Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Chemistry 86 (2004) 465–474
Food Chemistry
www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods

Comparison of donor–acceptor and alumina columns for the clean-up of polycyclic aromatic hydrocarbons from edible oils

Alejandro Barranco [a], Rosa M. Alonso-Salces [a], E. Corta [a], Luis A. Berrueta [a,*], Blanca Gallo [a], Francisca Vicente [a], Martin Sarobe [b]

[a] *Department of Analytical Chemistry, Faculty of Sciences, University of the Basque Country, P.O. Box 644, E-48080 Bilbao, Spain*
[b] *Unilever Foods España, S.A., P.O. Box 791, 48080 Bilbao, Spain*

Received 1 June 2003; received in revised form 3 October 2003; accepted 3 October 2003

Abstract

Two methods for clean-up and sample enrichment for the analysis of polycyclic aromatic hydrocarbons (PAHs) in edible oils are compared; a clean-up based on a donor–acceptor complex chromatography (DACC) column and a standardized method widely used in the food industry consisting in a low pressure column chromatography with alumina as stationary phase. Both methods are followed by a reversed-phase high-performance liquid chromatography with fluorescence detection for the separation and quantitation of each PAH. Certified materials were used in order to validate the methods. The limits of detection were lower than 1 ng/g and good selectivity was achieved in both cases. The DACC column clean-up is faster and better accuracies were obtained. The advantages and disadvantages of both methods are discussed.

Keywords: Polycyclic aromatic hydrocarbons; HPLC; Edible oils; Lipids clean-up; Off-line LC-LC; DACC

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are hazardous organic compounds that are ubiquitous and persistence environmental contaminants. They are of great health concern and are included in the European Union and in the Environmental Protection Agency (EPA) priority pollutant list due to their mutagenic and carcinogenic properties (IARC, 1983; USEPA, 1984). PAHs are mainly released from the incomplete combustion of organic matter and geochemical processes. Their presence in different environmental samples such as soil, sediments and water has been reported by numerous research papers (Bakker, Casado, Koerselman, Tolls, & Kollöfel, 2000; Manoli & Samara, 1999; Neilson, 1998; Yang, 2000). However, human exposure to PAHs is not only confined to occupational exposure. Several studies point out that food is the major contributor to this exposure, so their presence in food is a matter for concern and it requires continuous monitoring (Lodovici, Dolara, Casalini, Ciapellano, & Testolin, 1995; Phillips, 1999).

There are different sources of PAHs in food. The most significant are atmospheric exposure and some processes in the food industry, as smoking and thermal treatments of varying severity used in preparation and manufacture. Although the concentrations in edible vegetable oils are in the range of few micrograms per kilogram, they are an important source of dietary PAHs, because of the great consumption of these products (Guillén, Sopelana, Cid, & Partearroyo, 1996). Several organisations have proposed maximum values for PAHs in edible vegetable oils. For instance, the German Society for Fat Science proposed a value of 5 μg/kg as the limit value for total heavy PAHs (more than four benzene rings fused in their structure) and a value of 25 μg/kg for the sum of both, light and heavy PAHs (Speer, Steeg, Horstmann, Kühn, & Montag, 1990). Recently, Spain has set a maximum level in olive residue oil of 2 μg/kg for each compound of the next list and the

* Corresponding author. Tel.: +34-94-601-5505; fax: +34-94-464-8500.
E-mail address: qapbesil@lg.ehu.es (L.A. Berrueta).

0308-8146/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2003.10.030

sum of them cannot be above 5 μg/kg (benzo[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, benzo[e]pyrene, dibenzo[a,h]anthracene, benzo[g,h,i]perylene and indeno[1,2,3-c,d]pyrene) (Spanish Official Bulletin, 2001).

Analysis of PAHs in vegetable oils is problematic, owing to their lipophilic nature and the low levels in which they are present. To overcome these problems, several methods have been proposed. They generally consisted of a saponification or/and solvent extraction procedure, followed by a clean-up with low pressure column chromatography (with sorbents such as alumina, silica, Sephadex LH-20) and finally, a gas or liquid high performance chromatography in order to separate and quantify (Cert, Moreda, & Pérez-Camino, 2000; Lee, Novotny, & Bartle, 1981; Moret & Conte, 2000; Pupin & Toledo, 1996). These methods are time and solvent consuming and less suitable for routine analysis.

The isolation of a PAH fraction by HPLC has been introduced improving clean-up procedures. Untreated silica has been successfully used as stationary phase, because of its capacity to retain fat and other interferents; allowing the polyaromatic fraction to elute in a few millilitres of the appropriate mobile phase (an apolar solvent as hexane or pentane) (Moret & Conte, 1998). Recently, more specific sorbents have been introduced, based on a more selective mechanism due to the interaction of the sorbent with the π electrons of PAHs; which has been called donor–acceptor complex chromatography (DACC) (Brouwer, Hermans, Lingeman, & Brinkman, 1994; Funk, Frank, Oesch, & Platt, 1994; Stijn, Kerkhoff, & Vandeginste, 1996). PAHs are retained on the basis of a strong π–π interaction when a non-π-electron containing solvent is used as a mobile phase, eluting other components of the oil such as neutral lipids and tocopherols. Then, the PAH fraction is eluted with a proper solvent that neutralized the π–π interaction.

In this work, a deep study of DACC has been done and the optimum conditions found were applied to a great variety of edible vegetable oils. This method was exhaustively validated using certified reference materials, and the results were compared with a standardized clean-up method widely used in the food industry (Hendrikse & Dieffenbacher, 1991), consisting in a glass column with alumina. The advantages and disadvantages of both methods have been evaluated.

2. Materials and methods

2.1. Apparatus

The first HPLC unit consisted of two LKB (Barcelona, Spain) 5140 pumps, a high-pressure mixer LKB 2152-400, a HPLC controller LKB 2152, a Rheodyne (Cotati, CA, USA) 7125 sample injector with a fixed loop of 200 μl and a Perkin–Elmer (Norwalk, Connecticut, USA) 785A UV–Vis detector. A DACC column Chrompack (Middelburg, The Netherlands) ChromSpher 5π (80 × 3 mm i.d., 5 μm) column was used and the effluent was monitored at 280 nm. The system operated at room temperature and the flow rate was 1 ml/min. Solvents that constituted the mobile phase were A (hexane) and B (THF). The elution conditions applied were: 0–4.5 min, 95%A isocratic; 4.5–7 min, linear gradient 95%A–0%A; 7–13 min, 0%A isocratic; and finally, back to the initial conditions and recondition the column. The flow rate was 1 ml/min and the injection volume was 200 μl.

The collected fractions from clean-up columns (DACC or alumina) were evaporated to dryness using a Zymark (Hopkinton, MA, USA) Turbo Vap LV evaporator provided with nitrogen blowdown and a water bath at 28 °C.

The second HPLC system was used for the determination and quantitation of each PAH. The chromatographic system consisted on a Hewlett–Packard (Palo Alto, CA) Series 1100 system, equipped with a vacuum degasser, quaternary pump, autosampler and fluorescence detector connected to an HP Chemstation software. This system allows getting fluorescence spectra along the chromatogram and comparing spectra from different chromatograms, obtaining a match factor. Excitation and emission wavelengths were programmed as reported in Table 1. A reversed phase Vydac (Hesperia, CA) C18 (250 × 4.6 mm i.d., 5 μm) column and a Waters Nova-Pak C18 (20 × 3.9 mm i.d., 4 μm) guard column were used. A Perkin–Elmer oven LC 101 was used to maintain the temperature of the column constant at 35 °C. Solvents that constituted the mobile phase were A (acetonitrile) and B (water). The elution conditions applied were: 0–10 min, 50%A isocratic; 10–24 min, linear gradient 50%A–100%A; 24–35 min, 100%A isocratic; and finally, back to the initial conditions and recondition the column. The flow rate was 1 ml/min and the injection volume was 30 μl.

2.2. Reagents

Acetonitrile, tetrahydrofuran (THF), methanol, acetone, diethylether, dichloromethane, ethyl acetate, isopropanol (IPA) and hexane from Romil Chemical Ltd. (Heidelberg, Germany) were of HPLC grade. Water was purified on a Milli-Q system from Millipore (Bedford, MA). Nitrogen (>99.995%) was from Air Liquide (Madrid, Spain).

The standard PAHs mix from Supelco (Bellefonte, PA) consisted of a solution in acetonitrile-methanol (90:10, v/v) of naphthalene (Na) 500 μg/ml, acenaphthylene (Ap) 500 μg/ml, acenaphthene (Ac) 1000 μg/ml,

Table 1
Excitation and emission wavelength program for the RP-HPLC chromatograph

Time (min)	Excitation (nm)	Emission (nm)			PAH detected
		A	B	C	
0.0	275	330	390	500	Na, Ac, F
17.3	250	366	390	500	Phe
18.8	250	400	390	500	Ant
20.0	270	460	390	500[a]	Fl, Pyr, BaA, Chr
25.4	255[b]	410[c]	390	500	BbF, BkF, BaP
28.5	290[b]	410[d]	390	500	DbahA, BghiP, IP

[a] When the alumina clean-up is used, it is changed to 410 nm.
[b] When the alumina clean-up is used, it is changed to 300 nm.
[c] When the alumina clean-up is used, it is changed to 440 nm.
[d] When the alumina clean-up is used, it is changed to 420 nm.

fluorene (F) 100 μg/ml, phenanthrene (Phe) 40 μg/ml, anthracene (Ant) 20 μg/ml, fluoranthene (Fl) 50 μg/ml, pyrene (Pyr) 100 μg/ml, benzo[a]anthracene (BaA) 50 μg/ml, chrysene (Chr) 50 μg/ml, benzo[b]fluoranthene (BbF) 20 μg/ml, benzo[k]fluoranthene (BkF) 20 μg/ml, benzo[a]pyrene (BaP) 50 μg/ml, dibenzo[a,h]anthracene (DbahA) 200 μg/ml, benzo[g,h,i]perylene (BghiP) 80 μg/ml and indeno[1,2,3-c,d]pyrene (IP) 50 μg/ml. Stock solutions containing 0.25 μg/ml of BaP were prepared in hexane or acetonitrile and stored at 4 °C in darkness. These stock solutions were stable almost for three months. The different calibration solutions were prepared daily by appropriate dilutions of these stock solutions.

2.3. Samples

Samples of edible oils (crude and refined coconut oil, crude sunflower oil, palm oil, palm kernel oil, palm stearine, olive oil and residue olive oil) were commercially available.

In order to validate the method, two certified reference materials from BCR/IRMM (Geel, Belgium) were used. A highly refined coconut oil (CRM 459) which has been considered as a "blank" oil (certified values: <0.9 ng/g of Pyr, <0.6 ng/g of Chr, <0.2 ng/g of BkF, <0.3 ng/g of BaP, <0.2 ng/g of BghiP and <0.2 ng/g of IP) and a coconut oil artificially fortified with six selected PAHs in the lower nanogram per gram level (CRM 458; certified values: 9.4 ng/g of Pyr, 4.9 ng/g of Chr, 1.87 ng/g of BkF, 0.93 ng/g of BaP, 0.97 ng/g of BghiP and 1.00 ng/g of IP).

2.4. Clean-up with DACC column

Three-gram aliquots of edible oils were diluted until 5 ml with hexane, and 200 μl of this dilution was injected into the DACC column. The fraction ranged between 6 and 10 min was taken, evaporated at 28 °C under a nitrogen stream, reconstituted in 0.2 ml of acetonitrile, filtered through a Waters (Milford, MA) 0.45 μm GHP filter and 30 μl injected into the reversed-phase chromatograph.

2.5. Clean-up with alumina low-pressure column

A glass chromatographic column (300 × 15 mm) was half filled with *n*-hexane and 22 g of aluminum oxide, activity IV (addition of 10% of water to the aluminum oxide of activity I, shaked in a closed container and equilibrated for 24 h), was added rapidly. A layer of about 2 cm of anhydrous sodium sulphate was also added on top of the column and *n*-hexane was dropped out until it become level with the top of this layer.

About 1.5 ml of the diluted oil (2 g aliquots of edible oils were diluted until 10 ml with hexane) was transferred onto the column, and pure hexane was passed through it maintaining the flow rate at 1 ml/min approximately. The first 5 ml was discarded, and a fraction of 90 ml was collected in a 100-ml round bottom flask. This fraction was evaporated in a rotary evaporator to about 0.5–1 ml and transferred to a vial, washing the flask with a few milliliters of hexane. The evaporation was continued at 28 °C under a nitrogen stream to dryness. The residue was reconstituted in 0.2 ml of acetonitrile, filtered and 30 μl injected into the reversed-phase chromatograph.

3. Results and discussion

3.1. Optimisation of the clean-up with the DACC column

The retention times of PAHs in the DACC column were studied with different pure solvents as mobile phases at 1 ml/min. Standard solutions containing 0.25 μg/ml of benzo[a]pyrene were injected, and the order of elution strength of the different solvents were as follows: THF > dichloromethane ~ acetone > ethylacetate > acetonitrile ~ diethylether > methanol > IPA > hexane.

When IPA was used, a flow less than 1 ml/min was required, because it caused too much back-pressure in the system. With hexane as mobile-phase, the first four PAHs (naphthalene, acenaphthylene, acenaphthene and fluorene) eluted within 6 min and no more PAHs eluted until more than 15 min. On the other hand, with THF as mobile phase, all PAHs eluted within the less time interval of all solvents, only 2 min. Taking into account these results, hexane was chosen as the best solvent for applying the oil into the DACC column, because it is able to dissolve all oils assayed while most of PAHs were retained in the column. In order to elute PAHs, different mixtures of *n*-hexane and THF were evaluated using a crude coconut oil. Different fractions of 2.5 ml were collected, evaporated and the residue obtained was weighed in order to know if the fat coeluted with PAHs. In all cases, the four lighter PAHs (naphthalene, acenaphthylene, acenaphthene and fluorene) coeluted with fat and could not be analysed by this method. On the other hand, with more than 5% of THF, the other PAHs fraction went forward in the chromatogram and coeluted with the fatty components. For this reason, a 5% of THF was maintained until fatty components were eluted (4.5 min) and then, the percentage of THF was increased until 100%, in order to collect quickly the PAHs fraction with little dispersion.

In order to get a good sensitivity, different amounts of vegetable oils were assayed till a residue of fat was seen in the PAHs fraction collected. The greater amount of oil injected without fat coelution was 120 mg of oil (injection of 200 μl of a solution of 3 g of oil diluted until 5 ml with hexane) and all PAHs eluted from 6 to 10 min being the fat completely separated as Fig. 1 shows. These conditions were suitable for almost all the edible oils assayed, except for palm oil and palm stearine, which needed to be injected more diluted (0.75 g and 0.5 g diluted until 5 ml, respectively), in order to avoid oil precipitation into the column.

3.2. Optimisation of the clean-up with the alumina column

The standardized method (Hendrikse & Dieffenbacher, 1991), only proposed for benzo(a)pyrene, was adapted in order to be able to determine all 16 PAHs priorized by the EPA. First, a standard solution of PAHs was injected onto the column, and several fractions of 10 ml were collected using pure *n*-hexane as mobile phase. An aliquot of each fraction was taken, evaporated and reconstituted in 0.2 ml of acetonitrile, prior to its analysis by RP-HPLC.

Other mobile phases (*n*-hexane with different percentages of ethyl acetate) were also assayed. Fig. 2 shows the elution profiles of dibenzo[a,h]anthracene, which was the PAH more retained by alumina. However, when edible oils were injected onto the column, the more polar compositions of mobile phase lead to a worse separation between tryglycerides and PAHs and a residue insoluble in acetonitrile appeared after evaporation. Therefore, pure *n*-hexane was chosen. Under these operating conditions 300 mg of edible oil could be injected, and after passing through the column 90 ml of *n*-hexane, almost a complete recovery was achieved. Moreover, several washing solvents (THF, acetone and ethylacetate) were assayed in order to remove the lipids from the column, so the same column could be used several times. However, a loss in selectivity occurred and the results were not reproducible, being necessary a new packing of the column for each sample.

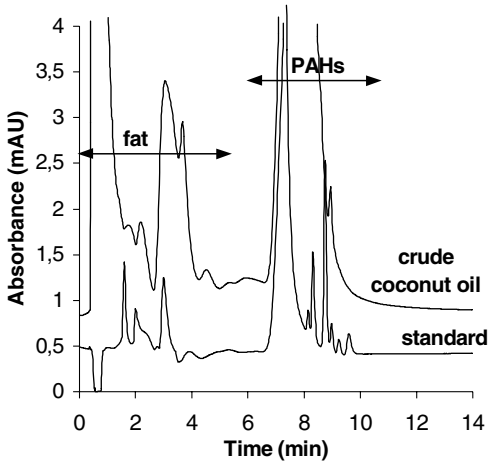

Fig. 1. Chromatograms obtained from the injection in the DACC column of 200 μl of a sample of crude coconut oil (3 g diluted with 5 ml of hexane) and a standard solution in hexane containing 0.25 μg/ml of BaP.

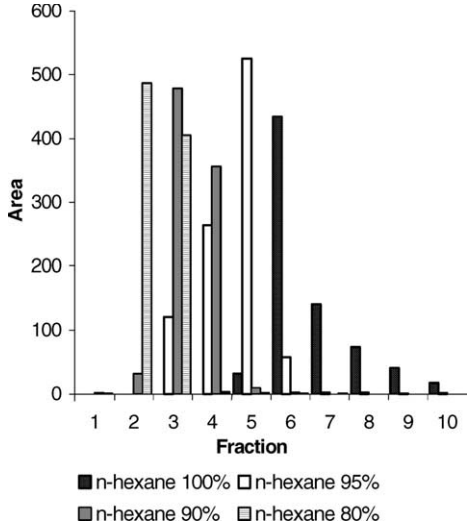

Fig. 2. Elution profiles of DbahA from the alumina low pressure column when 1.5 ml of a standard solution containing 0.25 μg/g of BaP in hexane was injected, using different mixtures of hexane and ethyl acetate as mobile phases.

3.3. Validation of methods

Both methods were validated following the Eurachem guide (Eurachem, 1998). The selectivity was assessed by evaluating the purity of PAHs chromatographic peaks, and comparing the excitation and emission spectra of each PAH peak in the chromatogram of different edible oils assayed with those obtained with standards solutions. In most cases, peak purity and the grade of match with the spectra of standards were greater than 95%. For instance, Fig. 3 shows the excitation spectra for the different PAHs found in a crude coconut oil cleaned up

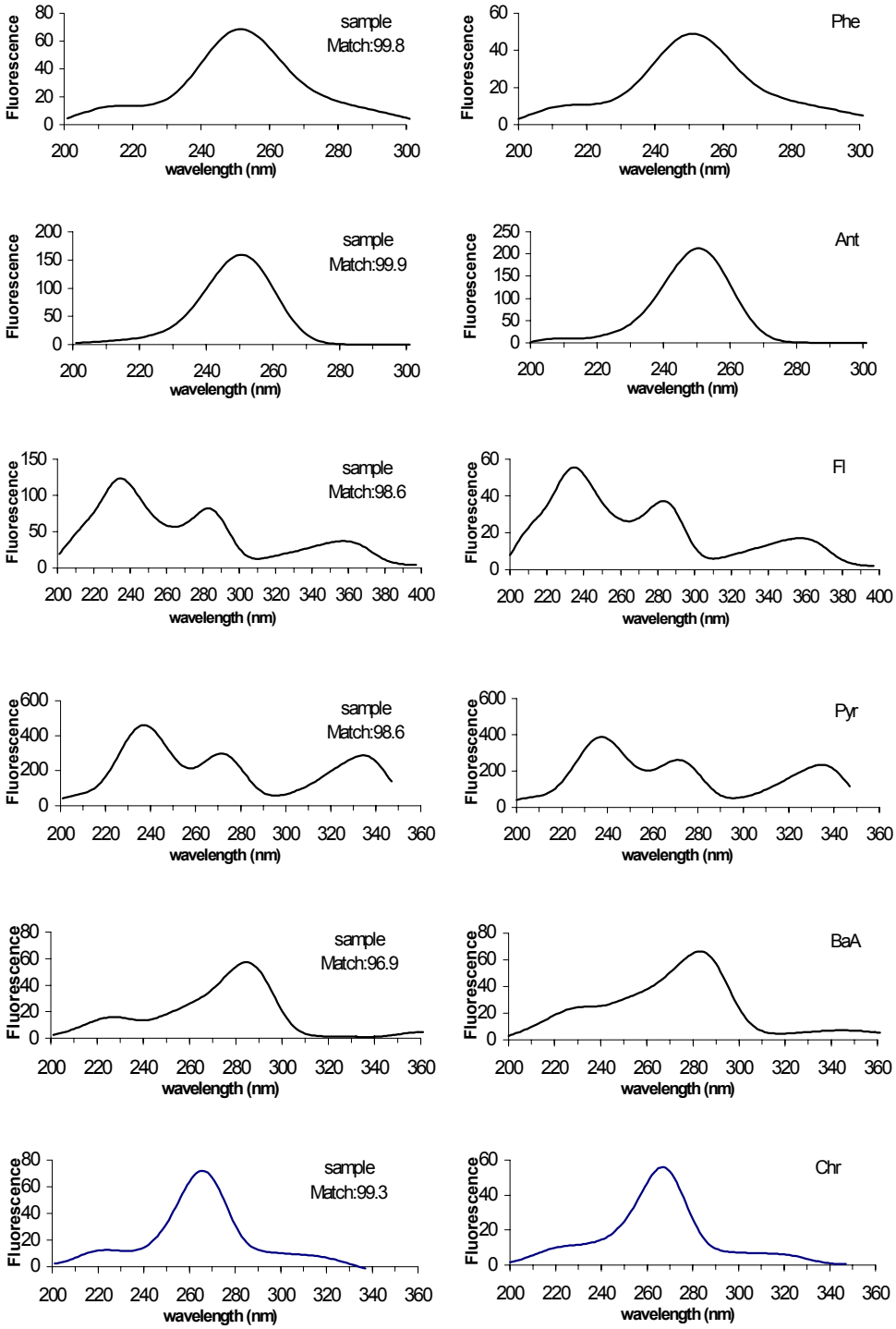

Fig. 3. Comparison of excitation spectra of PAH standards (on the right side) with the excitation spectra of the peaks with the same retention time of an extract of a crude coconut oil (on the left side). The match factor is given as percentage. PAHs abbreviations as indicated in Section 2.

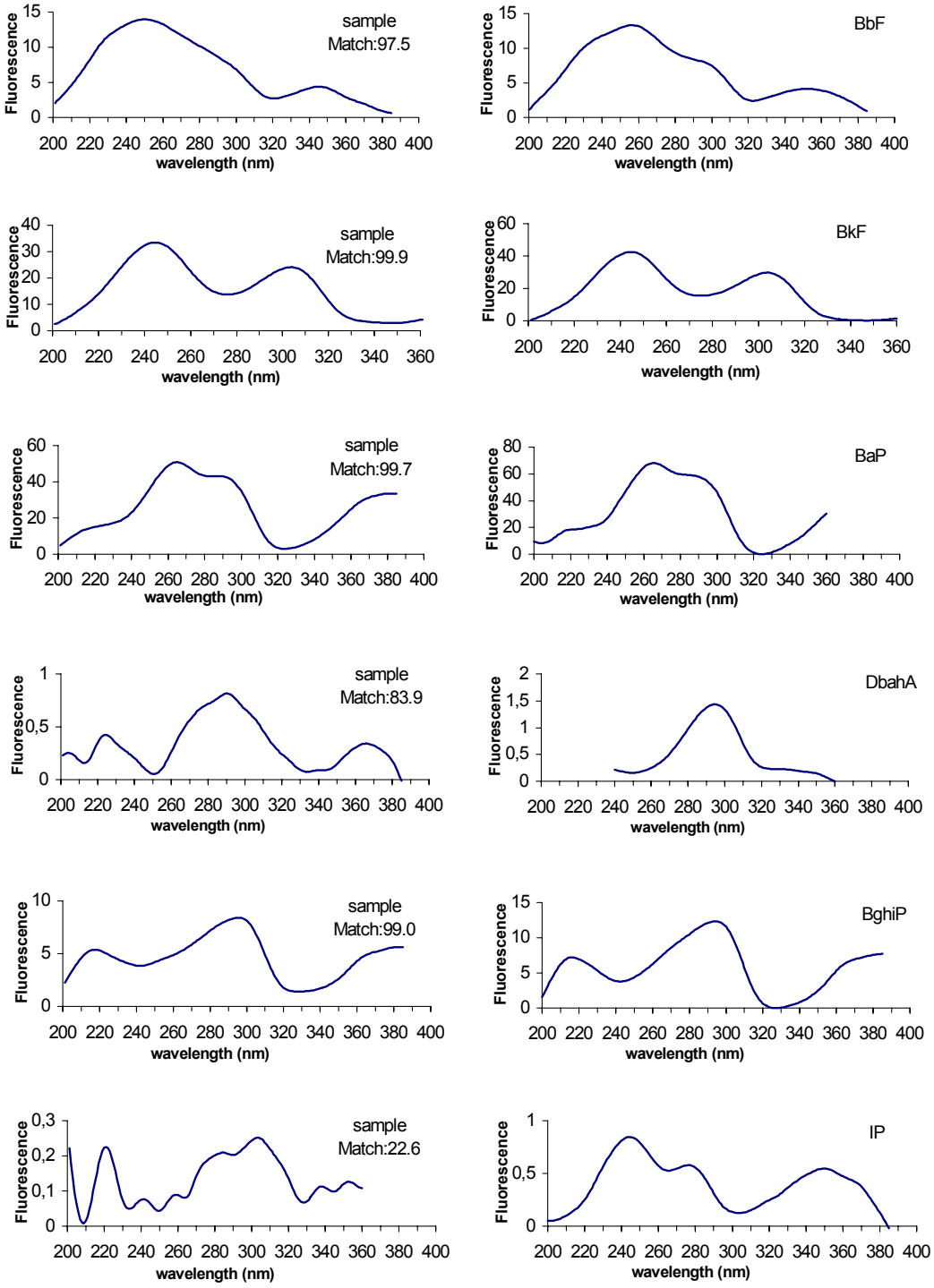

Fig. 3. (*continued*)

by the DACC column, and those of a standard solution. Dibenzo[a,h]anthracene and indeno[1,2,3-c,d]pyrene in this oil were below the limit of quantitation, so their spectra had little intensity and the match factors were very low. Similar match factors were obtained when the alumina column was used.

Selectivity was also assured applying the two methods to a certified reference material of a "blank" (highly refined) coconut oil (CRM 459), and to this blank fortified at a low concentration level. RP-HPLC chromatograms of extracts obtained after clean-up with the DACC and the alumina columns are given in Figs. 4 and

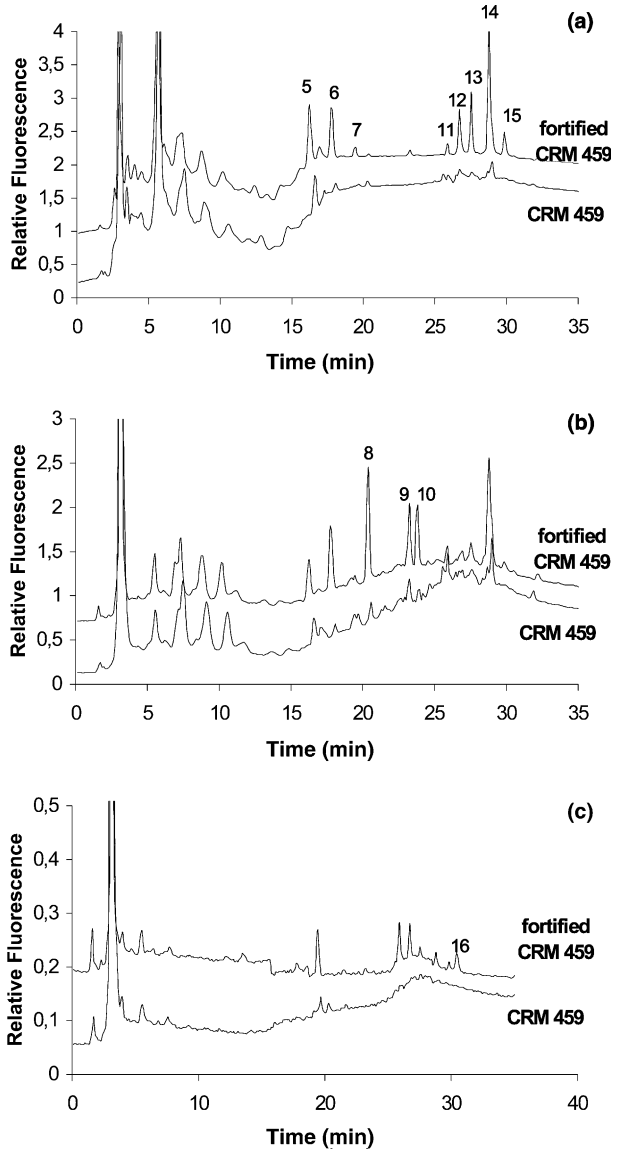

Fig. 4. Chromatograms of a highly refined coconut oil (CRM 459, PAHs certified values in Section 2) and this oil fortified at a level corresponding to 1.5 ng/g of BaP obtained with the DACC clean-up. (a) Fluorescence program as in Table 1, (b) excitation wavelength as in Table 1 and emission wavelength, always at 390 nm, and (c) excitation wavelength as in Table 1 and emission wavelength, always at 500 nm. Peaks: 5 ,Phe; 6, Ant; 7, Fl; 8, Pyr; 9, BaA; 10, Chr; 11, BbF; 12, BkF; 13, BaP; 14, DbahA; 15, BghiP; 16, IP.

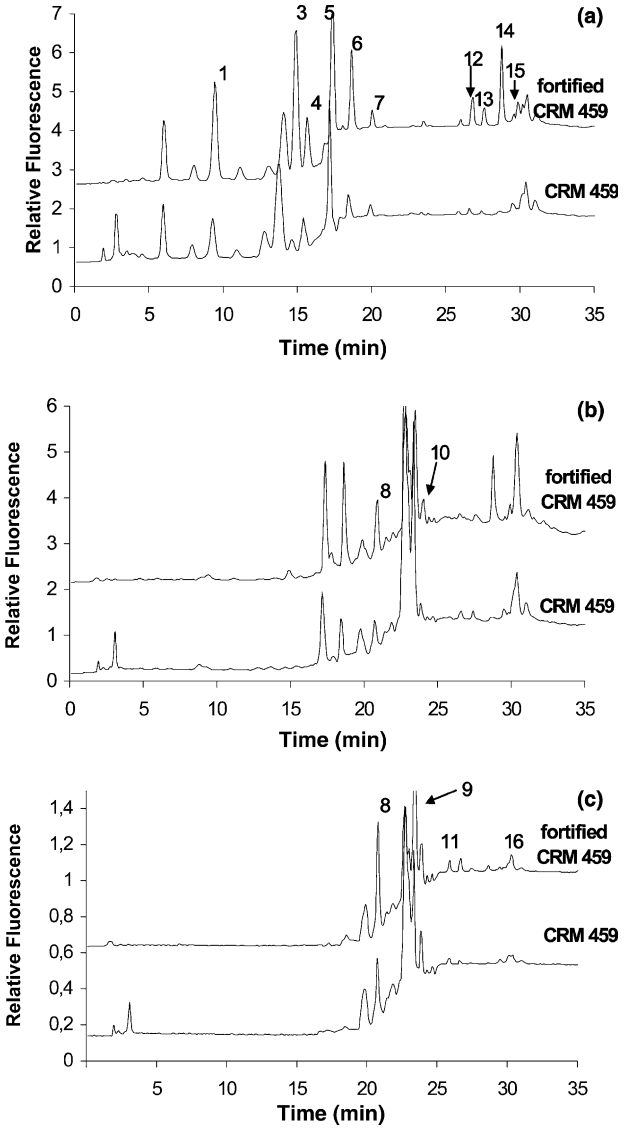

Fig. 5. Chromatograms of a highly refined coconut oil (CRM 459, PAHs certified values in Section 2) and this oil fortified at a level corresponding to 1.25 ng/g of BaP obtained with the alumina clean-up. (a) Fluorescence program as in Table 1, except for BbF, BkF, BaP ($\lambda_{exc} = 300$ nm and $\lambda_{emi} = 440$ nm) and DbahA and BghiP ($\lambda_{exc} = 300$ nm and $\lambda_{emi} = 420$ nm), (b) excitation wavelength as in Table 1 and emission wavelength, always at 390 nm, and (c) excitation wavelength as in Table 1 and emission wavelength, always at 500 nm, except for Pyr, BaA and Chr ($\lambda_{emi} = 410$ nm). Peaks: 1, Na; 3, Ac; 4, F; 5, Phe; 6, Ant; 7, Fl; 8, Pyr; 9, BaA; 10, Chr; 11, BbF; 12, BkF; 13, BaP; 14, DbahA; 15, BghiP; 16, IP.

5, respectively. CRM 459 is certified for six PAHs (see Section 2). Pyrene and other no certified PAHs were also found in little amounts, such as phenanthrene (0.5 ng/g), anthracene (~0.1 ng/g), fluoranthene (~0.4 ng/g), pyrene (~0.3 ng/g), benzo[a]anthracene (~0.3 ng/g) and dibenzo[a,h]anthracene (~0.2 ng/g). Some precaution is needed in relation with the possible presence of PAHs traces in the solvents used in the clean-up methodologies. In our case, little amounts of some PAHs were detected in the hexane used, in spite of its high quality. Therefore, reagents blanks were performed for both clean-up procedures in order to correct the quantities found in all samples treated along this work. This contamination due to solvents is more notorious in the case of alumina pretreatment, due to the greater volume of hexane required. Moreover, with the alumina clean-up, it was needed to change the wavelength program of the fluorescence detector in order to be able to quantify benzo[a]anthracene in the extract ($\lambda_{exc} = 300$ nm and $\lambda_{em} = 440$ nm from 25.4 to 28.5 min were used instead of those of Table 1). The chromatogram obtained from the alumina column shows interferences around 30 min,

which make difficult the quantification of benzo-[g,h,i]perylene and indeno[1,2,3-c,d]pyrene at low concentrations. In order to avoid these interfering peaks in the case of crude coconut oils, two fractions were collected separately: the first 30 ml containing the light PAHs and the next 60 ml with the heavy ones. However, in most oils there was no necessity of collecting two different fractions. On the other hand, with the DACC column no interferences appear and all PAHs can be determined without problems at low concentration levels.

The same reference material (CRM 459) was used to evaluate the limits of detection, limits of quantitation, linear ranges, recoveries and repeatabilities (within-day and between-day) of the methods. These results are given in the Tables 2–4. Limits of quantitation were less than 1 ng/g for most of PAHs in both methods; low enough to fulfil with the legal requirements. Limits of detection were also calculated in the cases of sunflower and olive oils, giving similar results to the coconut oil. The concentrations detected in all the oil samples analysed were inside the linear range except for the crude coconut and the olive residue oils, which had a large PAHs content, so it was necessary to dilute these samples. The precision of the method, expressed by the coefficient of variation (CV), was less than 10% in all cases.

A reference material of a coconut oil with certified concentrations for certain PAHs (CRM 458) was used to evaluate the accuracy of both methods. For the six certified PAHs, differences lower than 6% for DACC method and lower than 20% for the alumina method were achieved (Table 5). For the rest of PAHs the accuracy was assessed using the reference material of a ''blank'' coconut oil (CRM 459) which was fortified at a low concentration

Table 2
Limits of detection, limits of quantitation and the working linear ranges of both methods

PAHs	Limit of detection (ng/g)[a]		Limit of quantitation (ng/g)[b]		Linear range (ng/g)	
	DACC	Alumina	DACC	Alumina	DACC	Alumina
Ac		0.6		8		8–500
F		0.2		1		1–150
Phe	0.2	0.1	0.4	0.4	0.4–60	0.4–60
Ant	0.09	0.05	0.2	0.2	0.2–50	0.2–30
Fl	0.2	0.1	0.5	0.5	0.5–125	0.5–75
Pyr	0.2	0.1	0.5	0.3	0.5–250	0.3–150
BaA	0.2	0.06	0.5	0.2	0.5–125	0.2–75
Chr	0.2	0.06	0.5	0.2	0.5–125	0.2–75
BbF	0.1	0.07	0.3	0.2	0.3–30	0.2–30
BkF	0.1	0.03	0.3	0.1	0.3–30	0.1–30
BaP	0.2	0.08	0.6	0.3	0.6–75	0.3–75
DbahA	0.1	0.1	0.6	0.6	0.6–300	0.6–100
BghiP	0.2	0.2	0.4	0.6	0.4–120	0.6–120
IP	0.2	0.2	0.7	0.5	0.7–75	0.5–75

[a] Calculated as three times the SD of 10 independent sample blanks.
[b] Calculated as the lowest concentration that can be determined with an acceptable level of uncertainty (10%).

Table 3
Within-day ($n = 10$) and between-day ($n = 4$) repeatabilities of the clean-up with the DACC column

PAHs	Amount added (ng/g)[a]							
	Intra-day repeatability				Inter-day repeatability			
	1.5	12.5	75	Mean	1.5	12.5	75	Mean
Phe	6.0	5.0	5.6	5.5	9.4	3.5	6.6	6.5
Ant	8.5	1.9	3.5	4.6	9.8	6.6	4.3	6.9
Fl	7.2	8.7	4.2	6.7	7.4	4.7	5.9	6.0
Pyr	3.1	3.2	1.0	2.4	4.9	3.8	4.1	4.3
BaA	4.5	3.5	3.2	3.7	9.3	7.4	5.8	7.5
Chr	5.1	2.8	3.0	3.6	5.2	3.5	3.2	4.0
BbF	5.2	3.1	3.2	3.8	5.5	6.5	6.1	6.0
BkF	4.3	2.2	3.0	3.2	2.7	4.3	5.4	4.1
BaP	5.3	2.6	3.4	3.8	8.7	6.4	5.4	6.8
DbahA	4.3	2.2	4.2	3.6	4.3	2.0	6.3	4.2
BghiP	5.9	4.0	3.4	4.4	2.0	5.2	7.8	5.0
IP	7.0	3.5	3.3	4.6	5.3	2.0	7.9	5.1

The results are expressed as coefficient of variation (%).
[a] Amount added expressed as the concentration of BaP. The concentrations for other PAHs can be calculated from their relation in the standard mix.

Table 4
Within-day ($n = 10$) and between-day ($n = 4$) repeatabilities of the clean-up with alumina column

PAHs	Amount added (ng/g)[a]							
	Intra-day repeatability				Inter-day repeatability			
	1.25	12.5	75	Mean	1.25	12.5	75	Mean
Ac	9.4	4.9	5.2	6.5	3.3	0.8	6.7	3.6
F	8.5	5.7	6.3	6.8	5.0	1.4	3.1	3.2
Phe	8.8	5.8	3.2	5.9	9.2	7.5	8.5	8.4
Ant	5.5	4.1	3.5	4.4	9.9	1.3	5.8	5.4
Fl	6.2	3.9	3.0	4.4	8.0	4.0	4.6	5.5
Pyr	3.9	4.0	3.5	3.8	9.9	4.7	5.2	6.6
BaA	7.8	3.0	3.2	4.7	9.3	2.4	3.7	5.1
Chr	4.4	3.1	2.5	3.3	9.0	1.8	5.9	5.6
BbF	3.9	3.7	2.3	3.3	6.3	3.9	5.5	5.2
BkF	3.0	3.3	3.3	3.2	4.3	2.5	5.7	4.2
BaP	3.9	4.4	3.0	3.8	9.1	3.6	9.0	7.2
DbahA	2.6	2.5	2.5	2.5	2.3	2.5	4.6	3.1
BghiP	7.1	3.0	2.6	4.2	7.4	0.6	5.6	4.5
IP	3.0	4.3	3.3	3.5	3.7	4.9	5.9	4.8

The results are expressed as coefficient of variation (%).
[a] Amount added expressed as the concentration of BaP. The concentrations for other PAHs can be calculated from their relation in the standard mix.

Table 5
Accuracy of both methods

PAHs	Reference material CRM 458			Reference material CRM 459	
	Certified values (ng/g)	Measured (ng/g) ($n = 5$)[a]		DACC (%)[b]	Alumina (%)[c]
		DACC	Alumina		
Ac					67.3 ± 7.6
F					69.6 ± 9.6
Phe				87.8 ± 4.8	89.6 ± 6.2
Ant				92.6 ± 5.1	94.1 ± 5.8
Fl				105.4 ± 5.4	97.8 ± 7.1
Pyr	9.4 ± 1.5	9.47 ± 0.78	8.89 ± 0.67	96.3 ± 6.6	94.2 ± 8.1
BaA				97.3 ± 4.0	94.8 ± 6.3
Chr	4.9 ± 0.4	4.68 ± 0.42	3.94 ± 0.22	94.1 ± 2.2	91.5 ± 7.7
BbF				92.4 ± 4.2	103.2 ± 4.2
BkF	1.87 ± 0.18	1.95 ± 0.17	1.79 ± 0.06	102.8 ± 3.7	92.9 ± 5.4
BaP	0.93 ± 0.09	0.92 ± 0.09	0.83 ± 0.14	103.1 ± 6.7	93.3 ± 5.3
DbahA				93.1 ± 2.4	92.2 ± 4.2
BghiP	0.97 ± 0.07	0.97 ± 0.11	0.83 ± 0.09	95.3 ± 4.6	90.7 ± 7.1
IP	1.00 ± 0.07	1.01 ± 0.09	0.90 ± 0.13	98.2 ± 3.5	94.1 ± 6.9

[a] Mean ± confidence interval for a 95% confidence level.
[b] Mean ± SD ($n = 10$) expressed as % of recovery. Calculated with reference material CRM 459 fortified at 1.5 ng/g of BaP.
[c] Mean ± SD ($n = 5$) expressed as % of recovery. Calculated with reference material CRM 459 fortified at 1.25 ng/g of BaP.

level. The mean absolute recoveries were above 85% for all PAHs by DACC method and by alumina clean-up, except for the lowest molecular weight PAHs (acenaphthene and fluorene), that are more volatile and partially lost during the evaporation step.

Both methods were applied to other edible oils and the results obtained were comparable. As shown in Fig. 6, BaP quantities obtained with the two methods were highly correlated ($r = 0.9996$). The same occurred with other PAHs.

4. Conclusions

From the experimental results, it can be concluded that both methods are suitable for the determination of PAHs in edible oils. Both are comparable in sensitivity and precision. Although naphthalene, acenaphthylene, acenaphthene and fluorene are not possible to be determined with the DACC column, this method has better selectivity and accuracies specially for benzo[a]anthracene, chrysene, benzo[g,h,i]perylene and indeno[1,2,3-c,d]pyrene.

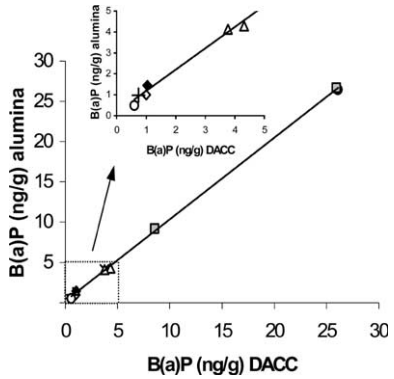

Fig. 6. Correlation of BaP contents in samples analysed by clean-up with the DACC column (*x*-axis) and clean-up with the alumina column (*y*-axis). ●, crude coconut; □, olive residue (two samples); △, crude sunflower (two samples); ◊, palm stearine; ◆, refined coconut; +, palm kernel; ○, olive oil.

Furthermore, DACC is less time consuming, since the whole procedure of the clean-up with the DACC column requires 70 min, approximately; whereas the clean-up with the alumina column takes more than 2 h. This fact makes the former better for routine analysis. Moreover, less amount of solvent and less work by the operator is needed.

Acknowledgements

These studies were supported by the Comisión Interministerial de Ciencia y Tecnología and the European Commission (Project No. 2FD1997-1999) and by Unilever Foods España, S.A. Alejandro Barranco wishes to thank the Basque Government for a PhD grant (Programa Realización de Tesis Doctorales en Empresas de los Departamentos de Industria, Comercio y Turismo y del Departamento de Educación, Universidades e Investigación).

References

Bakker, M. S., Casado, B., Koerselman, J. W., Tolls, J., & Kollöfel, C. (2000). Polycyclic aromatic hydrocarbons in soil and plant samples from the vicinity of an oil refinery. *The Science of the Total Environment, 263*, 91–100.

Brouwer, E. R., Hermans, A. N. J., Lingeman, H., & Brinkman, U. A. Th. (1994). Determination of polycyclic aromatic hydrocarbons in surface water by column liquid chromatography with fluorescence detection, using on-line micelle-mediated sample preparation. *Journal of Chromatography A, 669*, 45–57.

Cert, A., Moreda, W., & Pérez-Camino, M. C. (2000). Chromatographic analysis of minor constituents in vegetable oils. *Journal of Chromatography A, 881*, 131–148.

Eurachem (1998). *The fitness for purpose of analytical method: A laboratory guide to method validation and related topics*. LGC: Teddington.

Funk, M., Frank, H., Oesch, F., & Platt, K. L. (1994). Development of chiral stationary phases for the enantiomeric resolution of dihydrodiols of polycyclic aromatic hydrocarbons by π-donor–acceptor interactions. *Journal of Chromatography A, 659*, 57–68.

Guillén, M. D., Sopelana, P., Cid, C., & Partearroyo, M. A. (1996). Presencia de hidrocarburos aromáticos policíclicos en los alimentos que forman parte de la dieta de distintos paises europeos. *Alimentaria, 278*, 41–47.

Hendrikse, P. W., & Dieffenbacher, A. (1991). Determination of benzo[a]pyrene in oils and fats by reversed phase high performance liquid chromatography. Results of a collaborative study and the standardized method. *Pure and Applied Chemistry, 63*, 1659–1666.

International Agency for the Research on Cancer (1983). Chemicals, environment and experimental data. In *Monographs on the evaluation of the carcinogenic risk of chemicals to humans: Polynuclear aromatic compounds* (vol. 32). Lyon: IARC.

Lee, M. L., Novotny, M. V., & Bartle, K. D. (1981). *Analytical chemistry of polycyclic aromatic compounds*. New York: Academic Press.

Lodovici, M., Dolara, P., Casalini, C., Ciapellano, S., & Testolin, G. (1995). Polycyclic aromatic hydrocarbon contamination in the Italian diet. *Food Additives and Contaminants, 12*(5), 703–713.

Manoli, E., & Samara, C. (1999). Polycyclic aromatic hydrocarbons in natural waters: Sources, occurrence and analysis. *Trends in Analytical Chemistry, 18*(6), 417–428.

Moret, S., & Conte, L. (1998). Off-line LC-LC determination of PAHs in edible oils and lipidic extracts. *Journal of the High Resolution Chromatography, 21*, 253–257.

Moret, S., & Conte, L. S. (2000). Polycyclic aromatic hydrocarbons in edible oils and fats: Occurrence and analytical methods. *Journal of Chromatography A, 882*, 245–253.

Neilson, A. H. (1998). *The handbook of environmental chemistry* (vol. 3, part I). Berlin: Springer.

Phillips, D. H. (1999). Polycyclic aromatic hydrocarbons in the diet. *Mutation Research, 443*, 139–147.

Pupin, A. M., & Toledo, M. C. F. (1996). Benzo(a)pyrene in olives oils on the Brazilian market. *Food Chemistry, 55*(2), 185–188.

Spanish Official Bulletin BOE (2001). Orden de 25 de julio de 2001 por la que se establecen límites de determinados hidrocarburos aromáticos policíclicos en aceite de orujo de oliva. In: Spanish Official Bulletin (no. 178). Madrid: BOE. pp. 27397–27398.

Speer, K., Steeg, E., Horstmann, P., Kühn, Th., & Montag, A. (1990). Determination and distribution of polycyclic aromatic hydrocarbons in native vegetable oils, smoked fish products, mussels and oysters, and bream from the river Elbe. *Journal of the High Resolution Chromatography, 13*, 104–111.

Stijn, F. van, Kerkhoff, M. A. T., & Vandeginste, B. G. M. (1996). Determination of polycyclic aromatic hydrocarbons in edible oils and fats by on-line donor acceptor complex chromatography with fluorescence detection. *Journal of Chromatography A, 750*, 263–273 (Chrompack-Varian, Application Note 1347-HPLC rev:02).

US Environmental Protection Agency (1984). Guidelines establishing test procedures for the analysis of pollutants. Proposed regulations. In *Federal register* (vol. 49, p. 112). Washington: USEPA.

Yang, G. P. (2000). Polycyclic aromatic hydrocarbons in the sediments of the south China Sea. *Environmental pollution, 108*, 163–171.