JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Polycyclic Aromatic Hydrocarbons and Olive Pomace Oil

MARÍA D. GUILLÉN,* PATRICIA SOPELANA, AND GEMMA PALENCIA

Tecnología de Alimentos, Facultad de Farmacia, Universidad del País Vasco, Paseo de la Universidad No. 7, 01006 Vitoria, Spain

The occurrence of polycyclic aromatic hydrocarbons (PAHs) in five samples of olive pomace oil has been studied to determine the contamination degree of this type of oil and to evaluate if specific purification steps must be introduced during its manufacture. The PAHs present have been determined by gas chromatography−mass spectrometry. A high number of PAHs, with a wide range of molecular weights and in very high concentrations, have been found in four of the samples studied. A very high number of alkyl derivatives and, in many cases, in higher concentrations than their respective parent PAHs, have also been identified. One of the samples, however, presents a more reduced number of PAHs and in significantly lower concentrations than the others. These findings reveal that it is necessary to introduce adequate cleanup steps in the manufacturing process of olive pomace oil, which can give rise to oils with a relatively low content of PAHs. Some carcinogenic PAHs have also been identified, both unalkylated and alkylated.

KEYWORDS: Polycyclic aromatic hydrocarbons; olive pomace oil; gas chromatography; mass spectrometry

INTRODUCTION

The presence of polycyclic aromatic hydrocarbons (PAHs) in a wide variety of vegetable oils has been revealed by the studies of many authors (*1*−*9*). The occurrence of PAHs in edible oils is attributed mainly to environmental contamination of the vegetable raw material and to contamination coming from some operations carried out during their processing, such as seed drying or solvent extraction, although some authors indicate that the endogenous origin of some PAHs cannot be totally discarded (*5*, *10*). Among all of the vegetable oils, olive oil is highly appreciated not only for its excellent organoleptic and nutritious characteristics but also for its known healthy properties (*11*). For these reasons, its consumption is increasing continuously.

Different types of oils can be obtained from the fruit of the olive tree: virgin olive oil, obtained solely by mechanical or other physical means under conditions that do not lead to alterations in the oil and which has not undergone any treatment other than washing, decantation, centrifugation and filtration; refined olive oil, obtained from virgin olive oils by refining methods that do not lead to alterations in the initial glyceridic structure; olive oil, which is a blend of refined and virgin olive oil fit for consumption as it is; and olive pomace oil, which is obtained by treating the solid residue remaining after the extraction of olive oil (olive pomace) with solvents, excluding oils obtained from re-esterification processes and from any mixture of oils with other properties.

* Corresponding author (telephone 34-45-013081; fax 34-45-013014; e-mail knpgulod@vf.ehu.es).

Olive pomace oil contains proportions of the different acyl groups similar to those in olive and in virgin olive oils, slightly lower in oleic and higher in linoleic and saturated acyl groups (*12*); however, even though olive pomace is a rich source of antioxidants (*13*), its oil is slightly poorer than virgin olive oil in natural antioxidant components, probably due to their elimination during refining (*14*). It must also be noted that the manufacturing processes of each type of oil derived from the olives are different, to such an extent that it can determine the PAH contamination level of the final product. The influence of the manufacturing process on PAH contamination is clear in the case of some types of vegetable oils, such as coconut oil, where very high concentrations of this type of contaminant have been found in the crude product (without refining), which can also appear in the refined oil if no specific cleanup stage is carried out during its processing (*5*, *15*).

A wide range of PAH concentrations have been found in olive oils and in virgin olive oils (*1*, *3*, *4*, *6*, *8*, *9*, *16*, *17*); however, little attention has been paid to olive pomace oil, which is usually employed in the frying or cooking of foods and also in canned food. To the best of our knowledge, only two papers have been published on the occurrence of PAHs in this type of oil (*9*, *18*); moreover, they refer to only a reduced number of these toxic contaminants.

It must be pointed out that until now the European Union has not established legal limits for PAHs in edible oils. However, in Spain, a legal disposition was put into effect in July 2001 (*19*) limiting the concentrations of eight PAHs in olive pomace oils: benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*e*]pyrene, benzo[*a*]pyrene, indeno[1,2,3-*cd*]-pyrene, dibenz[*a*,*h*]anthracene, and benzo[*ghi*]perylene; a similar

10.1021/jf035259q CCC: $27.50
Published on Web 03/12/2004

approach was put into practice in Italy (*20*). Previously, DGF had proposed 25 μg/kg as a limit for total PAHs and 5 μg/kg for the sum of heavy PAHs (*10*) and, recently, the Canadian Food Inspection Agency has suggested correcting results on the basis of toxic equivalency factors (TEFs) (*21*).

Due to the few studies on the presence of PAHs in olive pomace oil, in this paper some samples of this oil have been studied. The study has been carried out following a classical scheme for PAH isolation and subsequent separation, identification, and quantification by gas chromatography−mass spectrometry (GC-MS) operating in scan and in selective ion monitoring (SIM) modes. The aim of this work is to study those PAHs present in the samples selected and capable of determination by GC-MS, to determine the degree of PAH contamination of olive pomace oil, and to assess the need to introduce adequate cleanup steps for PAHs during the manufacture of this oil.

MATERIALS AND METHODS

Samples. The samples are five different commercial olive pomace oils, designated OP1, OP2, OP3, OP4, and OP5. Samples OP1−OP4 were acquired prior to the year 2001, before the Spanish order of July 25th (*19*), and OP5 was acquired during the year 2002, after the previous order. These olive pomace oils are blends of refined olive pomace oil and virgin olive oil; the latter is added in a small proportion to improve the organoleptic properties of refined olive pomace oil. Each sample was studied in duplicate.

Reagents and Materials. The solvents employed were *n*-hexane for analyses, dimethyl sulfoxide (DMSO) for spectroscopy, cyclohexane and methanol, both of HPLC grade (99.9+%), and dichloromethane (99.8%). Other reagents and materials used were potassium hydroxide, sodium chloride, anhydrous sodium sulfate, and Supelclean LC-Si solid phase extraction (SPE) tubes, 3 mL (500 mg). All solvents, reagents, and materials mentioned 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).

Four groups of PAH standards were used for the identification and quantification of the PAHs present in the samples:

(1) A commercial mix of PAHs dissolved in a mixture of dichloromethane/benzene (75:25), contained 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 ~500 μg/mL. This mix was obtained from Supelco (Bellefonte, PA).

(2) Commercial individual cyclohexane solutions of 1,7-dimethylnaphthalene, 1,4-dimethylnaphthalene, 1,5-dimethylnaphthalene, 1-methylphenanthrene, 3,6-dimethylphenanthrene, 2,3-dimethylanthracene, 9,10-dimethylphenanthrene, 2-methylfluoranthene, 1-methylfluoranthene, 11*H*-benzo[*c*]fluorene, 1-methylpyrene, 6-methylbenz[*a*]anthracene, 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 were in concentrations of ~10 μg/mL. All of these solutions were purchased from Symta (Madrid, Spain).

(3) Pure PAHs included 1,6-dimethylnaphthalene, 2,6-dimethylnaphthalene, *o*-terphenyl, 2-methylanthracene, 9-methylanthracene, *m*-terphenyl, 11*H*-benzo[*a*]fluorene, 11*H*-benzo[*b*]fluorene, benzo[*e*]-pyrene, and perylene, all of which were obtained from Sigma-Aldrich (Steinheim, Germany); 2,3-dimethylnaphthalene, from Symta (Madrid, Spain); and *p*-terphenyl, from Merck (Darmstadt, Germany). All of these standards were used to prepare different solutions in dichloromethane or cyclohexane.

(4) Naphthalene-d_8, acenaphthene-d_{10}, phenanthrene-d_{10}, pyrene-d_{10}, *p*-terphenyl-d_{14}, chrysene-d_{12}, and perylene-d_{12} were used as internal standards. These were acquired as pure compounds from Supelco (Bellefonte, PA) except for pyrene-d_{10}, which was purchased from Symta (Madrid, Spain), and different solutions containing a mixture of them were prepared in dichloromethane or cyclohexane.

It must be pointed out that some of the PAH standards abovementioned are cancer suspect agents, so precautions must be taken in the handling of these compounds. The purity of all pure PAH standards ranged from 97 to 99.5%.

Methodology. Before the treatment of the samples for the determination of PAHs is begun, two aspects related to the procedure must be noted. First, it must be guaranteed that all of the glassware is free of PAHs. For this purpose, it is recommended that all the glass be cleaned with dichloromethane, several times, in an ultrasonic bath, concentrating the washing solvent and analyzing the concentrate by GC-MS in SIM mode to check for the absence of residual contamination. Second, the purity of the solvents employed should be carefully monitored in order to avoid the incorporation of impurities and even of additional PAHs to the samples of study.

The scheme of the methodology employed for the study of the oils is shown in **Figure 1**.

The extraction of PAHs from the oil samples was carried out by taking as the starting point the liquid−liquid partition scheme with DMSO described by Natusch and Tomkins (*22*); it must be pointed out that the ratio of 2 volumes of water to 1 volume of DMSO employed for these authors was changed to a ratio of water/DMSO of 2.4/1, because a better separation of phases in the subsequent extraction of PAHs with cyclohexane was observed with the latter proportion. This same ratio has also been used by García Falcón et al. (*23*) in the determination of benzo[*a*]pyrene in lipid soluble liquid smoke. *The cleanup procedure* by SPE silica tubes was performed according to that in a previous paper (*24*).

Samples OP3 and OP4 were subjected to an *alkaline treatment* after the whole procedure, because their analyses by GC-MS in scan mode showed that they contained fatty acids. For this purpose, ~11.2 g of potassium hydroxide dissolved in 100 mL of a mixture of methanol and distilled water (9:1 v/v) and boiling chips were added to the previous samples, and the whole mixture was refluxed for 4 h. The resulting mixture was diluted with 100 mL of methanol/water (8:2, v/v), and PAHs were again extracted with cyclohexane. This extract was concentrated to a smaller volume in a rotary evaporator at 40 °C, dried over anhydrous sodium sulfate, and concentrated to 1 mL in a rotary evaporator first and then under a nitrogen stream. This second extract was injected directly into the gas chromatograph. When the duplicates of the samples needing alkaline treatment were performed, this step was included after the extraction of PAHs with cyclohexane.

The identification and quantification of PAHs was carried out with a Hewlett-Packard gas chromatograph model HP 6890 series, equipped with a mass selective detector 5973 and a Hewlett-Packard Vectra XM series 4 computer. The column used was a fused-silica capillary column (60 m long × 0.25 mm i.d. × 0.25 μm film thickness), 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 (0.50 min hold), increased to 130 °C 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 into 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 in SIM mode is based on the measurement of the peak area corresponding 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 previously mentioned and was spiked with the same

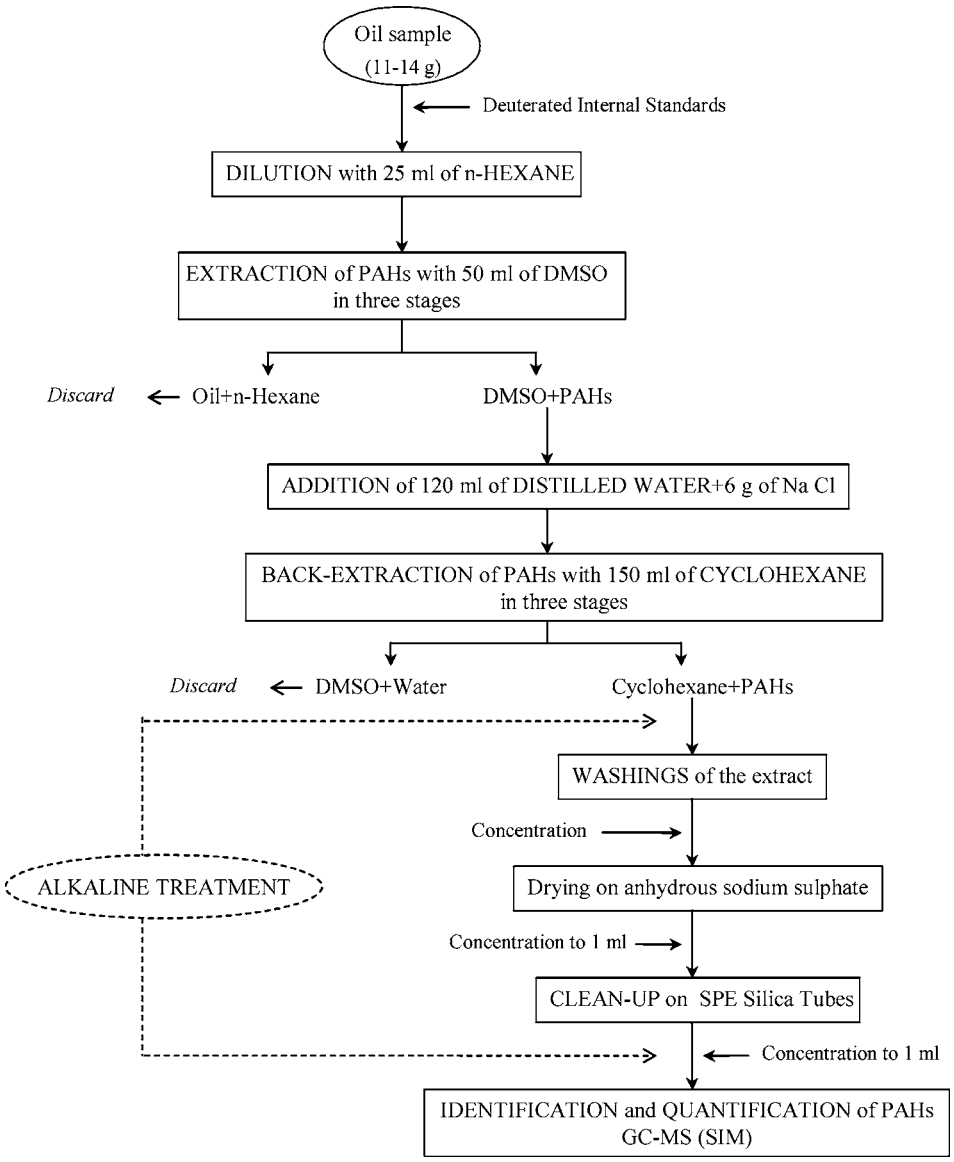

Figure 1. Scheme of the methodology employed for the study of the olive pomace oil samples.

internal standard solution as the one used to spike the samples. 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; and, finally, 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. The response of the detector in SIM mode to different concentrations of analytes is linear within 4 magnitude orders (~0.01−100 ng), with a correlation coefficient >0.99.

RESULTS AND DISCUSSION

In relation to the methodology, it is worthwhile mentioning that every procedure for the determination of any contaminant in low concentrations, as is usually the case of PAHs, should aim to obtain extracts as free as possible from compounds which can hinder the identification and quantification of the analytes of interest. Therefore, given that obtaining of clean extracts is often a difficult task in the study of complex matrices such as foodstuffs, the incorporation of additional interfering compounds at any stage of the process should be avoided or minimized. As has already been mentioned, it is necessary to emphasize the importance of the purity of the solvents employed for the extraction of PAHs because, given the large volumes often employed in these methodologies and the concentration degree in the final extracts, they can represent a significant source of interference. Nor must it be forgotten that many organic solvents are derived from coal tar or petroleum, materials very rich in PAHs, so the potential presence of PAHs should also be considered (*25*). However, as far as we know, there are very few studies dealing with the determination of PAHs in commercial solvents (*26*), and very little work has been done in the past decades. To verify if the solvents used for the determination of PAHs in oils contained PAHs, it was decided to study two commercial brands of hexane (H1 and H2) and two of cyclohexane (CH1 and CH2). Moreover, in the case of cyclohexane, samples from two bottles of the same brand were also taken (designated A and B), to determine if there were differences from one to another. The results obtained, expressed as micrograms per liter, are presented in **Table 1**. It can be observed that there are great differences in the PAH contents of the solvents studied, not only between solvents but also between brands of the same solvent, even though these differences are more noticeable in the case of hexane. Thus,

Table 1. PAHs Identified in Different Commercial Brands of *n*-Hexane (H1 and H2) and Cyclohexane (CH1 and CH2), and in Different Bottles of the Same Brand of Cyclohexane (A and B), and Their Concentrations, in Micrograms per Liter, Expressed as Mean Value ± Standard Deviation

PAH	H1	H2	CH1-A	CH1-B	CH2-A	CH2-B
naphthalene	0.27 ± 0.02	3.33 ± 0.06	0.09 ± 0.01	0.04 ± 0.01	0.25 ± 0.01	0.10 ± 0.01
2-methylnaphthalene	–[a]	5.09 ± 0.05	0.01 ± 0.00	0.01 ± 0.00	0.05 ± 0.02	0.02 ± 0.01
1-methylnaphthalene	0.05 ± 0.01	4.17 ± 0.07	0.01 ± 0.00	0.01 ± 0.00	–	0.02 ± 0.01
2,6-dimethylnaphthalene	–	11.16 ± 0.09	<0.01	<0.01	–	–
1,7-dimethylnaphthalene	–	13.44 ± 0.82	0.01 ± 0.00	<0.01	–	0.01[b]
1,6-dimethylnaphthalene	–	11.49 ± 0.14	0.01 ± 0.00	<0.01	–	0.01[b]
1,5-dimethylnaphthalene	–	2.85 ± 0.02	–	–	–	–
fluorene	–	1.34 ± 0.01	–	–	–	0.02 ± 0.01
phenanthrene	–	2.92 ± 0.04	0.01 ± 0.00	<0.01	0.03 ± 0.00	0.02 ± 0.01
3-methylphenanthrene	–	3.17 ± 0.04	–	–	–	–
2-methylphenanthrene	–	4.26 ± 0.04	–	–	–	–
9-methylphenanthrene	–	4.14 ± 0.02	–	–	–	–
1-methylphenanthrene	–	2.30 ± 0.00	–	–	–	–
dimethylphenanthrene or isomer **1**	–	1.38 ± 0.06	–	–	–	–
dimethylphenanthrene or isomer **2**	–	1.58 ± 0.00	–	–	–	–
dimethylphenanthrene or isomer **3**	–	1.20 ± 0.06	–	–	–	–
dimethylphenanthrene or isomer **5**	–	6.13 ± 0.04	–	–	–	–
dimethylphenanthrene or isomer **6**	–	2.77 ± 0.00	–	–	–	–
dimethylphenanthrene or isomer **7**	–	1.61 ± 0.04	–	–	–	–
dimethylphenanthrene or isomer **8**	–	0.84 ± 0.02	–	–	–	–
dimethylphenanthrene or isomer **9**	–	1.10 ± 0.02	–	–	–	–
dimethylphenanthrene or isomer **10**	–	0.33 ± 0.01	–	–	–	–
fluoranthene	–	–	<0.01	–	–	0.01 ± 0.01
pyrene	–	0.20 ± 0.02	<0.01	<0.01	–	0.01 ± 0.01
1-MFt[c] + 11*H*-benzo[*a*]fluorene	–	0.19 ± 0.00	–	–	–	–
methylfluoranthene or isomer **4**	–	0.14 ± 0.00	–	–	–	–
methylfluoranthene or isomer **5**	–	0.27 ± 0.01	–	–	–	–
1-methylpyrene	–	0.14 ± 0.00	–	–	–	–
o-terphenyl	–	–	0.01 ± 0.00	–	0.10 ± 0.01	0.01[b]
m-terphenyl	–	–	–	<0.01	–	–
p-terphenyl	–	–	0.02 ± 0.00	<0.01	–	0.02[b]
methylchrysene or isomer **3**	–	0.19 ± 0.01	–		–	–
2-methylchrysene	–	0.08 ± 0.00	–		–	–

[a] Not identified. [b] Identified in only one of the aliquots. [c] 1-Methylfluoranthene.

whereas in sample H1 only 2 PAHs have been identified, in H2, 29 PAHs have been found, in concentrations ranging from 0.08 μg/L of 2-methylchrysene to 13.44 μg/L of 1,7-dimethylnaphthalene. In contrast, the analysis of different bottles from the same brand reveals that there are very small differences in their PAH contents. These findings are in agreement with those of Lijinsky and Raha (*26*), who studied different commercial solvents, including hexane, and found that all of the solvents examined contained PAHs. They also observed that the concentrations of these compounds varied widely, even among solvents of the same grade from the same manufacturer, but the highest number and concentrations of PAHs were found in one sample of hexane, too. Nevertheless, unlike the results of these authors, which revealed the presence of high molecular weight PAHs in the solvents studied and, in some cases, carcinogenic ones, such as benz[*a*]anthracene or benzo[*a*]pyrene, all of the PAHs identified in the solvents here studied are compounds with two, thre, and four aromatic rings and, predominantly, with two and three rings. Moreover, PAHs larger than fluoranthene and pyrene have been found only in sample H2. It must be pointed out that no carcinogenic PAHs are present. Recently, Mottier et al. (*27*) have also pointed out the presence of traces of some of these compounds in the solvents used for the determination of PAHs in barbecued meat sausages. The highest contaminations were observed for naphthalene (1.5 μg/kg), phenanthrene (0.4 μg/kg), pyrene (0.5 μg/kg), and fluoranthene (0.2 μg/kg).

To obtain an idea about the presence of impurities in the solvents employed, samples of the two different brands of cyclohexane (CH1 and CH2) were also analyzed in scan mode. **Figure 2** shows the total ion chromatograms of samples CH1-A (**Figure 2a**) and CH2-A (**Figure 2b**). The first one corresponds to 45 mL of solvent and the second to 25 mL, concentrated to 1 mL in both cases. It can be observed that, although the volume of sample CH2-A is approximately half that of CH1-A, the amount of compounds in the former is far higher than in sample CH1-A. Moreover, in cyclohexane CH2, many of these compounds are linear hydrocarbons (LH), some of which interfere with the determination of some PAHs of interest, and they can be difficult to remove when their concentrations are high.

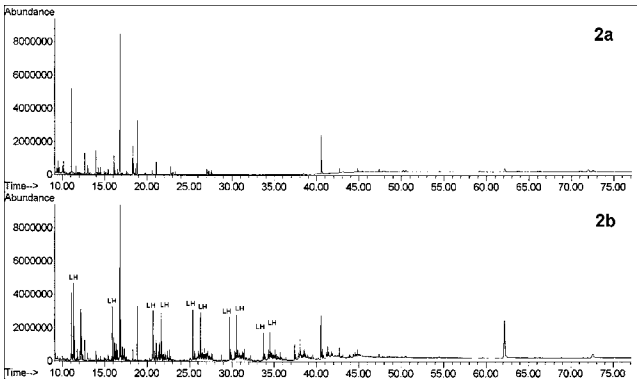

Figure 2. Total ion chromatograms corresponding to 45 mL of cyclohexane CH1-A concentrated to 1 mL (**a**) and to 25 mL of cyclohexane CH2, also concentrated to 1 mL (**b**). LH: Linear hydrocarbons.

It is clear from the above results that not only the purity of the solvents must be checked but also their PAH content, because the presence of variable concentrations of these compounds can lead to mistaken data, especially in the case of low PAH content samples. Therefore, it is recommended that commercial solvents be analyzed before use, to verify both their

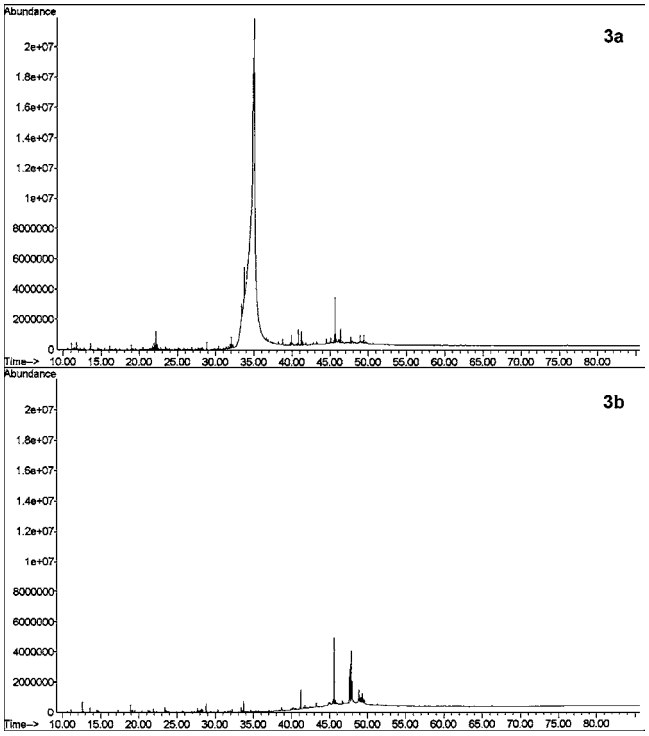

Figure 3. Total ion chromatograms of two extracts of sample OP4, one before the alkaline treatment (**a**) and the other, after (**b**).

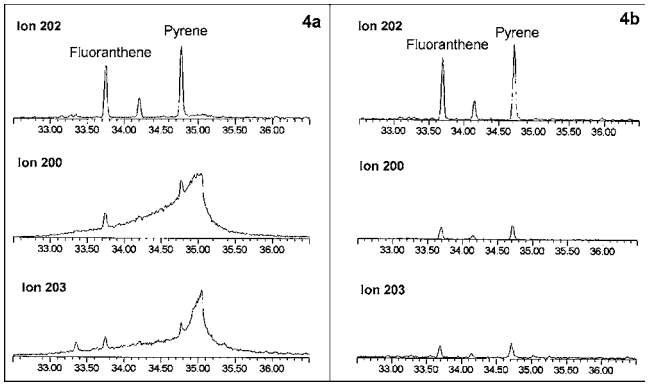

Figure 4. Chromatograms of the ions selected for the identification of fluoranthene and pyrene (202, 200, and 203), one before the alkaline treatment (**a**) and the other, after (**b**).

purity and the presence of PAHs, either to remove them or to be taken into account in quantification.

Apart from the compounds that can come from the solvents, there are others present in the samples in this study, such as fatty acids, which not only interfere in the determination of some PAHs by GC-MS but also are detrimental to the equipment. For these reasons, when acids are present in the oil extracts, an alkaline treatment is necessary to remove them. To illustrate the effect of this treatment, **Figure 3** shows two chromatograms of sample OP4, one before the alkaline treatment (**Figure 3a**) and the other afterward (**Figure 3b**). It can be observed that the large peak in **Figure 3a**, corresponding to a mixture of 9-octadecenoic acid (oleic acid) and hexadecanoic acid (palmitic acid), is removed from the extract after the alkaline treatment, as can be seen in **Figure 3b**. **Figure 4** shows the chromatograms of the three ions selected for the identification of fluoranthene and pyrene (202, 200, and 203), before (**Figure 4a**) and after (**Figure 4b**) the alkaline treatment. It is observed that the interfering ions with mass-to-charge ratios of 200 and 203, which come from the mixture of 9-octadecenoic and hexadecanoic acids previously mentioned and elute at the same time as fluoranthene and pyrene, disappear with the treatment, allowing a correct identification of these PAHs.

The recoveries of the deuterated internal standards added to the samples at the beginning of the process were >80% except for naphthalene-d_8 and acenaphthene-d_{10}, which were lower (51.94 and 61.13%, respectively). It must be pointed out that the recovery of naphthalene-d_8 is lower in the samples subjected to the alkaline treatment (38.11%). The detection limits of the heaviest PAHs in the oil samples are approximately 0.06 μg/kg for indeno[1,2,3-*cd*]pyrene and its isomers, 0.10 μg/kg for benzo[*a*]pyrene and its isomers, and, finally, 0.25 μg/kg for dibenzopyrenes.

The PAHs identified in the samples studied and their concentrations, in micrograms per kilogram, expressed as mean values ± standard deviation, are given in **Table 2**. The asterisked compounds have been identified by comparison with reference standards. This table also shows the total PAH concentrations, as well as the concentrations corresponding to parent PAHs and alkyl derivatives separately. It can be seen that a very high number of PAHs (71), with a wide range of molecular weights and aromatic ring numbers, have been identified; thus, compounds of both low molecular weight (naphthalene, acenaphthylene, and acenaphthene) and high molecular weight (coronene and dibenzopyrenes) can be observed. It must be pointed out that more than a half of the PAHs identified (38) are alkyl derivatives, of both light (naphthalene and phenanthrene) and heavy PAHs (chrysene, benzopyrene, or isomer). It is also worth pointing out that, in this work, a high number of PAHs have been identified which had not been studied by other authors earlier, such as the alkyl derivatives of benz[*a*]anthracene and chrysene or the dibenzopyrenes, which, paradoxically, include some of the compounds with the highest carcinogenic activities (*28*, *29*). Only Hopia et al. (*3*) included 1,2,3,4-dibenzopyrene (dibenzo[*a*,*l*]pyrene) in their study on vegetable oils. As far as we know, this is the first time that such an exhaustive study of the presence of PAHs in edible oils has been carried out. Although there are many studies dealing with the determination of PAHs in this type of food, most of them make reference to a limited number of these compounds, which usually varies between 6 and 16, all of them unalkylated (*1*, *4*, *6*−*8*, *16*, *17*). Moreover, there are studies in which benzo[*a*]pyrene is the only PAH determined (*30*−*32*). However, it must also be said that there are a few papers in which the number of PAHs is higher, between 20 and 38 (*2*, *3*, *5*); some of these latter, besides, include some alkylated PAHs (*3*, *5*), although these compounds are not taken into account by most of the authors. Despite this, the present study shows the great number and high concentrations of alkylated PAHs in olive pomace oil. Therefore, in view of this finding, it seems clear that the determination of these substituted PAHs cannot be overlooked.

As can be observed in Table 2, the highest number and concentrations of PAHs are found in samples OP1−OP4, which obviously have not been adequately purified during their manufacturing process. It must be noted that although the numbers of compounds identified in these samples are very similar (60−69), the total PAH concentrations vary widely from 831.75 μg/kg in sample OP4 to 3199.79 μg/kg in OP1. It is also observed that the total concentrations of alkyl derivatives in samples OP1, OP2, and OP4 are higher than those of parent PAHs, especially in the latter. With regard to individual PAH concentrations, sample OP4 has the highest concentrations of naphthalene and its alkyl derivatives; sample OP2 has the highest concentrations of phenanthrene, anthracene, fluoranthene, pyrene,

Table 2. PAHs Identifed in Olive Pomace Oil Samples, Together with the Major Ions Selected for Their Identification in SIM Mode (in Parentheses), Their Number of Rings (*n*), and Their Concentrations, in Micrograms per Kilogram, Expressed as Mean Value ± Standard Deviation (Ions in Bold Correspond to the Molecular Ions)

PAH	*n*	OP1	OP2	OP3	OP4	OP5
naphthalene* (**128**, 126, 102)	2	11.42 ± 1.77	4.95 ± 1.06	13.45 ± 1.01	24.88 ± 4.86	15.23 ± 0.51
methylnaphthalenes (**142**, 141, 115)	2	11.74	2.75	12.93	39.76	27.16
2-methylnaphthalene*		4.19 ± 1.06	1.58 ± 0.07	7.76 ± 0.58	22.05 ± 4.38	14.71 ± 0.95
1-methylnaphthalene*		7.55 ± 1.82	1.17 ± 0.04	5.17 ± 0.15	17.71 ± 3.71	12.45 ± 0.69
dimethylnaphthalenes (**156**, 141, 155)	2	12.56	4.78	23.00	175.49	137.21
2,6-dimethylnaphthalene*		2.71 ± 0.10	1.59 ± 0.23	7.25 ± 1.03	40.41 ± 13.42	31.20 ± 2.64
1,7-dimethylnaphthalene*		2.90 ± 0.68	1.51 ± 0.11	7.34 ± 0.33	47.21 ± 11.32	36.63 ± 3.57
1,6-dimethylnaphthalene*		6.37 ± 0.34	1.68 ± 0.07	6.71 ± 0.35	43.41 ± 15.53	32.95 ± 2.72
1,4- + 2,3-dimethylnaphthalene*		–[a]	–	–	22.25 ± 10.08	18.10 ± 1.64
1,5-dimethylnaphthalene*		0.58 ± 0.21	–	1.70 ± 0.01	10.23 ± 2.99	8.33 ± 0.76
dimethyl-/ethylnaphthalene		–	–	–	11.98 ± 4.05	10.00 ± 0.83
acenaphthylene***** (**152**, 151, 153)	3	1.08 ± 0.22	1.56 ± 0.04	4.41 ± 0.18	0.87 ± 0.13	0.43 ± 0.01
acenaphthene* (**154**, 153, 152)	3	0.45 ± 0.09	0.50 ± 0.02	1.10 ± 0.13	–	1.09 ± 0.01
fluorene* (**166**, 165, 163)	3	12.18 ± 0.54	1.76 ± 0.14	2.08 ± 0.13	3.27 ± 0.13	2.88 ± 0.08
phenanthrene***** (**178**, 176, 179)	3	46.92 ± 0.10	83.53 ± 1.97	35.49 ± 0.64	14.22 ± 0.22	8.25 ± 0.13
anthracene* (**178**, 176, 179)	3	6.32 ± 0.55	11.19 ± 0.69	3.95 ± 0.25	–	–
methylphenanthrenes/-anthracenes (**192**, 191, 189)	3	90.47	149.14	51.91	35.54	25.08
3-methylphenanthrene		28.39 ± 2.55	48.02 ± 0.66	16.23 ± 1.04	9.32 ± 0.51	6.17 ± 0.30
2-methylphenanthrene		15.77 ± 1.01	25.09 ± 0.80	9.78 ± 0.18	9.56 ± 0.71	7.16 ± 0.28
2-methylanthracene*		8.08 ± 1.32	12.16 ± 1.52	3.42 ± 0.13	–	–
9-methylphenanthrene		14.66 ± 1.19	27.88 ± 1.27	7.95 ± 0.33	8.86 ± 0.63	7.24 ± 0.35
1-methylphenanthrene*		23.57 ± 1.46	35.99 ± 0.50	14.53 ± 1.15	7.80 ± 0.46	4.51 ± 0.00
dimethylphenanthrenes/-anthracenes (**206**, 191, 205)	3	130.84	168.06	89.67	46.06	29.66
dimethylphenanthrene or isomer **1**		12.29 ± 0.19	17.29 ± 0.32	7.64 ± 0.24	4.17 ± 0.33	2.69 ± 0.06
dimethylphenanthrene or isomer **2**		16.68 ± 0.11	25.49 ± 0.69	10.60 ± 0.32	5.11 ± 0.59	2.81 ± 0.04
dimethylphenanthrene or isomer **3**		3.00 ± 0.03	4.33 ± 0.01	2.76 ± 0.08	2.70 ± 0.24	1.79 ± 0.01
dimethylphenanthrene or isomer **5**		14.94 ± 0.28	22.52 ± 1.46	12.92 ± 0.64	14.06 ± 0.81	11.68 ± 0.90
dimethylphenanthrene and/or isomer **6**		63.60 ± 1.30	98.43 ± 5.06	45.19 ± 0.58	14.38 ± 2.23	7.84 ± 0.06
dimethylphenanthrene or isomer **7**		13.23 ± 0.77	–	10.56 ± 0.25	5.64 ± 0.75	2.85 ± 0.01
dimethylphenanthrene or isomer **10**		7.10 ± 0.21	–	–	–	–
o-terphenyl* (**230**, 229, 215)	3	3.53 ± 0.92	1.99[b]	1.24 ± 0.29	2.54 ± 0.29	0.39 ± 0.09
fluoranthene* (**202**, 200, 203)	4	110.48 ± 7.52	154.24 ± 0.83	91.89 ± 2.26	13.86 ± 0.72	3.71 ± 0.17
pyrene* (**202**, 200, 203)	4	141.47 ± 18.76	211.76 ± 14.36	133.24 ± 4.45	17.41 ± 0.28	5.21 ± 0.12
methylfluoranthenes/-pyrenes (**216**, 215, 213)	4	149.97	212.20	81.13	17.32	5.65
2-methylfluoranthene*		11.49 ± 1.24	19.37 ± 1.34	6.30 ± 0.26	1.15 ± 0.23	0.31 ± 0.12
methylfluoranthene or isomer **1**		16.51 ± 0.04	52.12 ± 6.73	8.84 ± 0.06	1.74 ± 0.01	0.30 ± 0.01
methylfluoranthene or isomer **2**		31.84 ± 2.13	41.07 ± 5.76	19.81 ± 0.66	3.52 ± 0.27	0.81 ± 0.02
methylfluoranthene or isomer **3**		2.78 ± 0.38	3.33 ± 0.31	–	–	–
methylfluoranthene or isomer **4**		25.35 ± 1.56	27.39 ± 1.00	13.42 ± 0.28	3.26 ± 0.04	1.03 ± 0.02
methylfluoranthene or isomer **5**		30.70 ± 2.41	34.25 ± 2.67	16.29 ± 0.23	3.80 ± 0.06	1.87 ± 0.20
1-methylpyrene*		31.30 ± 2.28	34.67 ± 0.88	16.47 ± 0.30	3.85 ± 0.02	1.33 ± 0.11
1-MFt* + 11*H*-B[*a*]Fl*[c] (**216**, 215, 213)	4	31.07 ± 0.48	30.49 ± 4.63	18.73 ± 0.04	4.00 ± 0.43	1.61 ± 0.02
11*H*-benzo[*b*]fluorene* (**216**, 215, 213)	4	7.84 ± 0.53	13.48 ± 0.27	5.91 ± 0.64	1.15 ± 0.15	–
11*H*-benzo[*c*]fluorene* (**216**, 215, 213)	4	4.58 ± 0.33	–	3.28 ± 0.06	–	–
m-terphenyl* (**230**, 231, 228)	4	6.88 ± 0.10	–	5.62 ± 0.06	1.34 ± 0.12	0.61 ± 0.02
p-terphenyl* (**230**, 231, 228)	4	6.17 ± 0.18	8.41 ± 0.25	4.35 ± 0.07	1.02 ± 0.00	–
benz[*a*]anthracene*[d] (**228**, 226, 229)	4	123.67 ± 14.51	82.32 ± 7.52	62.19 ± 5.41	16.33 ± 1.01	1.22 ± 0.06
chrysene*[d] + triphenylene* (**228**, 226, 229)	4	339.88 ± 1.42	190.32 ± 1.34	169.35 ± 7.76	59.38 ± 2.63	4.67 ± 0.14
methylbenz[*a*]anthracenes/-chrysenes (**242**, 241, 239)	4	596.30	288.71	320.14	118.83	6.89
methylbenz[*a*]anthracene or isomer **1**		7.29 ± 0.43	–	–	–	–
methylbenz[*a*]anthracene or isomer **2**		43.00 ± 0.27	21.20 ± 1.99	23.19 ± 0.96	8.75 ± 0.33	–
methylbenz[*a*]anthracene or isomer **3**		26.91 ± 1.57	19.16 ± 6.31	27.25 ± 6.37	7.14 ± 0.06	–
3-methylchrysene* and/or isomer		320.60 ± 2.03	157.20 ± 33.71	172.67 ± 15.40	66.70 ± 0.43	5.74 ± 0.20
2-methylchrysene*		68.65 ± 1.44	31.14 ± 0.76	34.40 ± 1.80	13.78 ± 0.33	–
4-* or 6*[d]-methylchrysene		16.99 ± 0.63	8.07 ± 0.63	7.17 ± 0.35	2.94 ± 0.07	–
1-methylchrysene*		112.86 ± 3.00	51.94 ± 4.00	55.46 ± 5.22	19.52 ± 0.72	1.15 ± 0.01
dimethylbenz[a]anthracenes/-chrysenes (**256**, 241, 239)	4	693.50	331.90	306.15	78.31	–
dimethylbenz[*a*]anthracene or isomer **1**		23.62 ± 3.97	12.41 ± 0.09	–	–	–
dimethylbenz[a]anthracene or isomer **2**		60.51 ± 10.46	–	27.54 ± 0.13	–	–
dimethylbenz[a]anthracene and/or isomer **3**		609.37 ± 100.35	319.49 ± 5.92	278.61 ± 23.75	78.31 ± 2.45	–
benzofluoranthenes (**252**, 250, 126)	5	237.03	124.00	107.32	39.15	1.41
benzo[*b*]fluoranthene*[d]		108.63 ± 4.47	61.79 ± 1.73	50.64 ± 1.92	16.47 ± 0.60	0.66 ± 0.04
benzo[*j*+*k*]fluoranthenes*[d]		88.05 ± 5.30	40.54 ± 1.07	41.15 ± 0.91	18.36 ± 1.28	0.75 ± 0.02
benzo[*a*]fluoranthene		40.35 ± 1.64	21.67 ± 0.05	15.53 ± 0.91	4.32 ± 0.06	–
benzopyrenes (**252**, 250, 126)	5	182.65	111.80	105.59	36.49	1.64
benzo[*e*]pyrene*[d]		89.94 ± 0.82	57.64 ± 4.07	53.33 ± 0.93	19.74 ± 0.05	1.29 ± 0.04
benzo[*a*]pyrene***[d]**		92.71 ± 3.38	54.16 ± 2.90	52.26 ± 0.91	16.75 ± 1.07	0.35 ± 0.03
methylbenzopyrene or isomer (**266**, 265, 267)	5	11.66 ± 1.53	4.79 ± 0.22	–	–	–
perylene* (**252**, 250, 126)	5	20.72 ± 0.45	–	13.46 ± 0.29	3.89 ± 0.80	–
276[e] (**276**, 277, 274)		27.63 ± 1.81	12.84 ± 0.19	17.16 ± 0.78	6.39 ± 0.22	–
indeno[1,2,3-*cd*]pyrene*[d] (**276**, 277, 274)	5	42.81 ± 1.78	17.17 ± 1.17	30.55 ± 1.97	14.60 ± 1.04	–
dibenz[*a*,*h**[d] or *a*,*c**[d]]anthracene (**278**, 276, 279)	5	9.50 ± 0.35	3.45 ± 0.01	4.53 ± 0.35	2.19 ± 0.25	–

Table 2. (Continued)

PAH	*n*	OP1	OP2	OP3	OP4	OP5
benzo[*b*]chrysene* (**278**, 276, 279)	5	9.40 ± 0.49	3.08 ± 0.56	3.79 ± 0.04	2.03 ± 0.08	–
picene* (**278**, 276 279)	5	32.55 ± 2.06	12.65 ± 0.97	15.38 ± 0.51	7.47 ± 0.05	–
benzo[*ghi*]perylene* (**276**, 277, 274)	6	49.71 ± 0.29	21.95 ± 0.74	42.89 ± 5.42	18.78 ± 0.06	0.35 ± 0.03
anthanthrene*[d] (**276**, 277, 274)	6	11.16 ± 0.62	5.07 ± 0.81	8.49 ± 0.34	4.12 ± 0.22	–
coronene* (**300**, 301, 150)	7	12.89 ± 0.71	3.06 ± 1.43	12.60 ± 0.98	17.80 ± 2.26	–
dibenzopyrenes or isomers (**302**, 303, 300)	6	12.76	2.58	12.76	7.26	–
dibenzopyrene or isomer **1**		6.44 ± 1.90	1.73 ± 0.55	6.62 ± 1.17	2.59 ± 0.06	–
dibenzo[*a*,*e*]pyrene*[d]		2.45 ± 0.66	–	2.50 ± 0.04	1.84 ± 0.05	–
dibenzopyrene or isomer **2**		3.87 ± 1.48	0.85 ± 0.46	3.64 ± 0.13	2.83 ± 0.07	–
total		3,199.79	2,276.48	1,815.73	831.75	280.35
parent PAHs		1,502.75	1,114.15	930.80	320.44	231.65
alkyl derivatives		1,697.04	1,162.33	884.93	511.31	48.70

[a] Not identified. [b] Identified in only one of the aliquots. [c] 1-Methylfluoranthene + 11*H*-benzo[*a*]fluorene. [d] Compound with a certain degree of carcinogenicity, according to refs *28*, *29*, *41*, and *42*. [e] Compound with molecular weight equal to 276.

and their alkyl derivatives, and, from benz[*a*]anthracene onward, in general, the highest individual concentrations are found in sample OP1. If PAH concentrations in **Table 2** are observed carefully, a series of similarities can be found among samples OP1−OP4, where the highest concentrations correspond in all cases to PAHs with four and five rings. It can be seen that the sum of monomethyl derivatives of fluoranthene and pyrene is practically equal to the concentration of pyrene, except for sample OP3, and that the concentration of 3-methylchrysene and/or isomer is very similar to that of chrysene plus triphenylene. Among the concentrations of PAHs with five or more aromatic rings, there are also certain relationships that could be considered almost constant, such as the ratio between benzofluoranthenes and benzopyrenes, close to unity in all cases (OP1, 1.30; OP2, 1.11; OP3, 1.02; OP4, 1.07), or between benzo[*ghi*]perylene and indeno[1,2,3-*cd*]pyrene, which ranges from 1.16 in sample OP1 to 1.40 in OP3. Moreover, if only alkyl derivatives are considered, a certain pattern can also be observed in their concentrations. It must be noted that their individual concentrations, in general, are lower than those of their parent PAHs, with some exceptions, such as some dimethylnaphthalenes in sample OP4, 3-methylchrysene and/or isomer in samples OP3 and OP4, or dimethylphenanthrene and/or isomer **6** and dimethylbenz[*a*]anthracene and/or isomer **3** in all cases. Nevertheless, the sum of all of the alkyl derivative concentrations is, in general, higher than that of their parent PAHs. On the other hand, if total mono and dimethyl derivatives are considered separately, some differences can be observed between both groups of substituted PAHs; whereas the total concentrations of dimethyl derivatives are, in general, higher than those of parent PAHs in all of the samples, those of monomethyl derivatives vary depending on the parent PAH and on the sample considered. Finally, if monomethyl derivatives are compared to dimethyl derivatives, the latter are, in general, in higher proportions than the former, except for the derivatives of benz[*a*]anthracene or chrysene in samples OP3 and OP4. In view of all of these findings, it could be concluded that samples OP1−OP4 exhibit a characteristic PAH pattern, which reveals that the PAH contamination could come from the same source. Nevertheless, the PAH distribution observed in these olive pomace oil samples differs from the PAH profile found in other types of PAH contaminated matrices, such as liquid smoke flavorings (*24*) or smoked cheeses (*33*), where PAH concentrations, in general, decrease with the molecular weight of the compounds and the highest proportions correspond to naphthalene and its alkyl derivatives; however, in samples OP1−OP4, these PAHs are in low proportions (0.55−28.87%). The differences observed in samples OP1−OP4 in relation to other types of PAH-contaminated matrices could be attributed to a selective reduction of the lightest PAHs at the deodorization stage during the refining process, which has already been pointed out by several authors (*3*, *5*, *8*, *34*).

The high PAH concentrations found in samples OP1−OP4 can be due to the manufacture of olive pomace oil, which requires the elimination of the high water content of the olive pomace pulp (65−70%) before the extraction of the oil. It seems that high temperatures are needed during this process, so, taking into account that organic matter subjected to high temperatures gives rise to PAHs, large amounts of these contaminants can be found in the final product if they are not removed. The formation of PAHs during the heating of oils has been shown by the results of Chen and Chen (*35*). The solvents employed for the extraction of oils have also been suggested as another possible additional source of PAHs (*5*). However, on the basis of findings of several researchers (*25*, *34*), solvents used for extraction are not significant sources of PAH contamination in oils.

In relation to sample OP5, it can be said that both the number (43) and especially the total concentration of PAHs (280.35 μg/kg) are significantly lower than in samples OP1−OP4, revealing that, in this case, a successful cleanup process seems to have been carried out. Unlike that observed in samples OP1−OP4, in sample OP5 the total of alkylated PAHs is much lower than the sum of parent PAHs. It is also observed that not only has the total PAH concentration gone down but also the distribution of PAH concentrations has changed in relation to samples OP1−OP4. In sample OP5, the concentrations of naphthalene and its alkyl derivatives, which are the most abundant compounds (64.06%), are of a similar order or even higher than those of OP1−OP4. However, from phenanthrene onward PAH concentrations in sample OP5 decrease significantly in relation to the rest of the samples, to reach very low or undetectable levels in the case of the heaviest compounds. Therefore, from the comparison of samples OP1−OP4 and OP5, it appears that a correct cleanup of olive pomace oil can drastically reduce the concentrations of PAHs. The treatment with active carbon during the bleaching step seems to be the best method to remove the heaviest PAHs (*5*, *36*, *37*). Therefore, the use of active carbon in combination with deodorization which, as mentioned earlier, has been reported to have an effect on the lightest PAHs (*3*, *5*, *8*, *34*), can lead to oils with low PAH contents. A high reduction of PAH concentrations by treating the oil with active carbon has already been observed in coconut oil (*5*, *38*).

If the concentrations of those PAHs included in the Spanish order mentioned earlier (*19*), which have been identified in sample OP5 (benz[*a*]anthracene, benzo[*e*]pyrene, benzo[*a*]-

Table 3. Carcinogenicity and Other Parameters Related to the Carcinogenicity of PAHs

PAH	C1[a]	C2[b]	C3[c]	Iball[d]	RP[e]	PAH	C1[a]	C2[b]	C3[c]	Iball[d]	RP[e]
naphthalene	ND[f]	–	–	ND	ND	triphenylene	3	–	–	00	ND
fluorene	3	ND	ND	ND	ND	naphthacene	ND	–	ND	ND	ND
phenanthrene	3	–	–	00	ND	benzo[*b*]fluoranthene	2B	ND	ND	ND	0.141
1-methylphenanthrene	3	ND	ND	ND	ND	benzo[*j*]fluoranthene	2B	ND	ND	ND	0.061
1,4-dimethylphenanthrene	3	ND	ND	ND	ND	benzo[*k*]fluoranthene	2B	ND	ND	ND	0.066
anthracene	3	–	–	ND	ND	benzo[*e*]pyrene	3	–	+	02	0.004
5,10-dimethylanthracene	ND	ND	+	ND	ND	benzo[*a*]pyrene	2A	++++	++++	72	1.0
9,10-dimethylanthracene	ND	+	ND	ND	ND	1-methylbenzo[*a*]pyrene	ND	++++	ND	ND	ND
fluoranthene	3	ND	ND	ND	ND	2-methylbenzo[*a*]pyrene	ND	++++	ND	ND	ND
pyrene	3	–	ND	ND	0.081	3-methylbenzo[*a*]pyrene	ND	++++	ND	ND	ND
1-methylpyrene	ND	–	ND	ND	ND	4-methylbenzo[*a*]pyrene	ND	++++	ND	ND	ND
2-methylpyrene	ND	–	ND	ND	ND	5-methylbenzo[*a*]pyrene	ND	+++	ND	ND	ND
4-methylpyrene	ND	–	ND	ND	ND	6-methylbenzo[*a*]pyrene	ND	+++	ND	ND	ND
benzo[*a*]fluorene	3	ND	ND	ND	ND	7-methylbenzo[*a*]pyrene	ND	+++	ND	ND	ND
benzo[*b*]fluorene	3	ND	ND	ND	ND	10-methylbenzo[*a*]pyrene	ND	+	ND	ND	ND
benzo[*c*]fluorene	3	ND	ND	ND	ND	11-methylbenzo[*a*]pyrene	ND	++++	ND	ND	ND
cyclopenta[cd]pyrene	3	–	ND	ND	0.023	12-methylbenzo[*a*]pyrene	ND	++++	ND	ND	ND
benzo[*c*]phenanthrene	ND	+	ND	ND	ND	1,2-dimethylbenzo[*a*]pyrene	ND	++++	ND	ND	ND
1-methylbenzo[*c*]phenanthrene	ND	+	ND	ND	ND	1,3-dimethylbenzo[*a*]pyrene	ND	++++	ND	ND	ND
2-methylbenzo[*c*]phenanthrene	ND	+	ND	ND	ND	1,4-dimethylbenzo[*a*]pyrene	ND	++++	ND	ND	ND
3-methylbenzo[*c*]phenanthrene	ND	++	ND	ND	ND	1,6-dimethylbenzo[*a*]pyrene	ND	+++	ND	ND	ND
4-methylbenzo[*c*]phenanthrene	ND	++	ND	ND	ND	2,3-dimethylbenzo[*a*]pyrene	ND	++++	ND	ND	ND
5-methylbenzo[*c*]phenanthrene	ND	+++	ND	ND	ND	3,6-dimethylbenzo[*a*]pyrene	ND	+++	ND	ND	ND
6-methylbenzo[*c*]phenanthrene	ND	++	ND	ND	ND	3,12-dimethylbenzo[*a*]pyrene	ND	++++	ND	ND	ND
benz[*a*]anthracene	2A	±	±	07	0.145	4,5-dimethylbenzo[*a*]pyrene	ND	++++	ND	ND	ND
1-methylbenz[*a*]anthracene	ND	–	–	ND	ND	7,10-dimethylbenzo[*a*]pyrene	ND	–	ND	ND	ND
2-methylbenz[*a*]anthracene	ND	–	–	ND	ND	6-ethylbenzo[*a*]pyrene	ND	–	ND	ND	ND
3-methylbenz[*a*]anthracene	ND	–	–	ND	ND	perylene	3	–	–	ND	ND
4-methylbenz[*a*]anthracene	ND	ND	–	ND	ND	3-methylcholanthrene	ND	++++	ND	ND	ND
5-methylbenz[*a*]anthracene	ND	–	–	ND	ND	1,3-dimethylcholanthrene	ND	++	ND	ND	ND
6-methylbenz[*a*]anthracene	ND	++	++	ND	ND	2,3-dimethylcholanthrene	ND	++	ND	ND	ND
7-methylbenz[*a*]anthracene	ND	+++	+++	ND	ND	dibenz[*a*,*c*]anthracene	3	+	+	03	ND
8-methylbenz[*a*]anthracene	ND	–	++	ND	ND	dibenz[*a*,*h*]anthracene	2A	+++	++	26	1.11
9-methylbenz[*a*]anthracene	ND	–	+	ND	ND	dibenz[*a*,*j*]anthracene	3	+	ND	04	ND
10-methylbenz[*a*]anthracene	ND	ND	±	ND	ND	indeno[1,2,3-*cd*]pyrene	2B	ND	ND	ND	0.232
11-methylbenz[*a*]anthracene	ND	–	–	ND	ND	benzo[*b*]chrysene	ND	ND	–	ND	ND
12-methylbenz[*a*]anthracene	ND	++	++	ND	ND	7,12-dimethylbenzo[*b*]chrysene	ND	ND	–	ND	ND
5,12-dimethylbenz[*a*]anthracene	ND	ND	–	ND	ND	picene	ND	–	–	ND	ND
6,8-dimethylbenz[*a*]anthracene	ND	+++	++++	ND	ND	benzo[*ghi*]perylene	3	–	ND	ND	0.022
6,12-dimethylbenz[*a*]anthracene	ND	++++	++++	ND	ND	anthanthrene	3	±	ND	ND	0.320
7,12-dimethylbenz[*a*]anthracene	ND	+++++	++++	ND	ND	6-methylanthanthrene	ND	++	ND	ND	ND
8,12-dimethylbenz[*a*]anthracene	ND	ND	++++	ND	ND	6,12-dimethylanthanthrene	ND	++	ND	ND	ND
9,10-dimethylbenz[*a*]anthracene	ND	ND	++	ND	ND	coronene	3	–	ND	ND	ND
6,8,12-trimethylbenz[*a*]anthracene	ND	++++	ND	ND	ND	dibenzo[*a*,*e*]fluoranthene	3	ND	ND	ND	ND
7-ethylbenz[*a*]anthracene	ND	+	ND	ND	ND	dibenzo[*a*,*l*]pyrene	2B	++++	ND	33	ND
chrysene	3	±	+	05	0.0044	dibenzo[*a*,*e*]pyrene	2B	+++	+++	50	ND
1-methylchrysene	3	ND	–	ND	ND	dibenzo[*a*,*i*]pyrene	2B	++++	++++	74	ND
2-methylchrysene	3	ND	–	ND	ND	dibenzo[*a*,*h*]pyrene	2B	++++	++++	68	ND
3-methylchrysene	3	ND	–	ND	ND	dibenzo[*e*,*l*]pyrene	ND	–	ND	ND	ND
4-methylchrysene	3	ND	–	ND	ND						
5-methylchrysene	2B	+++	++++	ND	ND						
6-methylchrysene	3	±	–	ND	ND						
2,3-dimethylchrysene	ND	ND	–	ND	ND						
5,6-dimethylchrysene	ND	ND	++(?)	ND	ND						
5,12-dimethylchrysene	ND	ND	–	ND	ND						

[a] Carcinogenicity according to the classification of the IARC (*36*, *37*): 1, carcinogenic to humans; 2A, probably carcinogenic to humans; 2B, possibly carcinogenic to humans; 3, unclassifiable as to its carcinogenicity to humans; 4, probably not carcinogenic to humans. [b] Data from Cavalieri et al. (*28*): extremely active (+++++); very active (++++); active (+++); moderately active (++); weakly active (+); very weakly active (±); inactive (–). [c] Data from Loew et al. (*29*). [d] Iball index, from Braga et al. (*43*). [e] RP: relative carcinogenic potencies, adapted from Krewski et al. (*44*). [f] ND: no data.

pyrene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, and benzo[*ghi*]perylene), are compared with the limits established by this regulation, it is observed that none of them exceed the individual maximum limit of 2 μg/kg. Moreover, the total concentration of these PAHs (4.62 μg/kg) is also lower than the maximum of 5 μg/kg allowed for this sum.

To compare the concentrations of PAHs found in the olive pomace samples studied with the findings of other authors, we will take into account only the total of parent PAHs, because most of the studies do not include alkylated compounds, even though some of them should be taken into account due to their carcinogenicity, such as some methyl derivatives of benz[*a*]anthracene and chrysene (*29*). However, it must be pointed out that it is difficult to compare these results, because both the number of PAHs studied in each case and the techniques employed for their determination are different. If the PAH concentrations here obtained are compared with those in olive pomace oils from other authors (*9*, *18*), it can be observed that the total parent PAH concentrations of samples OP1−OP3 are in the range of the amount found by Barranco et al. (*9*) in olive pomace oil with a high PAH contamination level (1158.60 μg/kg). It must be noted that these authors also found that the highest concentrations corresponded to PAHs with four aromatic rings. Nevertheless, the total parent PAH concentrations in samples OP4 and OP5 (320.44 and 231.65 μg/kg, respectively), although much lower than those of samples OP1−OP3, are

considerably higher than that found by Barranco et al. (*9*) in the least contaminated samples of olive pomace oil (near 15.6 μg/kg) and the results of Weisshaar (*18*) (89.6 μg/kg, average value). The total parent PAH concentrations of the samples of this study, especially of OP1−OP3, are comparable to those found in other types of vegetable oils highly contaminated by PAHs, such as some samples of peanut oil (*2*) or grapeseed oil (*39*), and crude coconut oil (*5*, *15*, *40*).

To evaluate the possible risk derived from the consumption of any foodstuff contaminated with PAHs, not only their concentrations must be taken into account, but also the nature and, in consequence, the biological activity and carcinogenicity of the compounds involved in such contamination. In this sense, it must be pointed out that some of the PAHs identified in the samples of this study are considered to be carcinogenic according to data from differences sources (*28*, *29*, *41*−*43*), such as benz[*a*]anthracene, chrysene, benzo[*b*,*j*,*k*]fluoranthenes, benzo[*a*]pyrene, indeno[1,2,3-*cd*]pyrene, or dibenzo[*a*,*e*]pyrene. **Table 3** shows the carcinogenicity of a high number of PAHs, both unsubstituted and substituted, taken from different references and expressed in different units. It can be observed in this table that, among all of the PAHs included (104), 68 are alkylated compounds, of which 47 present a certain degree of carcinogenic activity. It is also worth pointing that there can be great differences among isomers and that sometimes the methyl derivatives of weakly carcinogenic PAHs may have strong carcinogenic properties depending on the position of the substitution, as in the case of benz[*a*]anthracene. It must be emphasized that, even though alkylated PAHs are always excluded from most of the studies on PAHs, the data in **Table 3** reveal that many of these compounds are considered to be very active in relation to their carcinogenic activity, especially those derived from PAHs with four and five rings. It must be noted that none of the alkyl derivatives of benz[*a*]anthracene or chrysene identified in the samples of this study coincide with the carcinogenic 6-methylbenz[*a*]anthracene, 7-methylbenz[*a*]-anthracene, 5-methylchrysene, or 7,12-dimethylbenz[*a*]anthracene, but the lack of reference compounds for the rest of the isomers does not allow us to ensure that they are, or are not, the other carcinogenic ones. In the same way, none of the dibenzopyrenes or isomers tentatively identified, apart from dibenzo[*a*,*e*]pyrene, coincide with dibenzo[*a*,*l*]pyrene, dibenzo-[*a*,*i*]pyrene, or dibenzo[*a*,*h*]pyrene, which are also very carcinogenic. Finally, it must not be forgotten that there are some PAHs, such as fluoranthene, pyrene, or benzo[*ghi*]perylene, which, despite not being carcinogenic per se, contribute to the incidence of some types of tumors produced by other carcinogens such as benzo[*a*]pyrene when administered together (*42*).

In the evaluation of the presence of PAHs suspected to be carcinogenic in any type of foodstuff, several aspects must be taken into account. First, despite the numerous studies on PAH carcinogenicity (*45*), it is difficult to know the exact effect of PAHs on humans because these studies are carried out with experimental animals and, so, it is difficult to extrapolate the results to humans. Second, the mechanism of carcinogenesis induction by PAHs is very complex and, given that these toxicants need a metabolic activation to act as carcinogens (*45*), all of the factors which can exert an effect on the metabolic reactions suffered by PAHs in the organism, either external or endogenous, will determine both the formation of the carcinogenic metabolites and their final effect. Therefore, the presence of carcinogenic PAHs does not imply the development of cancer.

As a summary, it can be said that this study shows the presence of a very high number of PAHs in very high concentrations in most of the olive pomace oil samples studied. Special attention should be given to the high number and concentrations of alkylated compounds, and the presence of carcinogenic PAHs such as dibenzopyrenes, which are not usually considered. It must also be pointed out that the highest PAH concentrations in samples OP1−OP4 correspond to the sum of benz[*a*]anthracene, chrysene plus triphenylene, and their alkyl derivatives, among which some unidentified compounds are observed, which could be carcinogenic.

The findings from this study reveal the great influence that the technological processes involved in the manufacturing of some foods, such as olive pomace oil, can have on the PAH contamination of the final product. The high concentrations of PAHs found in samples OP1−OP4 and the lower ones in OP5 show the importance of a purification step in the production of this type of oil, which can give rise to olive pomace oils with a low level of PAHs if it is carried out properly.

Although several carcinogenic PAHs have been identified in the samples of this study and this fact cannot be overlooked, it must also be noted that the carcinogenicity mechanism of PAHs is very complex and there are many factors which can determine their final effect. Among the factors that can have an influence on the metabolic activation of PAHs in the organism some dietary components such as antioxidants can be cited (*45*). Some natural antioxidants, although not in so high concentrations as in virgin olive oils, are present in olive pomace oil in higher levels than in other edible vegetable oils (*14*). Therefore, the absorption of these compounds together with PAHs may inhibit to a certain extent the oxidation reactions necessary for the metabolic activation of these toxicants.

Further studies on PAH toxicity are necessary but, meanwhile, caution must prevail and attempts must be made to minimize the exposure of humans to PAHs. It is necessary to focus the attention on those PAHs suspected to be more carcinogenic and, of course, alkylated PAHs must be considered in any study on PAHs. Consequently, any regulation on the level of PAHs in edible oils should include all of the carcinogenic PAHs, unalkylated or alkylated. Moreover, laws to limit the presence of PAHs in any type of foodstuff able to be contaminated should be implemented.

LITERATURE CITED

(1) Howard, J. W.; Turicchi, E. W.; White, R. H.; Fazio, T. Extraction and estimation of polycyclic aromatic hydrocarbons in vegetable oils. *J. Assoc. Off. Anal. Chem.* **1966**, *49*, 1236−1244.

(2) Kolarovic, L.; Traitler, H. Determination of polycyclic aromatic hydrocarbons in vegetable oils by caffeine complexation and glass capillary gas chromatography. *J. Chromatogr.* **1982**, *237*, 263−272.

(3) Hopia, A.; Pyysalo, H.; Wickström, K. Margarines, butter and vegetable oils as sources of polycyclic aromatic hydrocarbons. *J. Assoc. Oil Chem. Soc.* **1986**, *63*, 889−893.

(4) Welling, P.; Kaandorp, B. Determination of polycyclic aromatic hydrocarbons (PAH) in edible vegetable oils by liquid chromatography and programmed fluorescence detection. *Z. Lebensm. Unters. Forsch.* **1986**, *183*, 111−115.

(5) Larsson, B. K.; Eriksson, A. T.; Cervenka, M. Polycyclic aromatic hydrocarbons in crude and deodorized vegetable oils. *J. Assoc. Off. Anal. Chem.* **1987**, *64*, 365−370.

(6) Speer, K.; Steeg, E.; Horstman, P.; Kühn, Th.; Montag, A. Determination and distribution of polycyclic aromatic hydrocarbons in native vegetable oils, smoked fish products, mussels and oysters, and bream from the river Elbe. *J. High Resolut. Chromatogr.* **1990**, *13*, 104−111.

(7) Gertz, A.; Kogelheide, H. Untersuchung und Beurteilung von PAK in Speisefetten und -ölen. *Fat Sci. Technol.* **1994**, *96*, 175−180.
(8) Moret, S.; Piani, B.; Bortolomeazzi, R.; Conte, L. S. HPLC determination of polycyclic aromatic hydrocarbons in olive oils. *Z. Lebensm. Unters. Forsch. A* **1997**, *205*, 116−120.
(9) Barranco, A.; Alonso-Salces, R. M.; Bakkali, A.; Berrueta, L. A.; Gallo, B.; Vicente, F.; Sarobe, M. Solid-phase clean-up in the liquid chromatographic determination of polycyclic aromatic hydrocarbons in edible oils. *J. Chromatogr. A* **2003**, *988*, 33−40.
(10) Moret, S.; Conte, L. S. Polycyclic aromatic hydrocarbons in edible fats and oils: occurrence and analytical methods. *J. Chromatogr. A* **2000**, *882*, 245−253.
(11) Kris-Etherton, P. M.; Pearson, T. A.; Wan, Y.; Hargrove, R. L.; Moriarty, K.; Fishell, V.; Etherton, T. D. High-monounsaturated fatty acid diets lower both plasma cholesterol and triacylglycerol concentrations. *Am. J. Clin. Nutr.* **1999**, *70*, 1009−1015.
(12) Guillén, M. D.; Ruiz, A. Rapid simultaneous determination by proton NMR of unsaturation and composition of acyl groups in vegetable oils. *Eur. J. Lipid Sci. Technol.* **2004**, in press.
(13) Ibanez, E.; Palacios, J.; Senorans, F. J.; Santa-Maria, G.; Tabera, J.; Reglero, G. Isolation and separation of tocopherols from olive by-products with supercritical fluids. *J. Am. Oil Chem. Soc.* **2000**, *77*, 187−190.
(14) Garcia, A.; Brenes, M.; Garcia, P.; Romero, C.; Garrido, A. Phenolic content of commercial olive oils. *Eur. Food Res. Technol.* **2003**, *216*, 520−525.
(15) Swetman, T.; Head, S.; Evans, D. Contamination of coconut oil by PAH. *Int. News Fats, Oils Relat. Mater.* **1999**, *10*, 706−712.
(16) Menichini, E.; Bocca, A.; Merli, F.; Ianni, D.; Monfredini, F. Polycyclic aromatic hydrocarbons in olive oils on the Italian market. *Food Addit. Contam.* **1991**, *8*, 363−369.
(17) Van Stijn, F.; Kerkhoff, M. A. T.; Vandeginste, B. G. M. Determination of polycyclic aromatic hydrocarbons in edible oils and fats by on-line donor−acceptor complex chromatography and high-performance liquid chromatography with fluorescence detection. *J. Chromatogr. A* **1996**, *750*, 263−273.
(18) Weisshaar, R. Rapid determination of heavy polycyclic aromatic hydrocarbons in edible fats and oils. *Eur. J. Lipid Sci. Technol.* **2002**, *104*, 282−285.
(19) Orden de 25 de Julio de 2001, por la que se establecen límites de determinados hidrocarburos aromáticos policíclicos en aceite de orujo de oliva. *Bol. Of. Estado* (*B.O.E.*) **2001**, *178* (July 26).
(20) Ordinanza 18 Settembre 2001. Tenori massimi tollerabili di idrocarburi policiclici aromatici nell'olio di sansa di oliva e nell'olio di sansa di oliva raffinato. *Gazz. Uff.* **2001**, *225* (Sept 27).
(21) Canadian Food Inspectory Agency, Industry Advisory, Olive Pomace Oil, Sept 17, 2001.
(22) Natusch, D. F. S.; Tomkins, B. A. Isolation of polycyclic organic compounds by solvent extraction with dimethyl sulfoxide. *Anal. Chem.* **1978**, *50*, 1429−1434.
(23) García Falcón, M. S.; González Amigo, S.; Lage Yusty, M. A.; López de Alda Villaizán, M. J.; Simal Lozano, J. Determination of benzo[*a*]pyrene in lipid-soluble liquid smoke. *Food Addit. Contam.* **1996**, *13*, 863−870.
(24) Guillén, M. D.; Sopelana, P.; Partearroyo, M. A. Polycyclic aromatic hydrocarbons in liquid smoke flavorings obtained from different types of wood. Effect of storage in polyethylene flasks on their concentrations. *J. Agric. Food Chem.* **2000**, *48*, 5083−5087.
(25) Howard, J. H.; Fazio, T.; White, R. H. Polycyclic aromatic hydrocarbons in solvents used in extraction of edible oils. *J. Agric. Food Chem.* **1968**, *16*, 72−75.
(26) Lijinsky, W.; Raha, C. R. Polycyclic aromatic hydrocarbons in commercial solvents. *Toxicol. Appl. Pharmacol.* **1961**, *3*, 469−473.
(27) Mottier, P.; Parisòd, V.; Turesky, R. J. Quantitative determination of polycyclic aromatic hydrocarbons in barbecued meat sausages by gas chromatography coupled to mass spectrometry. *J. Agric. Food Chem.* **2000**, *48*, 1160−1166.
(28) Cavalieri, E. L.; Rogan, E. G.; Roth, R. W.; Saugier, R. K.; Hakam, A. The relationship between ionisation potential and horseradish peroxidase/hydrogen peroxide-catalyzed bindings of aromatic hydrocarbons to DNA. *Chem.-Biol. Interact.* **1983**, *47*, 87−109.
(29) Loew, G.; Poulsen, M.; Kirkjian, E.; Ferrell, J.; Sudhindra, B. S.; Rebagliati, M. Computer-assisted mechanistic structure−activity studies: application to diverse classes of chemical carcinogens. *Environ. Health Perspect.* **1985**, *61*, 69−96.
(30) Pupin, A. M.; Toledo, M. C. F. Benzo[*a*]pyrene in olive oils on the Brazilian market. *Food Chem.* **1996**, *55*, 185−188.
(31) Pupin, A. M.; Toledo, M. C. F. Benzo[*a*]pyrene in Brazilian vegetable oils. *Food Addit. Contam.* **1996**, *13*, 639−646.
(32) Vazquez Troche, S.; García Falcón, M. S.; González Amigo, S.; Lage Yusty, M. A.; Simal Lozano, J. Enrichment of benzo-[*a*]pyrene in vegetable oils and determination by HPLC-FL. *Talanta* **2000**, *51*, 1069−1076.
(33) Guillén, M. D.; Sopelana, P. Occurrence of polycyclic aromatic hydrocarbons in smoked cheeses. *J. Dairy Sci.* **2004**, in press.
(34) Cejpek, K.; Hajslová, J.; Kocourek, V.; Tomaniová, M.; Cmolík, J. Changes in PAH levels during production of rapeseed oil. *Food Addit. Contam.* **1998**, *15*, 563−574.
(35) Chen, B. H.; Chen, Y. C. Formation of polycyclic aromatic hydrocarbons in the smoke from heated model lipids and food lipids. *J. Agric. Food Chem.* **2001**, *49*, 5238−5243.
(36) Biernoth, G.; Rost, H. E. Occurrence of polycyclic aromatic hydrocarbons in edible oils and their removal. *Arch. Hyg. Bakteriol.* **1968**, *152*, 238−250.
(37) León Camacho, M.; Viera Alcaide, I.; Ruiz Méndez, M. V. Elimination of polycyclic aromatic hydrocarbons by bleaching of olive pomace oil. *Eur. J. Lipid Sci. Technol.* **2003**, *105*, 9−16.
(38) Biernoth, G.; Rost, H. E. The occurrence of polycyclic aromatic hydrocarbons in coconut oil and their removal. *Chem. Ind.* **1967**, *47*, 2002−2003.
(39) Balenovic, J.; Petrovic, I.; Perkovac, M. Determination of polycyclic aromatic hydrocarbons in vegetable oil. *Proceedings of Euro Food Chem VIII*, Vienna, Austria, Sept 18−20, Austrian Chemical Society: Vienna, Austria, 1995; Vol. 2, pp 275−281.
(40) Grimmer, G.; Hildebrandt, A. Hydrocarbons in the human environment. VI. Levels of polycyclic hydrocarbons in crude vegetable oils. *Arch. Hyg. Bakteriol.* **1968**, *152*, 255−259.
(41) IARC. Certain polycyclic aromatic hydrocarbons and heterocyclic compounds. In *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*; IARC: Lyon, France, 1973; Vol. 3.
(42) IARC. Polynuclear aromatic compounds, part 1: chemical, environmental and experimental data. In *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*; IARC: Lyon, France, 1983; Vol. 32.
(43) Braga, R. S.; Barone, P. M. V. B.; Galvao, D. S. Identifying carcinogenic activity of methylated polycyclic aromatic hydrocarbons (PAHs). *J. Mol. Struct. (THEOCHEM)* **1999**, *464*, 257−266.
(44) Krewski, D.; Thorslund, T.; Withey, J. Carcinogenic risk assessment of complex mixtures. *Toxicol. Ind. Health* **1989**, *5*, 851−867.
(45) Guillén, M. D.; Sopelana, P. Polycyclic aromatic hydrocarbons in diverse foods. In *Food Safety: Contaminants and Toxins*; D'Mello, J. P. F., Ed.; CABI Publishing: Oxon, U.K., 2003; pp 175−198.

Received for review October 28, 2003. Revised manuscript received January 28, 2004. Accepted February 6, 2004. We gratefully acknowledge the financial support of the MCYT (AGL2003-01838) and of the University of the Basque Country (9/UPV 00101.125.13667/2001). G.P. thanks the UPV for a predoctoral grant.

JF035259Q