rate for 30 min, and trapped on a Tenax TA trap at room temperature (Morales et al., 1994). Quantification was carried out using isobutyl acetate (3.33 ppm) as internal standard.

Off-Flavor Components. These were obtained by an accelerated thermoxidation process under the following conditions: 10 g of virgin olive oil sample was heated at 100 °C and purged by an oxygen stream of 22 L/h from 0 to 55 h. Each hour a new Tenax TA trap was used to concentrate the volatile compounds. Traps were sealed and stored until volatile compounds were analyzed.

The induction time was determined with a Rancimat apparatus (Laubli and Bruttel, 1986) under identical conditions to those described above. Rancimat was used to have a measure of the induction time by a well-established methodology. Aliquots of oil were periodically removed for peroxide value (EC, 1991) and fatty acid (León Camacho and Cert, 1994) determination.

Adsorbent traps were conditioned prior to use by heating them at 300 °C for several hours and again at 220 °C with passage of the carrier gas. Blank runs were periodically carried out during the study.

The desorption of volatiles trapped in the Tenax TA trap was carried out using a Chrompack thermal desorption cold trap injector (TCT). Desorption was carried out by heating the trap at 220 °C for 5 min. Volatiles were then transported by the carrier gas to a fused silica cold trap previously cooled at −110 °C with liquid nitrogen for 5 min, where they condensed. Finally, the samples were injected into the capillary GC system by flash heating the cold trap at 170 °C.

GC/MS Analysis. A Hewlett-Packard 5890 series II gas chromatograph coupled with a MS 30/70 mass spectrometer (VG Analytical, Manchester, U.K.) and a VG Model 11/250 data system were used for mass spectrometric analyses. A DB-Wax (J&W Scientific, Folsom, CA) fused silica capillary column (60 m × 0.25 mm i.d., 0.25 μm film thickness) was employed. The column temperature was held at 40 °C for 15 min and then was increased to 220 °C at 1 °C/min. The carrier gas (helium) flow rate was 1 mL/min. The end of the fused silica column was inserted directly into the ion source block. The spectra were recorded at an ionization voltage of 70 eV and an ion source temperature of 200 °C.

Sample components were verified by comparison of the mass spectral data with those of authentic reference compounds. When standards were not available, the components were identified by mass spectrum matching using the NBS mass spectral library collection.

HRGC/Olfactometry. To assess the aroma notes corresponding to olive oil volatile compounds, an high-resolution gas chromatography (HRGC)/olfactometry technique (Morales et al., 1994) was applied to virgin olive oil sample before oxidation. The effluent of the GC column was split 1 to 10 to the detector and the sniffing port, respectively. The odor-active regions of the eluate were evaluated, and their aroma notes were assigned by four assessors. The odor descriptions were noted on a form with a preprinted time scale; assessors did not see the chromatogram. Assessors basically agreed on the odors of volatiles, although different semantic terms where used to describe some of them. A consensus-building discussion was held with assessors to decide the final sensory descriptors. Table 1 shows the characterization of the volatile compounds by HRGC/olfactometry.

Sensory Analysis. The oxidative process previously described (off-flavor volatile concentration) was applied to aliquots of the same virgin olive oil. Samples were evaluated for flavor at different stages of the oxidation process (0−22 h of oxidation) by four trained assessors fully expert in evaluating virgin olive oil. An unstructured scale (100 mm) was used to score the samples, which were evaluated in triplicate.

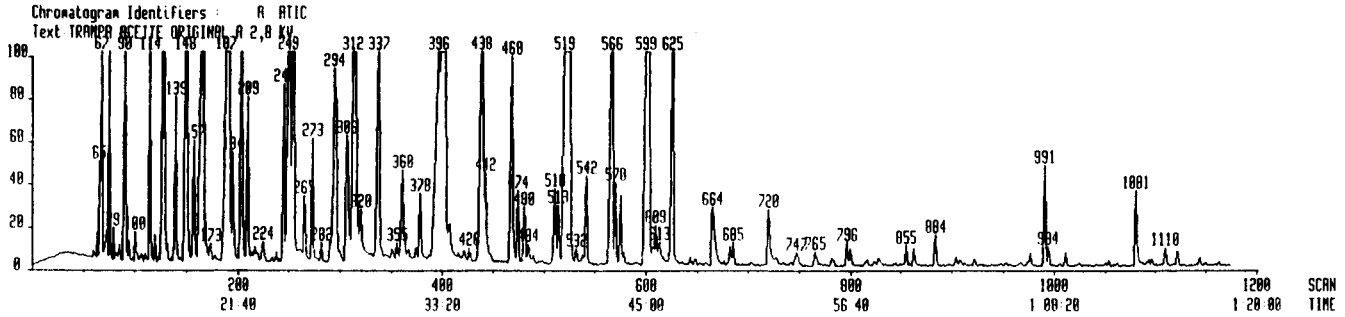

Figure 1. Mass chromatogram of virgin olive oil volatile compounds.

Assessors first qualified the samples by flavor descriptors, and then the flavor score was given. To determine the progression of off-flavors during oxidation, a hedonic scale was tentatively used to evaluate the samples. Assessors were asked how often they would use this olive oil in frying, according to the following 10-category food action scale (Schutz, 1965): 10, I would use this every opportunity I had; 9, I would eat this very often; 8, I would frequently eat this; 7, I like this, and I would eat it now and then; 6, I would eat this if available but would not go out of my way; 5, I do not like it but would eat it on occasion; 4, I would hardly ever eat this; 3, I would eat this only if there were no other food choices; 2, I would eat this if I were forced to; 1, never tried.

Statistical Analysis. The STATISTICA (1992) package was used to carry out the regression analyses. Polynomial regression was fitted by least squares under the following conditions: stiffness = 0.25 in order to have under control the local variation of the data, the lower the value, the more the sensitive; the order polynomial was 4; 10 was the base of logarithm. Linear regression was fitted to the points in the 2D scatterplot. Confidence bands have allowed us to know the probability that the "fine" fitted line falls into the bands. The statistical treatment was applied to quantitative data of total volatiles, hexanal, and nonanal. All data of total volatiles, hexanal, and nonanal were treated by polynomial regression. In each case, partial data were used for linear regression.

Two correlations were calculated: (i) between nonanal and hexanal, with the whole set of data; and (ii) between flavor scores and nonanal with a selected set of data (from 0−22 h).

RESULTS AND DISCUSSION

Virgin olive oil has a complex flavor owed mainly to the presence of many volatile compounds (Figure 1). Table 1 shows the main volatile compounds found in virgin olive oil, many of them responsible for its flavor. Most of them are produced through the lipoxygenase pathway and are always present in the headspace of virgin olive oils, although at different concentrations depending on the olive cultivar (Morales and Aparicio, 1993).

When samples were subjected to oxidation, the initial flavor disappeared in a few hours and then the oxidation process started producing a great amount of volatile compounds, some of them being present in the initial flavor. Figure 2 shows the evolution of the volatile compounds after the oil was subjected to oxidation for 52 h; Table 2 shows the volatile compounds possibly responsible for the virgin olive oil off-flavors and their scan numbers.

The evolution of the total volatile compounds responsible for virgin olive oil flavor during the thermoxidation process can be seen in Figure 3, where a regression coefficient $R = 0.91$ was obtained by the polynomial fit of the whole set of total volatile compounds vs time. A linear regression was also applied to a selected set of data, from 9 to 46 h (induction time), and the linear regression coefficient $R = 0.98$. During the first hour the total volatiles decreased as they were stripped by the gas flow rate, aided by the rise of temperature. At 5 h, it was possible to see (Figure 2A) a zone (from 300 to 900 scan number) where there were practically no volatiles in the oil. At this point, the oil could be considered a refined oil, as the initial volatiles produced mainly through enzymatic pathways have completely disappeared. A remarkable note is the lower amount of hexanal (249) (Figure 2A) at 5 h; it was one of the major peaks in the initial virgin olive oil flavor (Figure 1). From this time on, a further increase of new volatiles (breakdown products of lipid hydroperoxides) was observed while the oxidation process continued. A greater increase of the total volatile content, showing the progressive deterioration in the olive oil, corresponded to the induction time—determined by the Rancimat method as 46.4 ± 0.35 h (Figure 2E).

Once initial volatiles disappeared, the concentration of certain volatiles increased—2-farnesene greatly increased after 5 h of oxidation (Figure 2A). During the next hours the concentrations of several aldehydes increased, such as hexanal, produced by breakdown of linoleate 13-OOH, nonanal, and 2-decenal arising from oleate 9-OOH, and 2-heptenal by decomposition of linoleate 12-OOH (Figure 2B,C). Pentanal and heptanal, originated by decomposition of linoleate 13/11-OOH, and octanal from oleate 11-OOH were also produced in the next hours. 2-Undecenal from oleate 8-OOH also greatly increased. Almost all of these volatiles are responsible for virgin olive oil off-flavors, because their threshold levels for odor are very low, as can be seen in Table 3. This table also shows the aldehydes identified during the process—all of them are chemical compounds having sensory properties, and their odor thresholds (Meijboom, 1964) are low enough to contribute to olive oil off-flavor. Finally, the table shows their theoretical hydroperoxide isomer precursors (Frankel, 1985; Dobarganes et al., 1986; Kochhar, 1993).

The sensory descriptors evaluated by the assessors (Table 4) were compared with the evolution of the volatile compounds (Figure 2) and their sensory properties as they are described in the bibliography (Table 3). Figure 2A shows there are only a few volatiles after 5 h of oxidation, and assessors characterized this oil as "refined, fried oxidized" (Table 4). It is well-known that refined oils have no volatile compounds as they are deodorized.

After 11 h of oxidation (Figure 2B), the major volatile compounds are hexanal (scan number 249) and nonanal (scan number 606), which smell "fatty, waxy" (Table 3). The assessors basically characterized this oil as rancid. The possible synergy between these compounds and other volatiles present at lower concentration could explain why the assessors characterized this oil as rancid.

Hexanal (fatty), 2-heptenal (oxidized, tallowy), nonanal (fatty, waxy, painty), and decanal (penetrating,

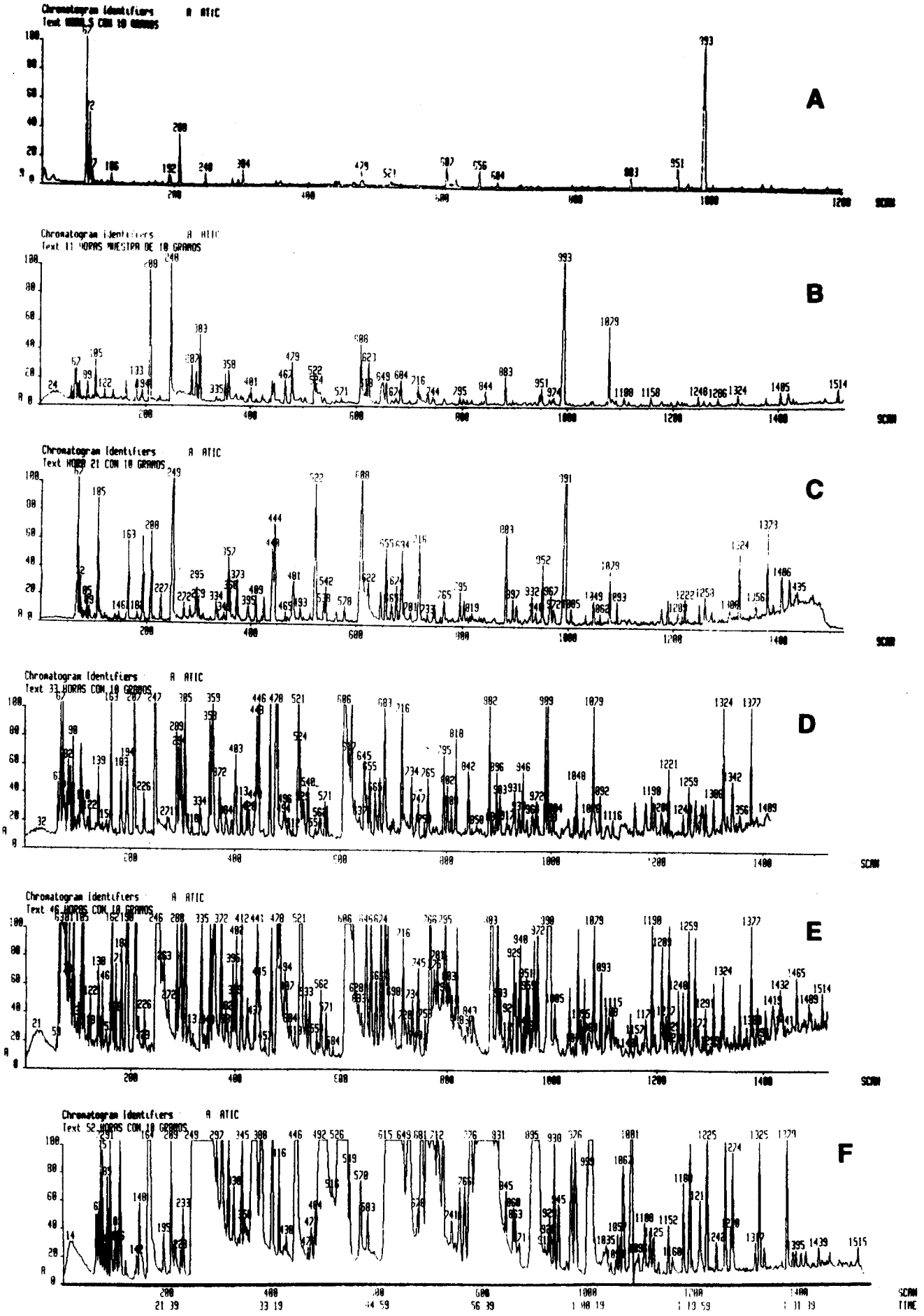

Figure 2. Mass chromatograms of oxidized olive oil volatile compounds: (A) after 5 h of oxidation; (B) after 11 h of oxidation; (C) after 21 h of oxidation; (D) after 33 h of oxidation; (E) after 46 h of oxidation; (F) after 52 h of oxidation.

Table 2. Volatile Compounds of Oxidized Virgin Olive Oil Samples

scan no.[a]	volatile compound	scan no.[a]	volatile compound
67	hexane	733	decanal
72	cyclohexane	747	2,5-octadien-2-one
105	tetrahydrofuran	765	2-nonenal
163	pentanal	795	octanol + hydrocarbon
191	chloroform	803	3,5-octadien-2-one
208	methyl benzene	819	undecanal
249	hexanal	883	2-decenal
295	4-methyl-2,3-dihydrofuran	897	ketone
334	1-penten-3-ol	904	nonanol
352	heptanal	932	pyrrole
373	*z*-methyl-*x*,*y*-dihydrofuran	952	terpenic hydrocarbon
395	2-hexenal	967	terpenic hydrocarbon
409	1,3-nonadiene	991	2-farnesene
440	1,3-nonadiene	996	2-undecenal
444	1,3-nonadiene	1005	2,4-decadienal
481	octanal	1049	2,4-decadienal
522	2-heptenal	1079	hexanoic acid
542	hexanol	1093	6-methyl-5,9-undecadien-2-one
578	3-hexenol	1178	heptanoic acid
608	nonanal	1190	an alcohol
645	2-octenal	1222	furan
655	1,3-dichlorobenzene	1258	octanoic acid
665	acetic acid	1324	nonanoic acid
674	1-octen-3-ol	1356	methyl heptanoate
684	2,4-heptadienal	1378	a ketone
716	2,4-heptadienal		

[a] Scan numbers correspond to Figure 2.

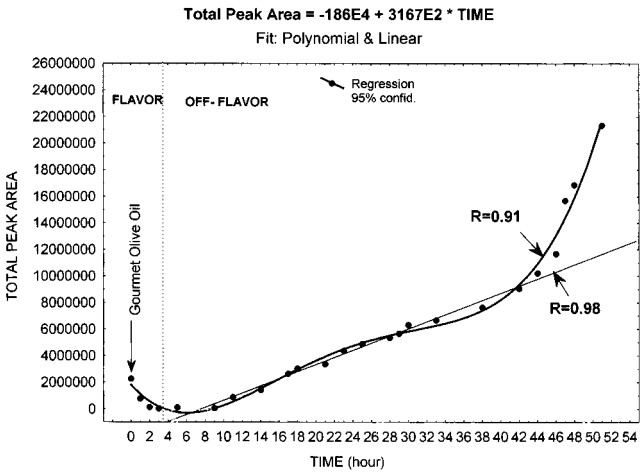

Figure 3. Total volatiles content vs time [polynomial fit of the whole set of data; linear fit of a selected subset of data (from 9 to 46 h)].

waxy) are the major volatiles at 21 h (Figure 2C), and their sensory descriptors completely agree with the sensory perceptions of the assessors for this oil, "unpleasant, rancid, penetrating".

After 21 h of thermoxidation, several aliphatic acids, such as hexanoic, nonanoic, octanoic, or heptanoic acid, appeared, being possibly formed by further oxidation of their corresponding aldehydes. Aliphatic ketones formed by autoxidation of unsaturated fatty acids also contributed to the undesirable flavors of virgin olive oil as they have low threshold values (Kochhar, 1993), e.g. 5-hepten-2-methyl-6-one and 3,5-octadien-2-one—characterized as fatty, fruity odor notes—were found in samples. 1-3-Nonadienes arising from linoleate 9-OOH—described as rancid, buttery (Evans et al., 1971)—and furans and alcohols such as 1-penten-3-ol, 2-pentanol, 1-octen-3-ol, and octanol were also found in oxidized samples. Aliphatic alcohols make a small contribution to the off-flavors because their flavor thresholds are significantly higher (Kochhar, 1993) than those of their corresponding aldehydes. The transformation into the alcohol of even a small amount of potent off-flavor aldehyde above the detection level will bring about considerable odor reduction (Eriksson et al., 1977; Kochhar, 1993).

Table 3. Threshold Values, Sensory Properties, and Main Hydroperoxide Isomer Precursors of Virgin Olive Oil Aldehyde Off-Flavor Components

volatile compound	sensory properties[a]	precursor[b]	odor threshold[c]
hexanal	fatty, powerful, oily, grassy	13-LOOH	0.32
nonanal	fatty, waxy, painty, citrus	9/10-OOOH	13.5
2-heptenal	oxidized, tallowy, pungent	12-LOOH	
2-decenal	painty, fishy, fatty	9-OOOH	
pentanal	woody, bitter, oily	13-LOOH	0.24
2,4-heptadienal	fatty, rancid (hazelnut), cinnamon	12-LnOOH	3.6
undecanal	fatty, tallowy		
heptanal	oily, fatty, heavy, woody, penetrating, nutty	11-LOOH	3.2
octanal	fatty, sharp, citrus	11-OOOH	0.32
2-nonenal	penetrating, fatty, waxy, beany, rancid	9/10-LOOH	3.2
decanal	penetrating, sweet, waxy, painty	8-OOOH	6.7
2,4-decadienal	powerful, fatty, citrus	9-LOOH	2.15
2-hexenal	sweet, fragrant, almond, fruity, green, leafy	12/13-LnOOH	10.0
2-octenal	brown beans, herbaceous, spicy	11-LOOH	
2-undecenal	fresh, fruity, orange peel	8-OOOH	

[a] Sensory characteristics found in the literature (Kochhar, 1993). [b] Theoretical hydroperoxide isomer precursor. OOOH, oleic acid hydroperoxide; LOOH, linoleic acid hydroperoxide; LnOOH, linolenic acid hydroperoxide. [c] Odor threshold values (mg/kg) in paraffin oil obtained from Meijboom (1964).

Table 4. Flavor Evaluation of Initial and Aged Virgin Olive Oil

time (h)	flavor descriptor[a]	flavor score[b]	hedonic scale[c]	peroxide value
0	pleasant, green, banana	7.1	8	3.3
0.5	less aromatic, heated olive oil	4.1	3	3.2
2	fried olive oil, slightly oxidized	3.6	3	8.2
5	used oil, refined-like, oxidized	2.1	2	15.1
9	rancid	0.9	2	18.3
12	rancid, metallic	0.9	1	23.3
15	unpleasant, rancid, metallic	0.6	1	26.7
18	old oil, rancid, unpleasant	0.5	1	27.8
22	unpleasant, rancid, penetrating	0.5	1	28.6

[a] Sensory descriptors assigned by assessors. [b] Scores based on 100 mm unstructured scale. [c] Punctuation according to the 10 point hedonic scale.

The change in fatty acid composition was determined by removing aliquots periodically during the oxidative process. Table 5 shows the fatty acid composition of the virgin olive oil sample from 0 to 33 h of oxidation; the quantification was based on the major saturated acid (C16:0) initially present, in order to show the real content of unaltered fatty acids (Dobarganes and Perez-Camino, 1988). As expected, mainly unsaturated fatty acids were altered during the process; thus oleic, linoleic, and linolenic acids were those most affected. The

Table 5. Change of Fatty Acids (Percent) during Oxidative Deterioration Process

	time				
fatty acid	0 h	5 h	11 h	21 h	33 h
C16:0	12.12	12.12	12.12	12.12	12.12
C16:1	1.26	1.07	1.24	0.99	0.64
C17:0	0.10	0.10	0.10	0.10	0.10
C17:1	0.23	0.20	0.20	0.18	0.11
C18:0	1.65	1.95	1.73	1.85	1.59
C18:1 (9ω)	70.42	72.5	72.14	59.23	32.49
C18:1 (7ω)	2.62	3.27	2.57	2.70	1.48
C18:2	8.55	7.90	8.64	3.00	0.19
C20:0	0.36	0.48	0.37	0.37	0.29
C18:3	0.57	0.47	0.55	0.08	0.01
C20:1	0.33	0.41	0.35	0.17	0.16

most important decrease was observed after 21 h of oxidation, when a higher amount of volatiles was detected (Figure 2C). Linolenic acid practically disappeared after 33 h of oxidation; linoleic acid content was drastically reduced, and oleic acid 9ω was also affected by the oxidative deterioration. These results were in accordance with the volatile compounds found in the oxidized samples, as many aldehydes were detected after 21 h of oxidation. Their concentrations increased further during the next hours, meaning that the main volatile decomposition products found in oxidized olive oils were produced from monohydroperoxides of the unsaturated oleic, linoleic, and linolenic fatty acids as the total content of unaltered fatty acids was reduced from 98.2% (0 h) to 80.8% (21 h) and later to 49.2% (33 h).

Some of the volatiles found in the oxidized samples were also present in the initial flavor. This is the case of hexanal, which, usually being associated with oxidation in refined vegetable oils, is present in the initial virgin olive oil flavor as it is produced from linoleic acid through the lipoxygenase pathway. In previous works authors have demonstrated that hexanal is an important flavor compound of virgin olive oil and it contributes to the sweet perception (Morales et al., 1996; Aparicio et al., 1996). On the other hand, it has been demonstrated that the hexanal content in virgin olive oil is positively correlated with the overall acceptability of potential and habitual consumers of virgin olive oil (McEwan, 1994). Figure 4 shows the relationship between the overall acceptability evaluated by a British panel (McEwan, 1994) and the hexanal concentration (determined according to the method described under Dynamic Headspace Volatile Concentration) for a set of virgin olive oil samples; the best valued samples had the highest concentrations of hexanal. In consequence, hexanal is not an adequate marker for the beginning of oxidation in the case of virgin olive oil, although it has been used with success in refined vegetable oils (Snyder et al., 1988; Warner et al., 1988).

As the main biochemical pathway, the lipoxygenase cascade (Figure 5), promotes the formation of C_6 volatile compounds from linoleic and linolenic hydroperoxides, rather than the C_9 compounds, nonanal was either not found or was found only at trace levels in virgin olive oil. Figures 6 and 7 show that nonanal and hexanal followed similar behavior during the oxidation; the shapes of both curves, after a polynomial fit of their whole set of data, were similar. A regression coefficient $R = 0.89$ was found for nonanal and $R = 0.84$ for hexanal. Both sets of data (nonanal and hexanal) were found to be highly correlated ($R = 0.99$). A linear regression was also applied to a partial set of data from 9 to 46 h of oxidation; $R = 0.98$ was obtained for nonanal and $R = 0.94$ for hexanal. Thus, an appropriate way to detect the beginning of oxidation could be an early

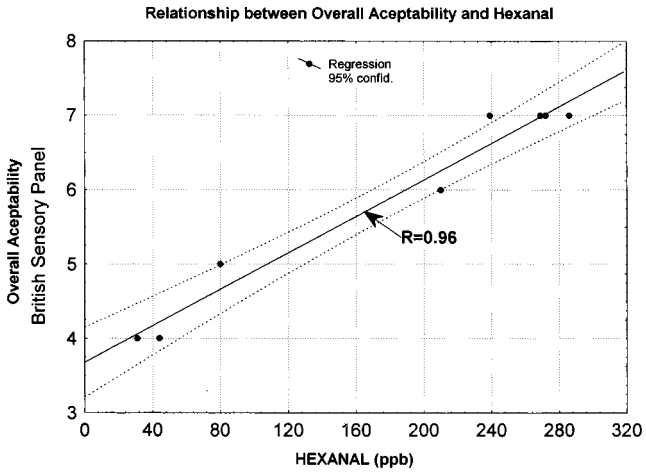

Figure 4. Hexanal content (parts per billion) in virgin olive oil samples scored with different acceptabilities.

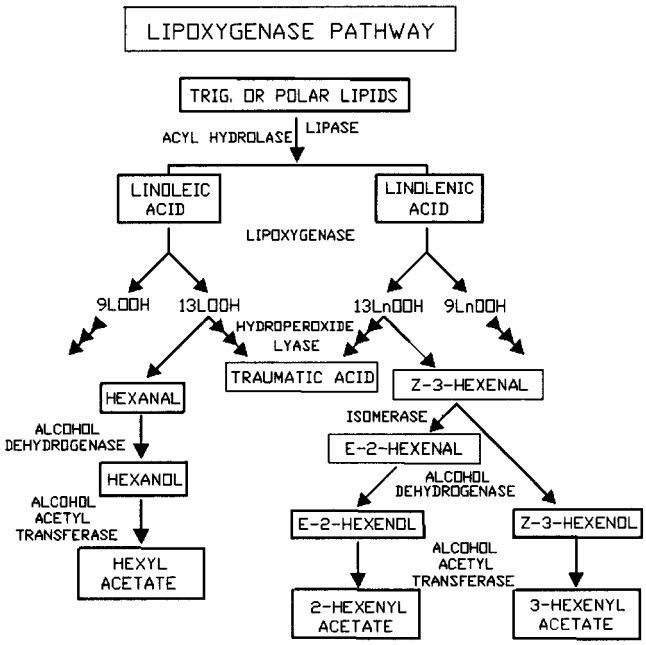

Figure 5. Scheme showing the lipoxygenase pathway in olives.

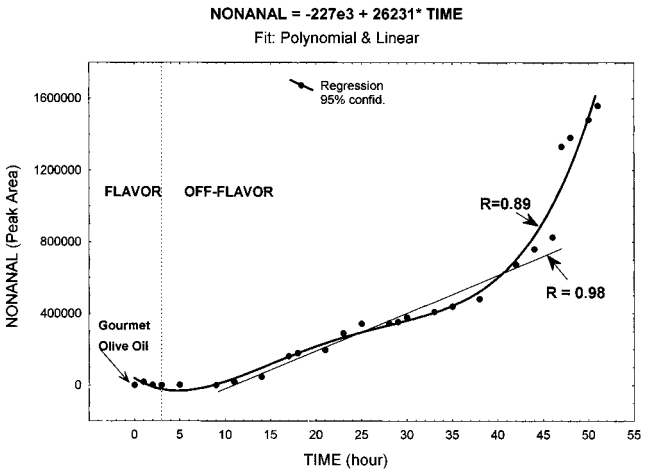

Figure 6. Nonanal content vs oxidation time [polynomial fit of the whole set of data; linear fit of a selected subset of data (from 9 to 46 h)].

measurement of nonanal. In fact, there is a good correlation ($R = 0.98$) between the amount of this compound and the oxidation time from 5 h to the induction period, and an equation has been formulated

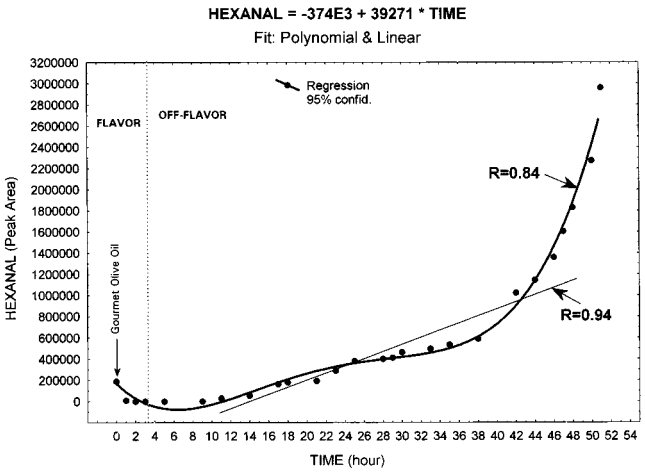

Figure 7. Hexanal content vs oxidation time [polynomial fit of the whole set of data; linear fit of selected subset of data (from 9 to 46 h)].

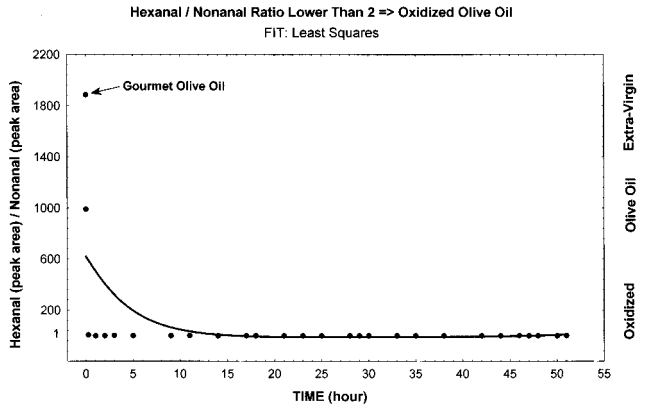

Figure 8. Hexanal/nonanal ratio vs oxidation time.

(nonanal = −227e3 + 26231 × time) for explaining the oxidation of a virgin olive oil until its induction period, with an acceptable predicted regression ($R = 0.88$). The ratio hexanal/nonanal was calculated for each point and plotted versus time (Figure 8). Initially, hexanal was very high, while nonanal was detected at trace level, but in the course of the oxidation both compounds had similar amounts. The ratio changes abruptly from thousands, for a gourmet oil, to lower than two for oxidized oils, whatever its level of oxidation. Once determined that the amount of hexanal does not result from the lipoxygenase cascade, it would be possible to predict the level of oxidation from the equation hexanal = −374e3 + 39271 × time, with a predicted regression $R = 0.84$.

Table 4 shows the sensory evaluation of a sample from 0 to 22 h when the oil was altered so much that sensory evaluation was not recommended. The initially pleasant sensory characteristics of the oil (pleasant, green banana) changed abruptly after 30 min of oxidation. All assessors agreed on the initial sensory description of the oil, its overall acceptability being given as 7.1 [extra virgin olive oil according to the EC regulation (EC, 1995)]. However, this description got progressively worse during olive oil oxidation. After 30 min, the initial value decreased to 4.1 [lampante according to the EC regulation (EC, 1995)], and assessors agreed that the oil did not smell like a virgin olive oil; this was due to loss of the fresh virgin olive oil volatiles. Surprisingly, the peroxide value (PV = 3.2) had not changed at this point of the process. The odor was described as rancid after 9 h of oxidation, and the peroxide value remained at 18.3, an accepted value for a virgin olive oil in the EC regulation (EC, 1995), as its maximum is 20. The hedonic evaluation also showed that the initially acceptable virgin olive oil was rejected in the initial steps of the oxidative deterioration, if we take into account that the oils with values lower than 5, in the food action scale, are not acceptable. After 12 h of oxidation, assessors stated that they would never have tried the oil. A good correlation ($R = -0.85$) between the flavor scores and the amount of nonanal from 0 to 12 h was detected. The coefficient abruptly dropped to $R = -0.51$ when we analyzed the oxidation process from 0 to 22 h. The latter result has a logical explanation since the amount of nonanal rose exponentially while the flavor score had reached its minimum.

CONCLUSIONS

This paper clarifies the evolution of the chemical compounds responsible for the flavor of virgin olive oil, produced mainly through biochemical pathways, and the formation of off-flavors, produced through oxidative pathways (Frankel, 1985; Dobarganes et al., 1986; Kochhar, 1993), indicating the main differences found between virgin olive oil flavors and off-flavors.

The volatile compounds identified in virgin olive oil flavor have been found to be quite different from those identified in virgin olive oil off-flavor. The explanation could be their different origins, mainly biochemical for flavors and chemical for off-flavors. The main differences that characterize off-flavors are the absence of C_6 aldehydes and alcohols (produced from linolenic acid), which contribute to the green flavor of virgin olive oil, the absence of esters contributing to fruity flavor, and the presence of many aldehydes with low odor thresholds contributing to the typical rancid odor of oxidized oils.

The maximum limit of peroxide value (<20) accepted by the EC regulation (EC, 1995) does not seem to agree with the sensory evaluation of assessors, as the latter rejected the oil as virgin olive oil when the peroxide value was <4, which is an acceptable value from the EC regulation point of view. On the other hand, the amount of hexanal does not allow oxidized olive oils to be distinguished from virgin ones, as this compound can come from the lipoxygenase cascade and oxidative pathways. The measurement of nonanal has clearly demonstrated its usefulness, as it does not appear in virgin olive oil but does appear in oxidized olive oils.

ACKNOWLEDGMENT

We acknowledge our indebtedness to assessors who carried out the sensory evaluation of the samples and to Mr. Leon for valuable assistance.

LITERATURE CITED

Aparicio, R.; Morales, M. T.; Alonso, M. V. Relationship between volatile compounds and sensory attributes of olive oils by the sensory wheel. *J. Am. Oil Chem. Soc.* **1996**, *73*, 1253−1264.

Dobarganes, M. C.; Perez-Camino, M. C. Fatty acid composition: a useful tool for the determination of alteration level in heated fats. *Rev. Fr. Corps Gras* **1988**, *35*, 67−70.

Dobarganes, M. C.; Rios, J. J.; Perez-Camino, M. C. Relationship between the composition of vegetable oils and the volatile components produced in their thermoxidation. *Grasas Aceites* **1986**, *37*, 61−67.

EC. Peroxide value determination. *Off. J. Commission Eur. Communities* **1991**, July 11, 8−9, Regulation n1 2568/91, Annex III.

EC. *Off. J. Commission Eur. Communities* **1995**, March 28, 3−4, Regulation n1 656/95.

Eriksson, C. E.; Qvist, J. A.; Vallentin, K. Conversion of aldehydes to alcohols in liquid foods by alcohol dehydrogenase. In *Enzymes in Food and Beverage Processing*; Ory, R. L., St. Angelo, A. J., Eds.; American Oil Chemists' Society: Champaign, IL, 1977; pp 132−142.

Evans, C. D.; Moser H. A.; List, G. R. Odour and flavour responses to additives in edible oils. *J. Am. Oil Chem. Soc.* **1971**, *48*, 495−498.

Flath, R. A.; Forrey, R. R.; Guadagni, D. G. Aroma components of olive oil. *J. Agric. Food Chem.* **1973**, *21*, 948−952.

Frankel, E. N. Chemistry of autoxidation: mechanism, products and flavor significance. In *Flavor Chemistry of Fats and Oils*; Min, D. B., Smouse, T. H., Eds.; American Oil Chemists' Society: Champaign, IL, 1985; pp 1−37.

Guth, H.; Grosch, W. Quantitation of potent odorants of virgin olive oil by stable-isotope dilution assays. *J. Am. Oil Chem. Soc.* **1993**, *70*, 513−518.

Kiritsakis, A. K.; Min, D. B. Flavor chemistry of olive oil. In *Flavor Chemistry of Lipid Foods*; Min, D. B., Smouse, T. H., Eds.; American Oil Chemists' Society: Champaign, IL, 1989; pp 196−221.

Kochhar, S. P. Oxidative pathways to the formation of off-flavours. In *Food Taints and Off-Flavours*; Saxby, M. J., Ed.; Blackie Academic & Professional: London, 1993; pp 150−201.

Laubli, M. W.; Bruttel, P. A. Determination of the oxidative stability of fats and oils: comparison between the AOM method and the Rancimat method. *J. Am. Oil Chem. Soc.* **1986**, *63*, 792−794.

León Camacho, M.; Cert, A. Suggestions for the application of some analytical methods included in the regulation EEC 2568/91 relative to the characteristics of the olive oils and olive pomace oils. *Grasas Aceites* **1994**, *45*, 395−401.

McEwan, J. A. Consumer attitudes and olive oil acceptance: the potential consumer. *Grasas Aceites* **1994**, *45*, 9−15.

Meijboom, P. W. Relationship between molecular structure and flavour perceptibility of aliphatic aldehydes. *J. Am. Oil Chem. Soc.* **1964**, *41*, 326−328.

Morales, M. T.; Aparicio, R. Characterizing some European olive oil varieties by volatiles using statistical tools. *Grasas Aceites* **1993**, *44*, 113−115.

Morales, M. T.; Aparicio, R.; Rios, J. J. Dynamic headspace gas chromatographic method for determining volatiles in virgin olive oil. *J. Chromatogr.* **1994**, *668*, 455−462.

Morales, M. T.; Alonso, M. V.; Rios, J. J.; Aparicio, R. Virgin olive oil aroma: relationship between volatile compounds and sensory attributes by chemometrics. *J. Agric. Food Chem.* **1995**, *43*, 2925−2931.

Morales, M. T.; Aparicio, R.; Calvente, J. J. Influence of olive ripeness on the concentration of green aroma compounds in virgin olive oil. *Flavour Fragrance J.* **1996a**, *11*, 171−178.

Morales, M. T.; Aparicio, R.; Rios, J. J. Evolution of flavor and off-flavor components of olive oil during processing and accelerated storage. *Abstracts of Papers*, 87th American Oil Chemists' Society Annual Meeting & Expo, Indianapolis; AOCS Press: Champaign, IL, 1996b; p 74.

Olías, J. M.; Pérez, A. G.; Ríos, J. J.; Sanz, L. C. Aroma of virgin olive oil: Biogenesis of the "green" odor notes. *J. Agric. Food Chem.* **1993**, *41*, 2368−2373.

Schutz, H. G. A food action rating scale for measuring food acceptance. *J. Food Sci.* **1965**, *30*, 365−374.

Snyder, J. M.; Frankel, E. N.; Selke, E.; Warner, K. Comparison of gas chromatographic methods for volatile lipid oxidation compounds in soybean oil. *J. Am. Oil Chem. Soc.* **1988**, *65*, 1617−1620.

STATISTICA. *Users Guide*, release 3.1; StatSoft: Tulsa, OK, 1992.

Tsimidou, M.; Papadopoulos, G.; Boskou, D. Determination of phenolic compounds in virgin olive oil by reversed-phase HPLC with emphasis on UV detection. *Food Chem.* **1992**, *44*, 53−60.

Warner, K.; Frankel, E. N.; Moulton, K. J. Flavor evaluation of crude oil to predict the quality of soybean oil. *J. Am. Oil Chem. Soc.* **1988**, *65*, 386−391.

Received for review August 5, 1996. Revised manuscript received March 20, 1997. Accepted March 31, 1997.[⊗] This work has been supported by AIR3-CT94-1967 and CICYT-Spain ALI-91-0786.

JF960585+

[⊗] Abstract published in *Advance ACS Abstracts,* June 1, 1997.