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Evidence of the Formation of Light Polycyclic Aromatic Hydrocarbons during the Oxidation of Edible Oils in Closed Containers at Room Temperature

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Solid phase microextraction (SPME) followed by gas chromatography−mass spectrometry (GC-MS) of the headspace composition of two sunflower oil samples was carried out; both samples were taken from the same original oil, stored for a prolonged time (112 months) in closed containers at room temperature under different air/oil volume ratios. Great differences in the headspace compositions of both samples were found due to the different oxidation levels reached. One of the most significant findings is that both contain monocyclic and light polycyclic aromatic hydrocarbons, the proportions of which are in line with the oxidation level of the sample. The determination of polycyclic aromatic compounds in the oil liquid matrix of both oil samples, carried out by means of a classical scheme of isolation, cleanup, separation, and quantification, showed that the concentrations of these compounds in the oil liquid phase also follow the oxidation degree reached by each sample, proving that this oxidation process at room temperature leads to the formation of these compounds.

KEYWORDS: Monocyclic and light polycyclic aromatic hydrocarbons; oxidation; room temperature; solid phase microextraction; storage; sunflower oil

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) make up a large group of compounds widely distributed in the environment and, in some cases, also in foods. The development of methods to determine these compounds in very diverse matrices and the study of their concentration and distribution in foods and in the diet of different countries have been the subject of research for a long time (*1, 2*).

The origin of the presence of these compounds in foods has also been widely studied, and in general, in addition to environmental contamination, the inadequate use of certain food-processing techniques has been put forward as the main cause of food contamination by PAHs (*3, 4*). Despite this, the possibility that these compounds form during metabolic processes in plants, seaweed, or bacteria has been suggested; thus, it has been observed that there is an increase in the PAH content of leaves during yellowing and germination (*5*). However, other investigators have rejected as a feasible mechanism the biosynthesis of PAHs by plants and have stressed the necessity for extreme precautions when isolating PAHs from plant extracts, because otherwise false positives can occur (*6*).

Some of these compounds are carcinogenic or cocarcinogenic, and their presence in foods is a cause for concern for human health, and for this reason regulations referred to maximum permitted limits of some of these compounds in specific foods have recently been established by European Union legislation (*7, 8*).

Due to the lipophilic nature of PAHs, oils and fats are prone to contamination by these toxic compounds. The origin of these contaminants in edible oils has been attributed mainly to the atmospheric pollution to which oily plants are exposed (*9*) and to contamination during processing. In the latter case, drying of the plant material with gases from direct combustion before oil extraction (*10, 11*), the solvents employed for oil extraction (*12*), contamination from mineral or lubricating oils (*13, 14*), and the use of high or very high temperatures in the processing (*15, 16*) have been suggested as origins of edible oils contamination by PAHs. In addition, the biosynthetic origin of PAHs in oily plants cannot be excluded either (*17*).

Furthermore, the formation of PAHs from lipids submitted to very high temperatures is well-known, and recently it has been reported that some edible oils rich in polyunsaturated acyl groups maintained at not very high temperatures (220 °C) during a certain period of time (2 h) can also form PAHs (*18*). However, results of studies on frying oils, submitted to repeated frying episodes between 170 and 205 °C, do not confirm these findings (*19*), and to the best of our knowledge, the formation

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Table 1. Time and Storage Conditions of R1 and R2 Sunflower Oil Samples

sample	storage (months)	air contact surface (cm^2)	air (cm^3)	oil (cm^3)	air/oil volume ratio
R1	112	15.2	10.9	898.6	0.012
R2	112	15.2	50.3	25.1	2.000

Table 2. Acyl Group Percentage of Sunflower Oil Samples R0, R1, and R2, Determined as in Previous Papers (*21, 22*)

	acyl groups (%)			
sample	linolenic	linoleic	oleic	saturated
R0	0.0	63.8	24.2	11.9
R1	0.0	63.2	24.4	12.4
R2	0.0	50.9	27.2	21.8

of PAHs in edible oil oxidation at room temperature has not been reported until now. In this context it should be mentioned that in a previous study carried out in our laboratory, using solid phase microextraction (SPME) followed by gas chromatography−mass spectrometry (GC-MS), of the headspace composition of edible oils rich in polyunsaturated acyl groups submitted to thermo-oxidative conditions (70 °C with aeration), the presence of PAHs was not observed, so it can be inferred that their formation was not produced under these conditions (*20*).

By contrast, in a study carried out in our laboratory on the headspace components of a very numerous groups of edible oils rich in polyunsaturated acyl groups and stored over different periods of time, at room temperature in closed receptacles, in the presence of limited amounts of air, it was observed that the headspaces of those samples at more advanced oxidation stages contained higher numbers and higher concentrations of certain PAHs than those in incipient oxidation stages, and a potential relationship between the oxidation degree reached by the sample and the concentration of PAHs seemed to exist.

These results raised the hypothesis of the possible formation of PAHs during the oxidation process of edible oils, at room temperature, in the presence of limited amounts of oxygen; however, those studies provided information only about the oil gas phase, and they were carried out with oils coming in some cases from different batches and/or brands. To prove the hypothesis above-mentioned, in this paper the study, by SPME/GC-MS, of the headspace of a sunflower oil of the same brand, batch, and bottle, stored for the same time, in two different closed containers, under different air/oil ratios, is carried out. This technique provides information about the oxidation degree of these oil samples by means of the nature and concentration of the secondary oxidation products present in their headspaces. In addition, the concentration of PAHs in the liquid phase of both sunflower oil samples is determined in order to know if it is different in both samples so as to prove the hypothesis above-mentioned.

MATERIALS AND METHODS

Samples. The study was carried out with a sunflower oil, named R0, acquired in a local supermarket. Different amounts of this oil were stored at room temperature, for 112 months, in different closed and impermeable containers, in the presence of different proportions of air. After storage under the conditions indicated in **Table 1**, the sunflower oil samples were named R1 and R2. The composition of the oil R0 in main components, when acquired, is shown in **Table 2**; this composition, expressed as a percentage of its main acyl groups, was determined from data from its ^{1}H nuclear magnetic resonance spectrum, using equations described in previous papers (*21, 22*).

Table 3. Some of the Compounds Detected in the Headspace of Sunflower Oil Samples R1 and R2 and Their Abundances Expressed as Area Counts Divided by 10^6

no.	compd (mol wt)[a]	ion considered	R1	R2
1	pentane (72)*	43	36.40	56.85
2	acetic acid (60)*	43	28.25	91.59
3	ethyl acetate (88)	43	3.76	9.34
4	propanoic acid (74)*	74	0.55	1.54
5	pentanal (86)*	44	17.40	38.49
6	1-pentanol (88)*	42	3.26	17.27
7	hexanal (100)*	56	122.41	123.68
8	*trans*-2-hexenal (98)*	41	2.51	13.84
9	2-heptanone (114)*	43	18.23	35.37
10	heptanal (114)*	70	2.80	3.95
11	nonadiene (124) (or isomer)	54	0.12	4.42
12	*trans*-2-heptenal (112)*	83	28.38	86.77
13	1-octen-3-ol (128)*	57	20.48	84.11
14	2-pentylfuran (138)*	81	42.72	129.82
15	2-octanone (128)*	43	1.47	5.35
16	hexanoic acid (116)*	60	125.79	790.51
17	3-octen-2-one (126)	55	1.81	7.89
18	*trans*-2-octenal (126)*	70	24.53	130.55
19	2-nonanone (142)*	43	0.51	5.81
20	*trans*-2-nonenal (140)*	41	0.95	11.78
21	2-decanone (156)*	58	0.01	2.55
22	5-butyl-2(5*H*)-furanone (140)	84	0.29	6.26
23	5-pentyl-2(3*H*)-furanone (154)	98	0.36	3.54
24	*cis,trans*-2,4-decadienal (152)*	81	3.56	38.58
25	*trans,trans*-2,4-decadienal (152)*	81	4.95	88.42
26	5-pentyl-2(5*H*)-furanone (154)	84	1.24	12.75
27	4-hydroxy-*trans*-2-nonenal (156)*	57	0.75	10.20
28	4,5-epoxy-2-decenal (168)*	68	0.17	1.75

[a] Asterisked compounds were acquired commercially and used as standards for identification purposes.

Extraction and Study of the Oil Headspace Components. *Extraction by SPME of the Headspace Components.* Vials containing 1 g of oil were introduced into a water bath maintained at 50 °C. After a period of sample equilibration (15 min), a fiber coated with DVB/CAR/PDMS (divinylbenzene/carboxen/polydimethylsiloxane, 50/30 μm film thickness), acquired from Supelco, was inserted into the headspace of the sample and was maintained for 60 min. Selection of the fiber type and extraction operating conditions was based on previous studies carried out in our laboratory.

GC Study of the Extracted Compounds. The extracted compounds retained on the SPME fiber were desorbed, separated, identified, and semiquantified in a gas chromatograph. To this aim 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 ChemStation program. The column used was a fused-silica capillary column (60 m long × 0.25 mm i.d. × 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 50 °C (5 min hold), increased to 280 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 ionization energy of 70 eV; the data acquisition mode employed was scan. After the first desorption, the fiber was routinely submitted to desorption conditions for a second time for its cleanup and at the same time to determine if the first process was complete.

Many of the component subjects of our study were identified by using standards. Therefore, asterisked compounds in **Tables 3** and **4** were acquired commercially from Aldrich, Sigma, and Fluka and used as standards for identification. Some other components were only tentatively identified; in the latter case, retention times, together with mass spectra, and matched with mass spectra of a commercial library

Table 4. Monoaromatic and Polyaromatic Hydrocarbons Detected in the Headspace of Sunflower Oil Samples R1 and R2 and Their Abundances Expressed as Area Counts Divided by 10^6

no.	compd (mol wt)[a]	ion considered	R1	R2
	Monoaromatic Hydrocarbons			
29	benzene (78)*	78	tr[b]	tr
	methylbenzene (toluene) (92)*	91	9.92	27.51
	dimethylbenzene (106)	91	0.17	0.25
	dimethylbenzene (106)	91	0.53	0.77
	dimethylbenzene (106)	91	0.12	0.22
	methylethylbenzene (120) (or isomer)	120	0.01	0.08
	1-methyl-2-(1-methylethyl)-benzene (134) (or isomer)	119	0.38	0.80
	1-methyl-4-propylbenzene (134) (or isomer)	105	0.02	0.11
	(1,1-dimethylethyl)-benzene (134) (or isomer)	119	0.02	0.25
	(2-methyl-2-propenyl)-benzene (132) (or isomer)	117	0.08	0.23
	(1,1-dimethylethyl)-benzene (134) (or isomer)	119	0.03	0.16
	(2-methyl-2-propenyl)-benzene (132) (or isomer)	117	0.03	0.19
	octylbenzene (190)	91	0.00	0.07
	Polyaromatic Hydrocarbons			
30	naphthalene (128)*	128	4.11	64.53
31	2-methylnaphthalene (142)*	142	0.84	20.24
32	1-methylnaphthalene (142)*	142	1.57	29.41
	1,1′-biphenyl (154)*	154	0.01	0.18
	dimethylnaphthalene (156)	156	0.00	0.05
33	dimethylnaphthalene (156)	156	0.00	0.09
34	1,6-dimethylnaphthalene (156)*	156	0.27	5.92
35	dimethylnaphthalene (156)	156	0.00	0.04
	2-methyl-1,1-biphenyl (168)	168	0.00	0.03
	9*H*-fluorene (166)*	166	0.13	1.92

[a] Asterisked compounds were acquired commercially and used as standards for identification purposes. [b] tr, traces.

higher than 85%, were taken as identification criteria (Wiley 275.L, Mass Spectral Database, Rev. D.01.00, June 2000) as in previous studies (*20*). The semiquantification of the components was based on the base peak area counts divided by 10^6.

Determination of PAH Content in the Oil. *Extraction and Isolation of PAHs.* The method followed begins with the addition of a mixture of deuterated PAH internal standards constituted by naphthalene-d_8, acenaphthene-d_{10}, phenanthrene-d_{10}, pyrene-d_{10}, *p*-terphenyl-d_{14}, chrysene-d_{12}, and perylene-d_{12} to approximately 10 g of the oil subject of study (R1 and R2 samples). After this, the sample is diluted with *n*-hexane, the PAHs are extracted with dimethyl sulfoxide (DMSO), and they are extracted again with cyclohexane from the DMSO solution; the extract is washed with water, dried on anhydrous sodium sulfate, and cleaned up by solid phase extraction tubes, as in previous papers (*16*). After cleanup, the extracts were submitted to alkaline treatment because its analyses by GC-MS in scan mode showed that they contained fatty acids which interfere with the determination of PAHs; the alkaline treatment was carried out in the way described in previous papers (*16*). Finally, the extract was concentrated to 1 mL, for chromatographic study. All glassware used was cleaned before use with dichloromethane in an ultrasonic bath several times, and the washing solvent was concentrated and analyzed by GC-MS in SIM mode to check the absence of residual contamination. The purity of the solvents was also checked to avoid the incorporation of impurities or even of additional PAHs to the samples subject to study.

Chromatographic Study. The separation, identification, and quantification of the PAHs were performed with the same gas chromatograph and the same column used for the study of the oil headspace composition. Operating conditions were as follows: the oven temperature was set initially at 50 °C (0.50 min hold), increased to 130 at 8 °C/min, and again increased to 290 °C at a rate of 5 °C/min (50 min hold); the temperatures of the ion source and the quadrupole mass analyzer were kept at 230 and 150 °C, respectively; helium with a purity of 99.999% was used as carrier gas at a constant flow of 1.0 mL/min; injector and transference line temperatures were held at 290 and 300 °C, respectively; pulsed splitless mode was used for injection with a pressure pulse of 30 psi, and 1 μL of each sample was introduced in the gas chromatograph. The data acquisition modes employed were scan and SIM. Scan mode was used to determine the type of compounds present in the samples, whereas SIM was used to identify and quantify the PAHs present. Identification of the compounds was based on their retention times and on the relative abundances of the specific ions selected for each PAH. Quantification was based on the measurement of the peak area correspondening to the most abundant ion of each compound and was carried out by the internal standard quantification method. For this purpose, a calibrant solution was prepared with the different PAH standards and was spiked with the same internal standard solution used to spike the samples. Thus, naphthalene-d_8 was used for quantification of naphthalene and its methyl derivatives, acenaphthene-d_{10} for acenaphthylene and acenaphthene, phenanthrene-d_{10} for phenanthrene, anthracene, and their methyl derivatives, pyrene-d_{10} for fluoranthene and pyrene, *p*-terphenyl-d_{14} for *m*-terphenyl, *p*-terphenyl, benzofluorenes, and methylfluoranthenes/pyrenes, chrysene-d_{12} for benz(*a*)anthracene, chrysene, and their methyl derivatives, perylene-d_{12} for benzofluoranthenes and benzopyrenes, and, last, benzo(*ghi*)-perylene-d_{12} for PAHs with higher molecular weights. The response factors of each compound relative to the internal standard chosen for its quantification were calculated for each sample.

Reagents and Materials Used. The solvents employed, *n*-hexane for analyses, DMSO for spectroscopy, cyclohexane and methanol, HPLC grade (99.9+%), and dichloromethane (99.8%), as well as other reagents and materials such as potassium hydroxide, sodium chloride, anhydrous sodium sulfate, and Supelclean LC-Si SPE (solid phase extraction) tubes, 3 mL (500 mg), are commercially available from Riedel-de Haën (Seelze, Germany), Merck (Darmstadt, Germany), Aldrich (Steinheim, Germany), Panreac (Barcelona, Spain), Symta (Madrid, Spain), and Supelco (Bellefonte, PA).

In addition, several solutions containing either individual or mixtures of PAH standards were used for identification and quantification purposes: a commercial mix of PAHs dissolved in a mixture of dichloromethane/benzene (75:25), containing naphthalene, acenaphthene, acenaphthylene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(*c*)phenanthrene, benz(*a*)anthracene, chrysene, 7,12-dimethylbenz(*a*)anthracene, benzo(*b*)fluoranthene, benzo(*j*)fluoranthene, benzo(*k*)fluoranthene, benzo(*a*)pyrene, 3-methylcholanthrene, indeno(1,2,3-cd)pyrene, dibenz(*a*,*h*)anthracene, benzo(*ghi*)perylene, dibenzo-(*a*,*l*)pyrene, dibenzo(*a*,*i*)pyrene, and dibenzo(*a*,*h*)pyrene in concentrations of approximately 500 μg/mL; commercial individual cyclohexane solutions of 1,7-dimethylnaphthalene, 1,4-dimethylnaphthalene, 1,5-dimethylnaphthalene, 1-methylphenanthrene, 2,3-dimethylanthracene, 9,10-dimethylphenanthrene, 2-methylfluoranthene, 1-methylfluoranthene, 11*H*-benzo(*c*)fluorene, 1-methylpyrene, 6-methylbenz(*a*)an-

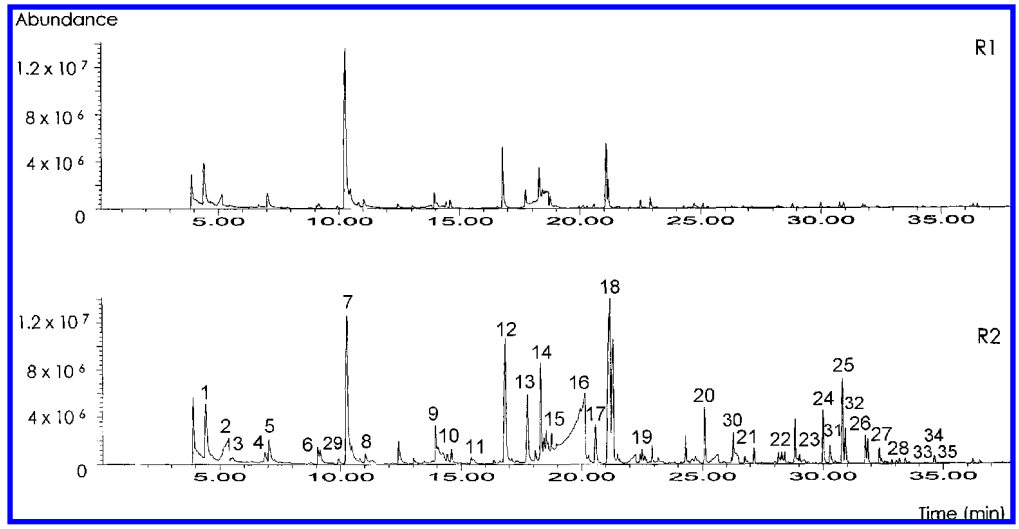

Figure 1. Chromatograms of the compounds extracted from the headspaces of R1 and R2 sunflower oil samples by solid phase microextraction (SPME). Some peaks are labeled according to the numbers in **Tables 3** and **4**.

thracene, 7-methylbenz(*a*)anthracene, 3-methylchrysene, 2-methylchrysene, 5-methylchrysene, 4-methylchrysene, 6-methylchrysene, 1-methylchrysene, dibenz(*a*,*j*)anthracene, benzo(*b*)chrysene, picene, anthanthrene, coronene, and dibenzo(*a*,*e*)pyrene, in concentrations of 10 μg/mL approximately, and of benzo(*ghi*)perylene-d_{12}, in a concentration of approximately 100 μg/mL; and different solutions of pure standards such as 2,6-dimethylnaphthalene, 2,3-dimethylnaphthalene, *o*-terphenyl, 2-methylanthracene, 9-methylanthracene, 3,6-dimethylphenanthrene, *m*-terphenyl, *p*-terphenyl, 11*H*-benzo(*a*)fluorene, 11*H*-benzo(*b*)fluorene, benzo(*e*)pyrene, and perylene, and of naphthalene-d_8, acenaphthene-d_{10}, phenanthrene-d_{10}, pyrene-d_{10}, *p*-terphenyl-d_{14}, chrysene-d_{12}, and perylene-d_{12} in dichloromethane or cyclohexane were also used. All of the standards, both solid and in solution, were obtained from Sigma, Aldrich, Supelco, and Symta.

All experiments were made in duplicate, and the results given in the several tables are average values.

RESULTS AND DISCUSSION

As commented above, different amounts of the original oil R0 were introduced into two bottles in the presence of different volumes of air and with the same air−oil contact surface (see **Table 1**), and these were kept closed for 112 months at room temperature. Under these storage conditions, the only degradative process suffered by the oil was its oxidation, and thus two different oil samples, named R1 and R2, each having a different oxidation level, were obtained from the original oil; as can be observed in **Table 2** the present compositions in acyl groups of these oil samples are fairly different due to the degradation in the oxidation process of a certain amount of their acyl groups, basically of linoleic acyl groups.

The headspace composition of the R1 and R2 samples, as mentioned above, was studied by extraction of their components by solid phase microextraction and posterior desorption of the extracted components in the injector of a gas chromatograph−mass spectrometer for their separation, identification, and semiquantification. **Figure 1** shows the gas chromatograms of the headspace components of both samples; it can be observed that both contain volatile compounds, these in higher number and in higher concentration in sample R2 than in sample R1, in agreement with the higher oxidation level reached by the first sample. The study of these chromatograms revealed that most of the compounds present in these headspaces are well-known as oil secondary oxidation products (*20*); among these there are alcohols, acids, esters, ketones, aldehydes, furan derivatives, and hydrocarbons. In **Table 3** some of these compounds are shown together with their area counts of the mass spectra base peaks, which provide information, valid for comparative purposes, about their concentrations in the headspace of these samples; these results show again that the R2 oil sample is at a much more advanced oxidation stage than the R1 oil sample, in agreement with data in **Table 1**.

In addition, study of the chromatograms given in **Figure 1** revealed that the headspace of both oil samples contained certain mono- and polyaromatic hydrocarbons. As **Table 4** shows, the main compounds of this type found are toluene and its methylated derivatives, from the monoaromatic hydrocarbons group; and naphthalene and its monomethyl derivatives 1,6-dimethylnaphthalene and fluorene, from the polyaromatic hydrocarbons group. These compounds were in higher concentration in the headspace of sample R2 than in that of sample R1, as **Table 4** shows. Although, to the best of our knowledge, the formation of aromatic hydrocarbons in oil oxidation processes at room temperature has not been described before, the results obtained indicate that the headspace of the sample at a more advanced oxidation stage, R2, contains a higher number and a also greater concentration of these compounds than that of the sample R1, which is at an earlier oxidation stage.

Because the R1 and R2 sunflower oil samples derive from the same sunflower oil, R0, their original aromatic hydrocarbon contents were the same; however, the current headspace of R2 is richer in these compounds than the current headspace of R1 is, and this could indicate that also the oil liquid phase of R2 is also richer in these compounds than that of R1, suggesting that these compounds are generated in the oxidation process.

It is known that polar compounds are formed in the oil oxidation processes; for this reason, the polarity of the liquid phase of the more oxidized oil sample R2 will be higher than that of R1; as aromatic hydrocarbons are nonpolar compounds, the higher the polarity of the oil liquid phase, the more intense the repulsive interactions between aromatic hydrocarbons and this phase are, facilitating their escape toward the gas phase. If these repulsive interactions were strong enough, samples with the same PAH content but different oxidation degrees could show different concentrations of these compounds in their headspaces; this will be due to the different intensities of the repulsive interactions between PAHs and the oil liquid phases, the polarity of which depends on its oxidation level. For these reasons, it could be thought that the higher concentration of aromatic hydrocarbons

Table 5. PAHs Identified in the R1 and R2 Sunflower Oil Samples, Their Concentrations, Expressed as Mean Value ± Standard Deviation, and the Ratio R_{LP} between the Concentrations of the Same PAH in the Liquid Phases of Both Samples

PAH	R1 (μg/kg)	R2 (μg/kg)	R_{LP}(R2/R1)
naphthalene	319.5 ± 1.5	5220.8 ± 3.6	16.4
2-methylnaphthalene	221.1 ± 6.8	5879.7 ± 85.9	26.6
1-methylnaphthalene	332.1 ± 14.8	6723.9 ± 52.7	20.2
2,6-dimethylnaphthalene	24.0 ± 0.6	69.1 ± 3.8	2.8
1,7-dimethylnaphthalene	26.5 ± 2.9	80.7 ± 1.0	3.0
1,6-dimethylnaphthalene	213.7 ± 10.5	5612.6 ± 245.8	26.2
1,4-dimethylnaphthalene	15.1 ± 0.1	46.9 ± 1.9	3.1
1,5-dimethylnaphthalene	7.0 ± 0.8	23.4 ± 3.5	3.3
acenaphthene	1.9 ± 0.7	11.8 ± 1.0	6.2
fluorene	216.3 ± 1.5	15601.9 ± 1105.0	72.2
phenanthrene	10.1 ± 0.1	42.7 ± 0.0	4.3
o-terphenyl	11.3 ± 0.3	256.4 ± 5.4	23.2
3-methylphenanthrene	7.3 ± 0.1	18.5 ± 0.8	2.5
2-methylphenanthrene	9.3 ± 0.4	20.3 ± 0.8	2.2
9-methylphenanthrene	9.1 ± 0.2	20.1 ± 0.5	2.2
1-methylphenanthrene	6.1 ± 0.6	12.4 ± 0.2	2.0
3,6-dimethylphenanthrene	10.9 ± 0.4	21.9 ± 1.4	2.0

found in the headspace of R2 could be due to the higher polarity of its liquid phase.

To clarify if the higher concentration of PAHs in the headspace of sample R2 was due to a higher concentration of these compounds in its liquid phase, and was not a consequence of its higher polarity, the concentration of the PAHs present in the liquid phases of R1 and R2 oil samples was determined; this determination was carried out following the classical scheme that includes isolation of the PAHs by extraction, cleanup, saponification, cleanup again if necessary, and separation, identification, and quantification of the PAHs isolated, by GC-MS operating in SIM mode as in previous studies (*16*).

Table 5 shows the PAHs detected together with their concentrations expressed in micrograms per kilogram of oil. The results obtained confirm that the concentration of PAHs in sunflower oil sample R2 is much higher than it is in sunflower oil sample R1; these results prove that these compounds were generated during oxidation at room temperature, because both samples contained the same PAH concentration before oxidation and because the oil samples did not undergo any other process.

It is noteworthy that only PAHs of low molecular weight were detected in both oil samples, with only two or three aromatic rings, with or without alkyl substituents. The concentrations found, as **Table 5** shows, are very different for each PAH, indicating that these oxidative conditions favor the formation of some of these compounds. This is observable in each sample and also in the comparison of both samples. Therefore, although the presence of the two methylnaphthalene derivatives is significant in samples R1 and R2, the formation of 1-methylnaphthalene is more favored than that of 2-methylnaphthalene; likewise, the formation of 1,6-dimethylnaphthalene is much more favored than that of the other dimethylnaphthalene derivatives. Values of the ratios between the concentrations of the same PAH in the liquid phases of R2 and R1 samples, named R_{LP}, are also given in **Table 5**. These values indicate that the yield of each PAH under the storage conditions of sample R2 is R_{LP} times higher than under those of sample R1; the highest R_{LP} values are shown by fluorene, 1,6-dimethylnaphthalene, 2-methylnaphthalene, *o*-terphenyl, 1-methylnaphthalene, and naphthalene.

It is also worth noting that the increase in the liquid phase polarity, due to oxidation, has little influence on the escape of PAHs toward the gas phase. In most of these PAHs (naphthalene, 2-methylnaphthalene, 1-methylnaphthalene, and 1,6-dimethylnaphthalene), the R_{GP} ratios between their proportions in the headspace of samples R2 and R1, expressed in area counts of the base peak of their mass spectra in the chromatograms given in **Figure 1** (R_{GP} of naphthalene = 15.7; R_{GP} of 2-methylnaphthalene = 24.0; R_{GP} of 1-methylnaphthalene = 18.6; R_{GP} of 1,6-dimethylnaphthalene = 21.9) are of an order similar to that of the R_{LP} ratios (R_{LP} of naphthalene = 16.4; R_{LP} of 2-methylnaphthalene = 26.6; R_{LP} of 1-methylnaphthalene = 20.2; R_{LP} of 1,6-dimethylnaphthalene = 26.2).

To the best of our knowledge, this is the first time that the formation of light PAHs and also of monoaromatic hydrocarbons in the oxidation process of edible oils at room temperature has been described. The formation of both light polycyclic aromatic and monoaromatic hydrocarbons in sunflower oil stored at room temperature for prolonged periods of time could be explained in two ways: either by the generation, during oxidation, of unsaturated alkyl free radicals, which, in the presence of limited amounts of oxygen, may evolve to give aromatic cycles; or by reactions between secondary oxidation products that are unsaturated. Pathways for the aromatic hydrocarbon formation from cyclopentadienyl radicals generated in combustion processes (*23, 24*) as well as from different secondary oxidation compounds have been described (*18*). It should also be stated that a previous study has shown that alkylbenzene and naphthalene derivatives form during the autoxidation at room temperature of methyl arachidonate (*25*).

As has been mentioned, the presence of monoaromatic and polyaromatic hydrocarbons in vegetable oils is well-known, and in most cases this has been attributed either to environmental contamination or to inadequate processing (*13, 14, 16*). However, it has also been pointed out that biosynthetic pathways for monoaromatic hydrocarbon formation cannot be ruled out (*26, 27*). Likewise, although the formation of PAHs has been mainly associated with the effect of high temperatures on organic matter, including lipids, other origins (*28*) have also been pointed out, such as plant or living being metabolism (*5, 29–33*) or organic matter degradation under oxygen-deficient conditions (*34*). All of these results support the idea that the formation routes of mono- and polyaromatic hydrocarbons can be significantly varied.

Finally, it should be added that the formation of PAHs of low molecular weight during sunflower oil storage is a cause of safety concern because naphthalene has been recently considered as "*possibly carcinogenic to humans*" (group 2b) by the International Agency for Research on Cancer (IARC) (*35*). In this context, it should be mentioned that European Union laws to guarantee edible oil safety have established limits on concentrations of only heavy PAHs (*7, 8*) without considering that some oils can be free of heavy PAHs but can contain important concentrations of naphthalene.

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