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Study of both Sunflower Oil and Its Headspace throughout the Oxidation Process. Occurrence in the Headspace of Toxic Oxygenated Aldehydes

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The static headspace composition of sunflower oil throughout the oxidation process at 70 °C with circulating air is studied by means of solid-phase microextraction followed by gas chromatography−mass spectrometry (SPME-GC-MS); at the same time the liquid phase of the same oil is studied by means of Fourier transform infrared (FTIR) spectroscopy. Each technique provides complementary information about the process; FITR spectroscopy detects changes in the functional groups of the liquid matrix in a global way and SPME/GC-MS provides information about the different components present in the volatile phase during the oxidation process. Concordance between the timing of the changes produced in both liquid and gaseous phases is observed, as well as agreement and complementarity in the results obtained from both phases. The formation of some well-known genotoxic and cytotoxic oxygenated aldehydes in this process and their presence in the oil headspace are proved.

KEYWORDS: Sunflower oil; oxidation process; Fourier transform infrared spectroscopy; liquid phase; solid-phase microextraction; gas chromatography−mass spectrometry; headspace composition; oxo-, hydroxy-, and epoxyalkenals

INTRODUCTION

Oxidation of edible oils is a subject of great interest because it causes the loss of shelf life due to the formation of compounds, some of which give the typical rancid odor and others of which have harmful effects on human health (*1*). The methods most commonly used to study oxidation processes in foods are based on the determination of parameters related to the concentration in the oil of either primary or secondary oxidation products. However, these wet methods give only a partial view of the sample, and it is necessary to use more than one in order to have more complete information about the status of the sample (*2*, *3*).

In previous papers the usefulness of Fourier transform infrared (FTIR) spectroscopy to characterize edible oils (*4*−*7*) as well as to study its oxidation process (*8*−*11*) has been shown. This technique provides information about the status of the oil from the values of the frequency and/or absorbance of the bands of the different functional groups present in the oil liquid phase throughout the oxidation process. In addition, small volatile and semivolatile compounds formed in this process and dissolved in the oil contribute to the bands of the total liquid phase. Using this technique, three stages have been distinguished in the oxidation process of edible oils and fats associated with the generation of hydroperoxides, their degradation, and the formation of secondary oxidation products (*8*). In addition, close relationships have been found between the information provided by FTIR spectroscopy and by classical oxidation parameters such as peroxide (PV) and anisidine values (AV) (*11*).

Foods headspace composition can be studied using several techniques, one of which is solid-phase microextraction (SPME) followed by gas chromatography−mass spectrometry (GC-MS). This technique provides a great deal of information after a careful study of the chromatographic results (*12*−*15*); it is simple and can be very useful to study not only the volatile and semivolatile oil components but also their evolution throughout the oxidation process. Among these volatile and semivolatile components are the compounds responsible for the oil flavor as well as many other secondary oxidation products generated under oxidative conditions.

In recent studies on the oxidation process of refined sesame oil (an oil rich in linoleic acyl groups) by ^{1}H NMR we have shown the formation of genotoxic and cytotoxic oxygenated aldehydes (*16*), which have been considered as possible causative agents of different diseases such as cancer, atherosclerosis, and Alzheimer's, (*17*−*24*). These compounds have low molecular weight, and for this reason, they could be thought to be present in the headspace of the oil submitted to oxidative conditions.

Therefore, in this paper the study of the oil liquid phase by means of FTIR spectroscopy as well as of the oil gas phase by SPME/GC-MS is undertaken throughout the oxidation process.

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Table 1. Compounds Detected in the Sunflower Oil Headspace at Different Days of the Oxidation Process and Their Abundances Expressed as Area Counts Multiplied by 10^{-6}

no.	compound[a]	Bp[b]	day 1	day 5	day 8	day 11
	acids		**0.123**	**1.861**	**10.779**	**5.203**
1	formic acid (46)	46	0.000	0.831	1.910	1.124
2	acetic acid (60)*	43	0.041	0.177	0.345	0.261
3	butanoic acid (88)*	60	0.001	0.011	0.025	0.014
4	pentanoic acid (102)*	60	0.011	0.057	0.289	0.176
5	hexanoic acid (116)*	60	0.053	0.716	7.467	3.281
6	heptanoic acid (130)*	60	0.008	0.032	0.206	0.101
7	octanoic acid (144)*	60	0.009	0.021	0.209	0.101
8	nonanoic acid (158)*	60	0.000	0.016	0.328	0.145
	alcohols		**0.033**	**0.862**	**2.600**	**0.695**
9	1-pentanol (88)*	42	0.016	0.080	0.962	0.091
10	1-hexanol (102)*	56	0.000	0.057	0.085	0.046
11	1-heptanol (116)*	70	0.005	0.021	0.086	0.015
12	1-octen-3-ol (128)	57	0.000	0.687	1.354	0.507
13	1-octanol (130)	56	0.011	0.016	0.113	0.036
	hydrocarbons		**0.446**	**1.317**	**11.992**	**2.024**
14	pentane (72)*	43	0.429	0.950	10.750	1.613
15	hexane (86)*	57	0.000	0.122	0.097	0.000
16	hexene (84)	41	0.000	0.017	0.027	0.000
17	heptane (100)*	43	0.000	0.183	0.987	0.403
18	styrene (104)*	104	0.000	0.022	0.025	0.004
19	3-dodecene (168)	55	0.000	0.005	0.091	0.000
20	kaur-16-ene (272)	91	0.017	0.018	0.015	0.005
	furan derivatives		**0.112**	**0.563**	**7.607**	**3.185**
21	2-butylfuran (124)	81	0.000	0.018	0.148	0.088
22	2-pentylfuran (138)	81	0.112	0.501	7.328	3.097
23	2-hexylfuran (152)	81	0.000	0.044	0.131	0.000
	nitrogen derivatives		**0.159**	**0.165**	**0.044**	**0.050**
24	2-ethyl-benzenamine (121)	106	0.061	0.009	0.000	0.000
25	3-(1-methyl-2-pyrrolidinyl)-pyridine (162) (nicotine)	84	0.098	0.156	0.044	0.050
	phenolic derivatives		**0.106**	**0.028**	**0.027**	**0.005**
26	methyl-di-*tert*-butylphenol (220)	205	0.028	0.016	0.015	0.000
27	ethyl-di-*tert*-butylphenol (234)	219	0.078	0.012	0.012	0.005
	ketones		**0.000**	**0.348**	**5.802**	**2.401**
28	2-propanone (58)*	43	nd	nd	nd	nd
29	2-butanone (72)*	44	nd	nd	nd	nd
30	2-pentanone (86)*	43	0.000	0.037	0.092	0.122
31	2-hexanone (100)*	43	0.000	0.000	0.069	0.066
32	2-heptanone (114)*	43	0.000	0.071	0.689	0.520
33	ketone 41 (31), 43 (100), 55 (13), 71 (44), 99 (42)	43	0.000	0.042	1.233	0.238
34	ketone 41 (20), 43 (100), 55 (25), 71 (53), 75 (71), 99 (46)	43	0.000	0.038	0.901	0.048
35	2-octanone (128)*	43	0.000	0.015	0.119	0.101
36	3-octen-2-one (126)	55	0.000	0.033	0.644	0.501
37	2-nonanone (142)*	43	0.000	0.000	0.052	0.055
38	3-nonen-2-one (140)	55	0.000	0.094	1.548	0.523
39	2-decanone (156)*	58	0.000	0.006	0.037	0.035
40	6-undecanone (170)	43	0.000	0.000	0.097	0.052
41	6-dodecanone (184)	43	0.000	0.012	0.321	0.141
	saturated aldehydes		**0.493**	**1.922**	**20.725**	**3.508**
42	butanal (72)*	44	nd	nd	nd	nd
43	pentanal (86)*	44	0.086	0.379	2.178	0.606
44	hexanal (100)*	44	0.102	1.058	16.507	2.356
45	heptanal (114)*	44	0.056	0.126	0.204	0.077
46	octanal (128)*	43	0.043	0.098	0.280	0.093
47	nonanal (142)*	57	0.166	0.115	1.221	0.297
48	decanal (156)	41	0.023	0.025	0.097	0.045
49	dodecanal (184)	43	0.017	0.121	0.238	0.034
	monounsaturated aldehydes		**0.168**	**1.568**	**9.342**	**3.462**
50	*trans*-2-hexenal (98)*	41	0.000	0.067	0.147	0.053
51	*trans*-2-heptenal (112)	41	0.051	0.608	2.078	0.700
52	*trans*-2-octenal (126)	41	0.042	0.576	2.865	1.160
53	*trans*-2-nonenal (140)	41	0.022	0.075	0.160	0.058
54	*trans*-2-decenal (154)	43	0.028	0.105	1.558	0.523
55	*trans*-2-undecenal (168)	70	0.025	0.106	2.444	0.951
56	*trans*-2-dodecenal (182)	41	0.000	0.031	0.090	0.017
	diunsaturated aldehydes		**0.231**	**2.001**	**35.005**	**4.718**
57	*cis*,*trans*-nona-2,4-dienal (138)	81	0.000	0.059	0.275	0.063
58	*trans*,*trans*-nona-2,4-dienal (138)*	81	0.000	0.123	1.111	0.225
59	*cis*,*trans*-2,4-decadienal (152)*	81	0.050	0.376	7.876	0.706
60	*trans*,*trans*-2,4-decadienal (152)*	81	0.171	1.416	25.631	3.700
61	*trans*,*trans*-2,4-undecadienal (166)	81	0.010	0.027	0.112	0.024

Table 1. (Continued)

no.	compound[a]	Bp[b]	day 1	day 5	day 8	day 11
	oxygenated aldehydes and other compounds		**0.110**	**2.098**	**54.823**	**15.861**
62	*trans*-2,3-epoxyhexanal (114)	71	0.000	0.041	0.171	0.044
63	41 (48), 43 (21), 55 (46), 56 (12), 70 (32), 83 (100), 84 (16), 97 (6)	83	0.000	0.049	0.203	0.049
64	41 (45), 55 (63), 70 (67), 82 (35), 83 (100), 84 (14), 95 (3)	83	0.000	0.151	0.969	0.374
65	5-ethyldihydro-2(3*H*)-furanone (114)	85	0.000	0.063	0.527	0.362
66	*trans*-2,3-epoxyoctanal (142)	71	0.000	0.144	2.438	0.476
67	42 (12), 55 (100), 70 (46), 83 (65), 97 (21), 98 (71), 111 (65)	55	0.000	0.053	0.070	0.037
68	41 (45), 43 (33), 55 (60), 70 (84), 83 (100), 96 (23), 97 (10), 109 (6)	83	0.000	0.031	0.093	0.109
69	55 (60), 57 (30), 84 (100), 85 (34), 97 (14), 111 (58), 122 (5), 140 (2)	84	0.000	0.018	0.180	0.086
70	4-oxo-*trans*-2-nonenal (154)*					
	43 (72), 55 (94), 70 (53), 83 (65), 98 (84), 111 (6), 125 (100), 154 (3)	125	0.021	0.310	4.003	0.604
71	hydroxy-2-octenal (142)	57	0.006	0.076	0.625	0.161
72	43 (42), 55 (100), 70 (56), 83 (33), 98 (59), 111 (28), 125 (68), 154 (11)	55	0.000	0.040	0.421	0.107
73	butyldihydro-2(3*H*)-furanone (142)	85	0.017	0.037	0.498	0.171
74	41 (27), 55 (90), 70 (51) 83 (51), 98 (100), 111 (69), 125 (14), 154 (23)	98	0.000	0.032	0.796	0.299
75	*trans*-2,3-epoxydecanal (170)	71	0.000	0.000	0.301	0.198
76	4,5-epoxy-*trans*-2-nonenal (154)	68	0.000	0.021	0.548	0.132
77	43 (73), 55 (71), 71 (12), 84 (100), 99 (19), 111 (5), 125 (45), 126(13)	84	0.010	0.351	7.938	3.000
78	4-hydroxy-*trans*-2-nonenal (156)*	57	0.024	0.324	10.734	3.361
79	5-pentyldihydro-2(3*H*)-furanone (156)	85	0.012	0.032	0.642	0.440
80	4,5-epoxy-*trans*-2-decenal (168)	68	0.005	0.067	5.161	0.892
81	4,5-epoxy-*trans*-2-decenal (168) (isomer)	68	0.015	0.251	17.608	3.774
82	6-pentyl-5,6-dihydro-2*H*-pyran-2-one (168)	97	0.000	0.007	0.897	1.185

[a] Compounds marked with an asterisk were acquired commercially and used as standards for identificative purposes. [b] Base peak of the mass spectrum of each compound.

The purpose of the paper is double: on the one hand, to match the changes observed by both techniques in order to determine if at each of the three stages observed by infrared spectroscopy there is a characteristic composition of the oil headspace and, on the other hand, to study the possible presence of harmful compounds in the gas phase and its evolution throughout the oxidation process. To the best of our knowledge only two papers have reported the presence of the genotoxic 4-hydroxy-*trans*-2-nonenal in edible oils at frying temperatures (*25*, *26*); the detection of this compound was carried out by high-performance liquid chromatography (HPLC) after derivatization with 2,4-dinitrophenylhydrazine. However, the presence of compounds of this nature in the headspace of oil submitted to oxidative conditions has not been previously described.

MATERIALS AND METHODS

Samples and Standards. Sunflower oil was acquired from a local supermarket. Compounds such as 4-hydroxy-*trans*-2-nonenal, acquired from Merck (Whitehouse Station, NJ), 4-oxo-*trans*-2-nonenal, acquired from Cayman (Ann Arbor, MI), and all compounds asterisked in **Table 1**, acquired from Aldrich (Milwaukee, WI), were used as standard compounds with identification purposes.

Sample Oxidation. Ten grams of oil was weighed in polystyrene Petri dishes of 80 mm diameter and 15 mm height and placed in a Selecta convection oven, with circulating air, the temperature of which was maintained at 70 °C with a stability of ±0.5%. The Petri dishes were introduced into the oven without their lids to facilitate the exposure of the sample to the circulating air. The oxidation was carried out in duplicate. The oxidation process was monitored by peroxide value (PV), FTIR spectroscopy, and SPME/GC-MS on a daily basis.

Spectral Acquisition. The infrared spectra were recorded on an FTIR Bruker Vector 33 (Bruker Optic Gmbh) interfaced to a personal computer operating under Opus NT software (version 2.0). A film of a small amount of sample (~2 μL) was deposited between two disks of KBr, avoiding the presence of air, as in previous studies (*4*, *6*−*11*). Duplicate spectra were collected daily until the sample became so viscous that it was impossible to deposit a film of the sample between the KBr disk. All spectra were recorded from 4000 to 500 cm^{-1} with a resolution of 4 cm^{-1}. For each spectrum 32 interferograms were co-added before Fourier transformation and zero-filled to give a data point spacing of ~1.9 cm^{-1}. The measurement accuracy in the frequency data is better than 0.01 cm^{-1} due to the laser He−Ne internal reference of the instrument. The frequency value for each band was obtained automatically by the equipment software. The assignment of the bands to the specific functional group vibration mode was made by comparison with previous studies of edible fats and oils (*5*, *8*).

Extraction of Oil Headspace Components by SPME. Vials containing 1 g of oil at different days of the oxidation process were introduced into a water bath maintained at 40 °C. After a period of sample equilibration (15 min), the fiber was inserted into the headspace of the sample and was maintained for 60 min. A fiber of poly(dimethylsiloxane) (100 μm film thickness), acquired from Supelco, was used.

GC-MS Study. Fibers with the adsorbed compounds were injected into a Hewlett-Packard gas chromatograph model HP 6890 series II, equipped with a mass selective detector 5973 and a Hewlett-Packard Vectra XM series 4 computer operating with the ChemStation program. The column used was a fused-silica capillary column (60 m long × 0.25 mm inner diameter × 0.25 μm film thickness; from Hewlett-Packard), coated with a nonpolar stationary phase (HP-5MS, 5% phenyl methyl siloxane). The operation conditions were the following: the oven temperature was set initially at 40 °C (5 min hold) and increased to 280 °C at 4 °C/min (2 min hold); the temperatures of the ion source and the quadrupole mass analyzer were kept at 230 and 150 °C, respectively; helium was used as carrier gas at a pressure of 16.5 psi; injector and detector temperatures were held at 220 and 280 °C, respectively; splitless mode was used for injection with a purge time of 1.5 min. The fiber was maintained in the injection port for 10 min. Mass spectra were recorded at an ionization energy of 70 eV. After the first desorption, the fiber was routinely desorbed for a second time in order to determine if the first process was complete.

Many components were identified by using standards commercially acquired, which are the asterisked compounds in **Table 1**. Many other components were only tentatively identified. In this latter case, retention times, together with mass spectra, and matching with mass spectra of a commercial library of >85% were taken as identification criteria (Wiley 138.L, Mass Spectral Database, Wiley 1990) as in previous studies (*14*, *27*, *28*). Due to the overlapping of the peaks of many compounds, semiquantification was based on arbitrary units of the base peak ion area counts divided by 10^6.

PV Determination. PV was determined according to the standard 965.33 AOAC iodometric method (*29*). The data are presented as means of duplicate determinations for each day of the experiment.

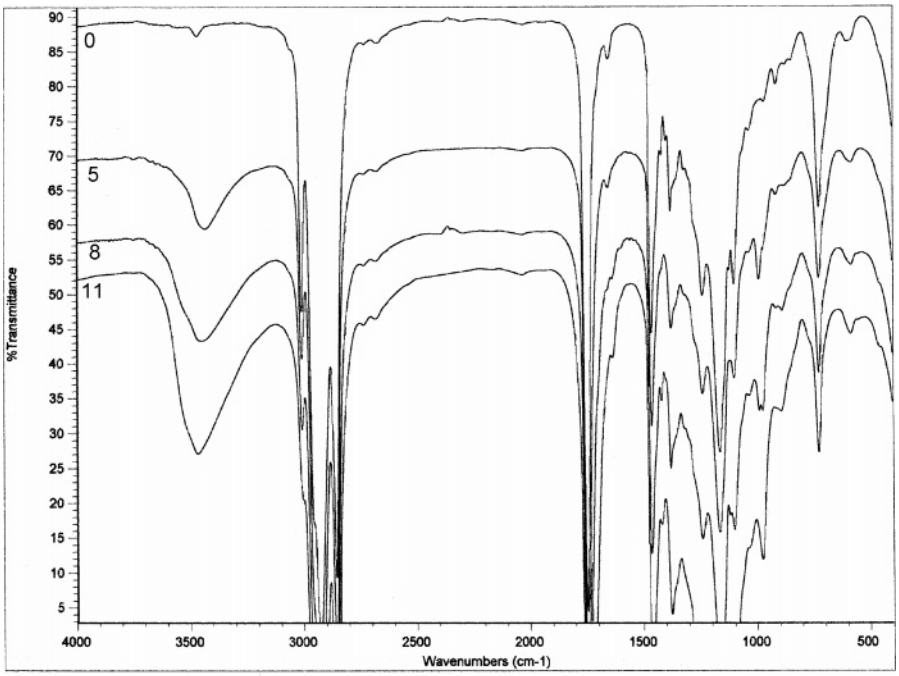

Figure 1. Fourier transform infrared spectra of the sunflower oil at days 0, 5, 8, and 11 under oxidative conditions.

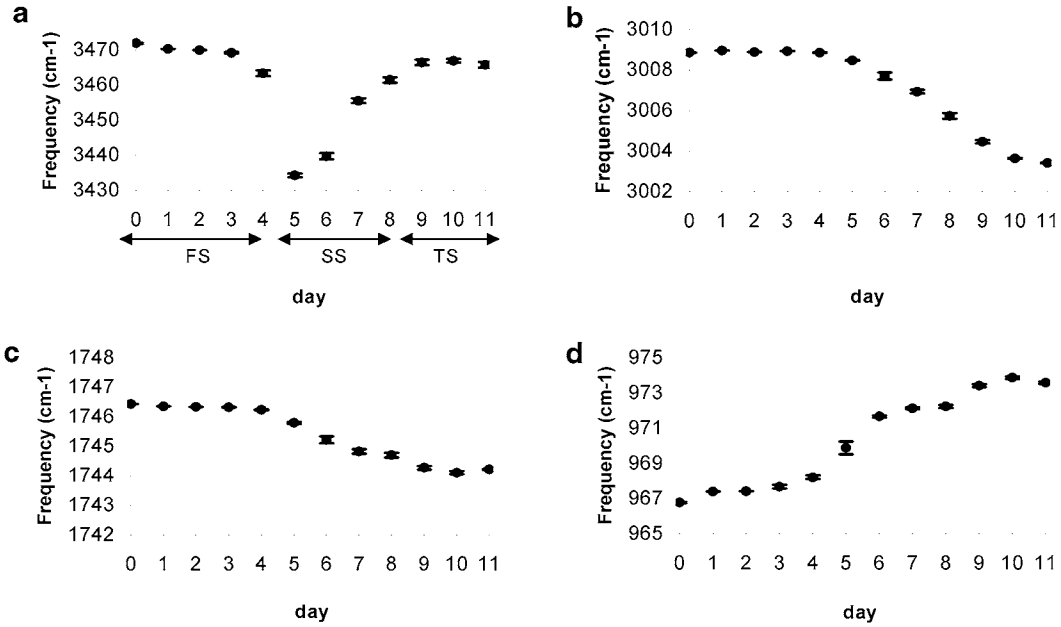

Figure 2. Frequency values of the bands near 3470 cm^{-1} (**a**), 3008 cm^{-1} (**b**), 1746 cm^{-1} (**c**), and 967 cm^{-1} (**d**) versus time for sunflower oil under oxidative conditions.

RESULTS AND DISCUSSION

Some Significant Changes in the Sunflower Oil FTIR Spectrum throughout the Oxidation Process. In previous papers the assignment of the FTIR spectrum bands of edible oils (*4*, *5*) and their changes throughout the oxidation process have been commented on (*8*, *9*); the evolution of the oils' infrared spectra during the oxidation process has been shown to follow a general pattern with differences among samples due to both the rate and magnitude of the changes produced.

Figure 1 shows the FTIR spectra of the sunflower oil at 0, 5, 8, and 11 days under oxidative conditions. In the region between 3600 and 3100 cm^{-1} there is, in nonoxidized oils (**Figure 1**, day 0), a band near 3470 cm^{-1}, associated with the overtone of the glyceride ester carbonyl absorption (*8*). In the sunflower oil under oxidative conditions this small band becomes more intense and broad on day 5 (**Figure 1**) with a maximum of the frequency near 3440 cm^{-1} due to the presence of hydroperoxides. The period of time from the beginning of the experiment to when the frequency of this band begins to diminish has been previously named the first stage, FS (*8*); at the end of this period the deformation of the band is clearly perceptible with the naked eye.

The frequency of this band remains near 3440 cm^{-1} while there is a certain concentration of hydroperoxides in the sample; the period of time from the shift of the frequency of this band to values near 3440 cm^{-1} to its return to values near 3470 cm^{-1} has been named the second stage, SS. Before the end of the FS a band near 987.5 cm^{-1} appears and remains until the end of the SS (**Figure 1**, day 8). This band, which has been associated with bending vibrations of CH *trans,trans* and *cis,trans* con-

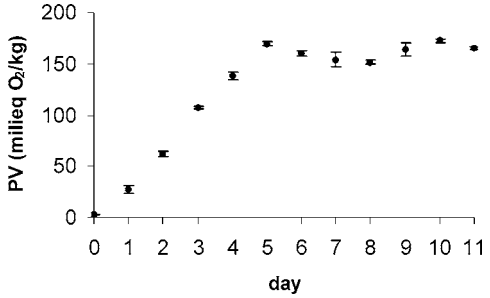

Figure 3. Peroxide values (PV) of the sunflower oil under oxidative conditions.

jugated diene groups of hydroperoxides (*30*−*32*), reaches the maximum of 988.2 cm^{-1} at day 5, just when the PV (see **Figure 3**) also reaches maximum values, showing that hydroperoxide groups are associated with conjugated double bonds, as has been commented on by other authors (*33*).

The third stage, TS, describes the period of time between the return of the frequency of the band to the original values near 3470 cm^{-1} and the total polymerization of the sample (**Figure 2a**, days 8−11). **Figure 2a** shows these three stages as being clearly distinguishable. These same stages are also observed from the changes in the intensity of some bands, as has previously been shown (*9*). In parallel, the peroxide values of this oil determined daily show an evolution similar to that observed in the FTIR spectra, reaching maximum values at day 5 as **Figure 3** shows in agreement with previous studies (*11*).

Therefore, the length of the FS period could be considered as a measure of the oxidative stability of the oil; the shorter the FS period is, the lower the oxidative stability of the oil sample will be and the more quickly the hydroperoxides will appear. For this sunflower oil the duration of the FS is 4 days. Differences between edible oils have been shown by the length of the FS, SS, or TS intervals (*8*), which are basically functions of the presence or absence of minor components having antioxidant ability and of the proportions of monounsaturated, polyunsaturated, and saturated acyl groups in the oil.

Throughout the oxidation process, changes are also observed in the band at ∼3008 cm^{-1} owing to the stretching vibration of *cis*-CH olefinic groups; during the FS period the frequency value of this band remains almost unaltered. After this period, a clear and pronounced diminution in the frequency value of this band is produced as a consequence of the diminution of *cis* double bonds (due to their isomerization to *trans* groups and/or their breakdown to produce secondary oxidation products). **Figure 2b** shows the evolution of the frequency of this band, which almost disappears at very advanced oxidation stages (see **Figure 1**). In relation to these changes, the band at ∼1654 cm^{-1}, associated with the stretching vibration of the carbon−carbon double bonds of *cis* olefins, practically disappears at the end of the SS (see **Figure 1**, day 8).

Changes are also observed in the band at ∼1746 cm^{-1}, due to the ester carbonyl functional group of the triglycerides. Its frequency remains practically unchanged during the FS period, after which its frequency begins to decrease, reaching values near 1744 cm^{-1} at the end of SS period (**Figure 2c**). This change has been associated with the appearance of aldehyde functional groups (*34*, *35*), causing an absorption at 1728 cm^{-1} that overlaps with the band of the ester functional group. It should be noted that some of these aldehydes are responsible for the oxidized odors. In addition, at advanced stages of the oxidation

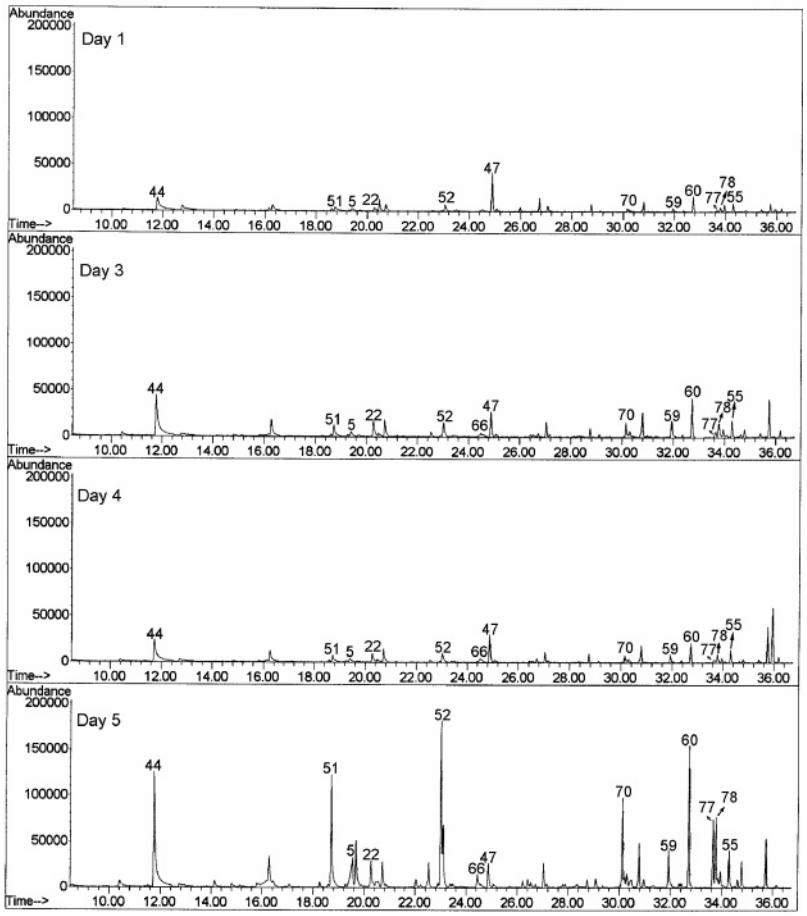

Figure 4. Chromatograms of the compounds extracted from the sunflower oil headspace by SPME at days 1, 3, 4, and 5 under oxidative conditions.

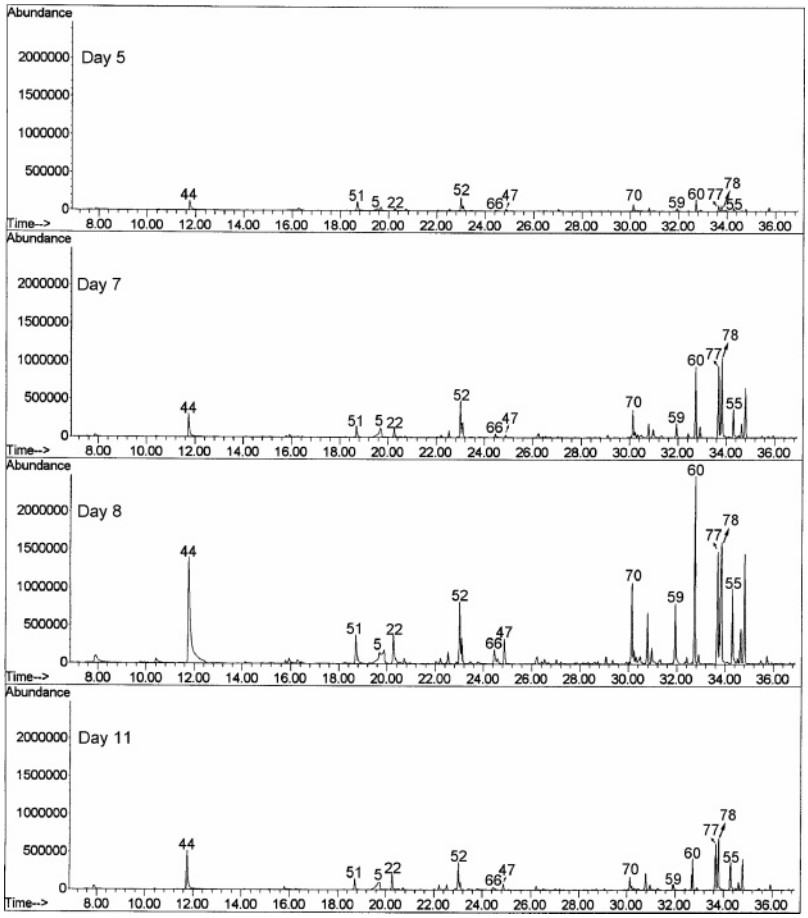

Figure 5. Chromatograms of the compounds extracted from the sunflower oil headspace by SPME at days 5, 7, 8, and 11 under oxidative conditions.

process (days 7−11) a very weak band appears at ∼1630 cm^{-1}, which has been assigned to α,β-unsaturated aldehydes and ketones (*35*). Furthermore, the frequency of the band originally at 967 cm^{-1} (see **Figure 1**, day 0), which is associated with bending vibrations of CH groups of isolated *trans*-olefins, suffers a slight increase during FS, after which it becomes more noticeable, reaching values near 973 cm^{-1} at the end of the TS (see **Figure 2d**). Bands at 973 cm^{-1} have been assigned to secondary oxidation products such as aldehydes or ketones supporting isolated *trans* double bonds (*35*).

Evolution of the Components Detected in the Sunflower Oil Headspace throughout the Oxidation Process. Simultaneously with the study of the changes produced in the sunflower oil liquid phase during the oxidation by FTIR spectroscopy, the changes occurring in the sunflower oil gas phase were studied by SPME-GC-MS. **Figures 4** and **5** show the chromatograms of the components extracted from the sunflower oil headspace on different days of the process. It can be observed that on day 1 the oil headspace contains some components in very low proportions that are also present at similar concentrations on the following days until day 4. This could be due to the degradation of the small amount of hydroperoxides contained in the oil at the beginning of the experiment. In this period of time, which coincides with the above-named first stage, hardly any changes can be observed in the oil headspace composition. This is in agreement with the results obtained by FTIR spectroscopy and shows that over this period of time, in the liquid phase, only hydroperoxides are formed in the triglyceride structure, producing the shift of the band at 3470 cm^{-1} toward 3440 cm^{-1}. Nevertheless, this change produced in the liquid phase does not have repercussions in the gas phase.

However, curiously, the detected compounds in the oil headspace during the FS period are basically secondary oxidation products in very low proportions the concentrations of which remain almost constant in this period. The compounds detected are acids from formic to nonanoic, mainly hexanoic and acetic; a reduced number of primary alcohols from 1-pentanol to 1-octanol, and one secondary alcohol, 1-octen-3-ol; some saturated and unsaturated hydrocarbons in low proportions, such as kauer-16-ene; two phenolic derivatives having antioxidant ability; two nitrogenated compounds, 2-ethylbezenamine and nicotine, which may be contaminants; three 2-alkylfurans, the main one being 2-pentylfuran, probably derived from 2,4-nonadienal; a significant number of saturated and unsaturated ketones, which are undetectable or in very low proportions; saturated aldehydes from butanal to dodecanal, principally hexanal followed by pentanal and nonanal; *trans*-2-unsaturated aldehydes from *trans*-2-hexenal to *trans*-2-dodecenal; 2,4-alkadienals from 9 to 11 carbon atoms, mainly 2,4-decadienals; a very important group of oxygenated aldehydes, among which are epoxyalkanals, and some of the well-known genotoxic and cytotoxic epoxyalkenals, oxoalkenals, and hydroxyalkenals (*18*, *19*, *36*, *37*); and finally some furanones and one pyranone. It should be pointed out that 4-oxo-*trans*-2-nonenal and 4-hydroxy-*trans*-2-nonenal have been identified by means of standard compounds; 2,3-epoxyalkanals and 4,5-epoxy-*trans*-2-decenals have been identified by comparison with the mass spectra given by Buettner and Schieberle (*38*). In addition, 4,5-epoxy-*trans*-2-alkenals have been identified in our laboratory, by GC-MS and by ^1H nuclear magnetic resonance (NMR), as the main compounds produced in the oxidation of 2,4-decadienal at 70 °C with aeration.

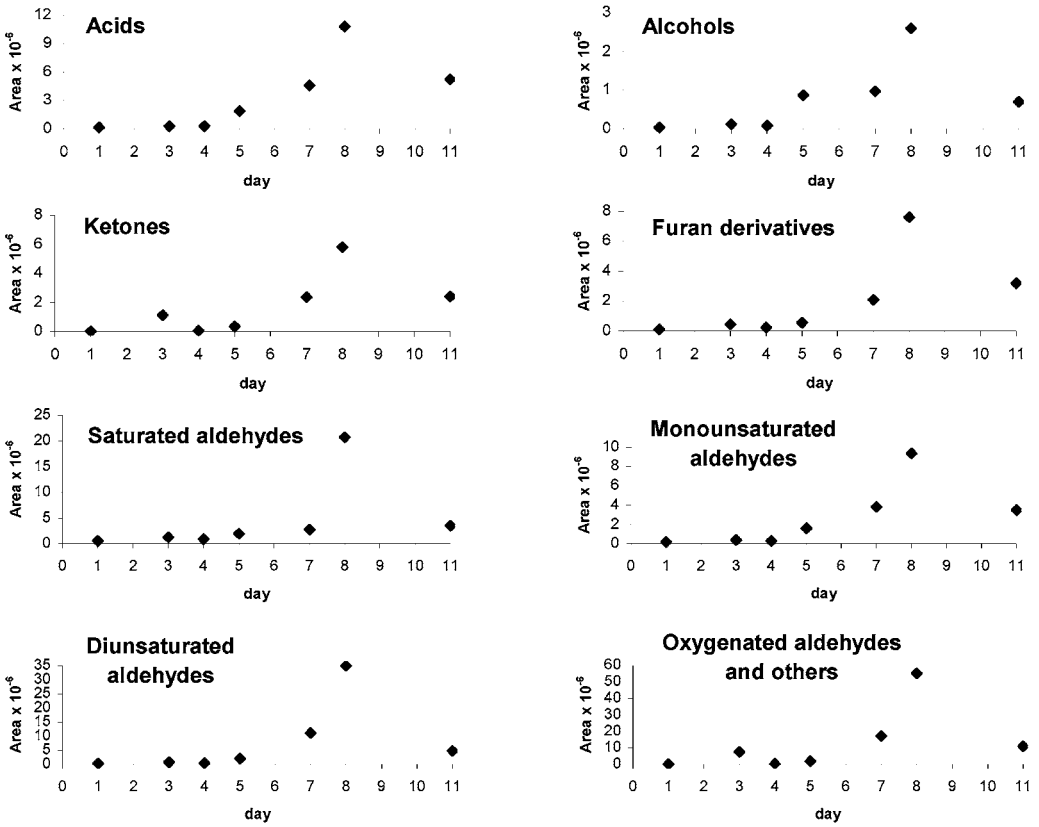

Figure 6. Evolution of the area counts, divided by 10^6, of the main groups of compounds in the headspace of sunflower oil at different days of its oxidation process.

During the first stage, the composition of the sunflower oil headspace hardly changes; however, from day 4 to day 5, as **Figure 4** shows, a slight increase in the area of the peaks of most of the components detected on the preceding days is observed, and finally, from day 5 to day 8 a very sharp increase is produced, as **Figures 5** and **6** show. As in the first stage, the results obtained from the studies of the oil liquid and gas phases in the second stage are not only coincident but also complementary. In this stage the infrared results indicate that hydroperoxides, formed in the FS, gradually degrade. This is shown by the return of the band at 3440 cm^{-1} to the original frequency value near 3470 cm^{-1}, probably due to the degradation of some hydroperoxides, and by the disappearance of the band at 988 cm^{-1}, typical of *trans*,*trans*-conjugated olefinic double bonds associated with hydroperoxides. At the same time, the disappearance of the bands near 3008 and 1654 cm^{-1} takes place.

The degradation of hydroperoxides produces secondary oxidation products, which is reflected in the shifting of the band at 1746 cm^{-1} toward lower wavenumbers due to the appearance of aldehydes and in the appearance of the bands near 1630 and 973 cm^{-1} assigned to α,β-diunsaturated and to isolated *trans*-unsaturated carbonylic derivatives. This is in agreement with the increasing concentration of the components in the headspace during this period of time, which accumulate successively up to day 8.

In the second stage an increase in the concentration of most of the compounds detected in the headspace on the four first days is produced; however, this increase is not of the same order for all compounds, as **Table 1** shows. It can be observed that among acids formic and hexanoic acids undergo the highest increase in their concentrations. The concentration of alcohols does not increase significantly; the greatest increase occurs in 1-octen-3-ol. Despite the high number of ketones formed, their concentrations are not especially significant, except for compound **33**, tentatively considered as ketone, and the peak identified as 3-nonen-2-one. As **Table 1** shows, there is a noticeable increase of 2-pentylfuran concentration as well as of some saturated aldehydes such as hexanal and nonanal, of some unsaturated aldehydes such as *trans*-2-heptenal, *trans*-2-octenal, *trans*-2-decenal, and *trans*-2-undecenal, and of some diunsaturated aldehydes such as the 2,4-decadienals. It is also worth noting the great increase in the concentration of some genotoxic and cytotoxic oxygenated aldehydes such as 4-oxo-*trans*-2-nonenal, 4-hydroxy-*trans*-2-nonenal, and two 4,5-epoxy-*trans*-2-decenals isomers.

From day 8 the concentration of the majority of the components previously detected decreases abruptly, probably because most of the hydroperoxides formed in the previous stages have already been degraded and also because the oil sample is totally polymerized on day 12. Although the concentration of all compounds in **Table 1** decreases in this period of time, this lessening is relatively smaller in *trans*-2-alkenals than in the other kinds of compounds. This period of time between days 9 and 12 coincides with the third stage (*8*). These results show that in this stage, too, there is agreement between results obtained from the study of both the oil liquid and gas phases.

It is should be noted that oxygenated aldehydes have been detected on day 1 at very low but detectable proportions by SPME/GC-MS operating in scan mode. The concentration of these compounds increases as the oxidation process advances, reaching important proportions on day 8. Most of these oxygenated aldehydes are also produced in cells and tissues during the peroxidation process of omega-6 acids due to oxidative stress, and some of them, such as 4-hydroxy-*trans*-2-nonenal, have been considered to be possible causative agents of different diseases such as cancer or Alzheimer's among others

(*21*, *36*, *37*, *39*). In addition, other studies have shown that these harmful compounds can be also absorbed from the diet (*40*−*42*). This should be taken into account when concentration limits of these compounds in oils and foods are established to safeguard human health.

It should be also noted that, to the best of our knowledge, this is the first time that the presence of these toxic compounds has been detected in the headspace of sunflower oil submitted to oxidative conditions, and it is also worth mentioning that this detection has been made by simple GC/MS. Only two previous papers have reported the presence of one of these toxic compounds (4-hydroxy-*trans*-2-nonenal) in oils submitted to thermal stress, and in those studies the detection was made by HPLC after derivatization with 2,4-dinitrophenylhydrazine (*25*, *26*).

Edible oils under oxidative conditions are transformed into complex mixtures of secondary oxidation products, most of which are stable. However, some of these volatile compounds are unstable at high temperatures and can evolve, during their study by gas chromatography, to give other compounds. In this context it should be pointed out that 4-hydroxy-*trans*-2-nonenal and 4-oxo-*trans*-2-nonenal seem to be present in the oil headspace in the first days of the oxidation process at very low proportions, increasing their concentrations as the oxidation advances. However, a study carried out in our laboratory on the same oxidation process of sunflower oil by ^{1}H NMR at room temperature has proved that in this process 4-hydroperoxy- and 4-hydroxy-*trans*-2-alkenals together with some other oxygenated aldehydes were present in the oil liquid phase, whereas 4-oxo-*trans*-2-alkenals were not detected. The absence of 4-oxo-*trans*-2-nonenal in the oil liquid phase and its detection in the oil headspace by means of SPME/GC-MS suggest the absence of this compound not only in the liquid phase but also in the gas phase; its detection in the gas phase could be explained by the formation of this compound in the chromatographic column as a consequence of the high temperature. The fact that 4-hydroperoxy-*trans*-2-alkenals are present in the liquid phase of oxidized sunflower but are not detected in its gas phase suggests that these compounds are degraded in the chromatographic column due to the effect of the high temperature, to yield 4-oxo-*trans*-2-alkenals, among other compounds. The formation of 4-oxo-*trans*-2-alkenals from hydroperoxy-*trans*-2-alkenals (*36*) as well as the degradation of hydroperoxy-*trans*-2-alkenals in the chromatographic run to give 4-oxo-*trans*-2-alkenals (*43*) has been previously described and reinforces the results obtained in this study.

In conclusion, the results obtained from the FTIR spectroscopy study are in total agreement and are complementary with those obtained from the study by SPME/GC-MS. It is worth noting the presence, in the sunflower headspace from the first day of the experiment, of secondary oxidation compounds, at very low proportions, the concentrations of which do not increase until the second stage, at the end of which they reach their highest concentrations. However, the amount of compounds formed during the oxidation is not of the same order for all secondary oxidation products, the main ones being some saturated, *trans*-2-monounsaturated, *trans,trans*-2,4-diunsaturated and some oxygenated aldehydes. Although 4-oxo-*trans*-2-nonenal has been detected in the headspace of the sunflower oil through the oxidation process by SPME/GC-MS, the absence of compounds of this nature in the oil liquid phase, as ^{1}H NMR studies have shown, suggests that these compounds might be formed during the chromatographic run. The identification, among the headspace components, of various harmful oxygenated aldehydes is of great importance. These compounds have not been reported in edible oils oxidation studies until now but are well-known in many human health studies. These results open a new perspective in the control and study of oil and fat safety and suggest the need for regulations concerning the presence of this type of compounds in foods.

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