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PAHs content in sunflower, soybean and virgin olive oils: Evaluation in commercial samples and during refining process

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Abstract

Polycyclic aromatic hydrocarbons (PAHs) are environmental carcinogenic compounds that may contaminate vegetable oils and their levels can be reduced by refining. In order to understand the influence of the refining steps, the content of 15 PAHs was assessed throughout alkaline refining in soybean, sunflower and olive oil samples. Eight commercial brands of these oils were also analysed. The analytical method involved a liquid–liquid extraction, a solid-phase clean up (C_{18} and Florisil) followed by RP-HPLC with fluorimetric detection. The total PAHs content in the studied samples can be considered generally low. The light PAHs (2–4 rings) were predominant. Virgin olive oils showed the highest values (max. 26 μg/kg). An evident decrease of PAHs contents during alkaline refining was observed (71%, 88% and 85% in sunflower, soybean and olive oils, respectively) being more pronounced in light PAHs. Neutralization and, particularly, deodorization were the more effective steps contributing to the PAHs decrease. Bleaching was responsible for a slight increase in the PAHs content in soybean and olive oils.

Keywords: Polycyclic aromatic hydrocarbons (PAHs); Sunflower oil; Soybean oil; Virgin olive oil; Refining; HPLC

1. Introduction

PAHs are a diversified family of more than 100 lipophilic organic contaminants composed by two or more fused aromatic rings (Guillén, 1994). These compounds are mostly formed by incomplete combustion of organic matter as a consequence of a series of natural and anthropogenic processes (Moret & Conte, 2002). Due to their multiple potential sources of contamination, PAHs are ubiquitously distributed in nature (Baan, Steenwinkel, van den Berg, Roggeband, & van Delft, 1994). Therefore, human exposition is virtually unavoidable, which raises an important public health concern due to their recognized carcinogenic activity. Exposition to PAHs was epidemiologically associated with an increased risk of skin and lung cancer (Baan et al., 1994). Sixteen PAHs are actually classified as priority pollutants by Environmental Protection Agency (EPA) on the basis of their occurrence and carcinogenicity, being six with 4–6 rings classified as heavy PAHs (Environmental Protection Agency, 1983).

Diet is the major non-occupational source of PAHs for non-smokers (Lodovici, Dolara, Casalini, Ciappellano, & Testolin, 1995), being meat and meat products, cereals, and oils and fats the principal sources (Dennis et al., 1991; Ibáñez et al., 2005). Due to their lipophilic nature, PAHs contaminate oils and fats that are a significant dietary source, either directly or indirectly by their incorporation into other foods such as cereal-based products (Dennis et al., 1991). Another aspect that emphasizes the significance of oils and fats as a PAHs vehicle is that lipids could raise their intestinal absorption (Starvic & Klassen, 1994). Vegetable oils, natively free of PAHs, are contaminated mainly by environmental pollution of the vegetable raw material, and by contamination from seed drying, solvent extraction, soil burn, package material, mineral oils

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residues and migration from contaminated water or soils (Larsson, Eriksson, & Cervenka, 1987; Speer, Steeg, Horstmann, Kühn, & Montag, 1990). A high number of PAHs of a wide range of molecular weights is present in vegetable oils, of which many are alkylated compounds, although they are ignored by legal regulations (Guillén & Sopelana, 2004).

The European Union (Commission Regulation No. 208/2005) has recently set maximum levels of 2 ppb for benzo[*a*]pyrene in oils and fats intended for direct consumption or use as an ingredient in foods (Moret, Purcaro, & Conte, 2005). Some countries (Spain, Italy, Portugal and Greece) have established limits for the concentration of the following eight heavy PAH: benzo[*a*]anthracene, benzo[*e*]pyrene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, dibenz[*a*,*h*]anthracene, benzo[*ghi*]perylene, indeno[1,2,3-*cd*]pyrene. A maximum limit value of 2 ppb for each single PAH and 5 ppb for the sum of the eight heavy PAHs was established (Moret et al., 2005). Some organizations establish their own recommendations, such the German Society for Fat Science (GSFS) that suggests that total PAHs in edible oils should not surpass 25 μg/kg and heavy PAHs should be below 5 μg/kg (Cejpek, Hajslova, Kocourek, Tomaniova, & Cmolik, 1998).

PAHs contamination in crude edible oils varies widely, but refined vegetable oils generally present low levels than the crude ones, which can be attributed, at least in part, to the reduction observed through refining (Cejpek et al., 1998). The deodorization strongly reduces "light" PAHs (up to four aromatic rings), while bleaching with activated charcoal is an effective strategy to reduce the higher condensed "heavy" PAHs (5–6 rings) (Dennis et al., 1991; Larsson et al., 1987). It is important to know the extension of the contamination of vegetable oils with PAHs, but also the influence that each step of the refining process has on it. In this context, only few papers have measured the PAHs content through refining (Barranco et al., 2004; Cejpek et al., 1998; Moret, Piani, Bortolomeazzi, & Conte, 1997), and the majority focuses only in raw and refined samples (Biernoth & Rost, 1967; Larsson et al., 1987; Sagredos, Sinha-Roy, & Thomas, 1988).

The aims of this study were to determine the content of 15 (EPA) PAHs in commercial samples of virgin olive oil, refined sunflower and soybean oils, and evaluate their behaviour during the alkaline refining processing steps.

2. Materials and methods

2.1. Samples

Refined soybean (three samples) and sunflower (three samples) oils, and virgin olive oils (two samples) commercially available in Portugal were analysed for 15 EPA PAHs levels (one bottle for each brand). By law, virgin olive oil can only be obtained by mechanical or other physical processes, in thermal conditions avoiding alterations, and cannot undergo any treatment besides washing, decantation, centrifugation and filtration. The soybean, sunflower and olive oils sampled raw and at different steps of the refining process (neutralization, bleaching, and deodorization) were provided by refining industries from Portugal and Spain. In the olive oil refining process the neutralization was made at 30–40 °C and the bleaching at 95–100 °C with both bleaching earth and activated charcoal (4 kg/T). The deodorization was accomplished at 190–200 °C and 200–300 Pa with the use of direct stream (15 kg/h) in order to eliminate volatile compounds and pigments. The neutralization temperature of the other vegetable oils was higher (80–90 °C), as well as that one of deodorization (sunflower oils, 230 °C, and soybean oil, 240 °C). Activated earth was used in the bleaching step but in the soybean oil activated charcoal was also included (1 kg/T). Once in the laboratory, samples were stored in darkness in near-full bottles at room temperature pending analysis within in a maximum period of three months.

2.2. Standard mixture

A mixture of 16 EPA priority pollutant PAHs (Ultra Scientific PM-831A, USA), was used for the identification and quantification of the PAHs present in the samples and included naphthalene 502.1 μg/ml, acenaphthene 1002.4 μg/ml, acenaphthelene 501.2 μg/ml, fluorene 100.2 μg/ml, phenanthrene 40.2 μg/ml, anthracene 20.0 μg/ml, fluoranthene 50.2 μg/ml, pyrene 100.0 μg/ml, benz[*a*]anthracene 50.2 μg/ml, chrysene 50.2 μg/ml, benzo[*b*]fluoranthene 20.0 μg/ml, benzo[*k*]fluoranthene 20.0 μg/ml, benzo[*a*]pyrene 50.2 μg/ml, dibenz[*a*,*h*]anthracene 200.4 μg/ml, benzo[*ghi*]perylene 80.2 μg/ml and indeno[1,2,3-*cd*]pyrene 50.2 μg/ml. This certified standard mixture was stored at −20 °C in darkness to avoid volatilization and photodegradation. Stock solutions containing 50 μg/l were prepared by dilution of this standard mix in tetrahydrofuran and methanol and stored at −20 °C in darkness.

2.3. Reagents and materials

Methanol (Fluka, Switzerland), *n*-hexane, acetonitrile, dichloromethane, tetrahydrofuran, toluene (all from Merck, Germany) and acetone (Aldrich, Germany) were all of HPLC grade. The glassware was washed with detergent and water, rinsed with ethanol, acetone and *n*-hexane (2 times) and dried at 90 °C before use. Water used for chromatographic analysis was deionised (Seraldest LFM 20 from Seral, Germany), filtered through a 0.45 μm membrane and subsequently degassed. The purity of all analytical material and reagents was checked by blank tests at the beginning, middle and end of analysis of each subset of samples.

2.4. Extraction and purification

The initial isolation procedure involved a liquid–liquid extraction. Aliquots (2.5 g) of oil samples were diluted with

10 ml of acetonitrile–acetone 60:40 (v/v), shacked 30 s with vortex (half speed), sonicated during 5 min and centrifuged 5 min at 4000 rpm. The top layer was transferred into a conical tube and evaporated under a nitrogen flow at 35 °C, in a Reacti-Vap™ Pierce® (USA) evaporating unit. This extraction process was repeated twice and the extracts were combined in the same conical tube. The solvents were evaporated avoiding total dryness, otherwise volatile PAHs would be lost.

The solid phase extraction (SPE) clean-up was performed in a 12-port Visiprep solid-phase extraction Vacuum Manifold from Supelco® (USA). The SPE cartridges used were Sep-Pak® C18 (12 cc, 2 g) and Sep-Pak® Florisil (6 cc, 500 mg), both from Waters® (USA). The C18 bonded phase cartridges were activated with methanol and acetonitrile, while the Florisil were with dichloromethane and hexane. The residual fat material from the liquid–liquid extraction was dissolved in 2 ml of acetonitrile–acetone 60:40, shacked 15 s with vortex (half speed) and centrifuged (4000 rpm, 30 s). The top layer was then transferred to the C18 cartridge. This operation was repeated twice more. Five milliliter of acetonitrile/acetone 60:40 were eluted through the cartridge and vacuum was applied. The solvents eluted were evaporated under a nitrogen stream with caution to avoid the volatilization of the more sensitive PAHs. The residue was then dissolved in 1 ml of hexane and transferred to the previously conditioned Florisil bonded phase cartridge. A mixture of hexane–dichloromethane 75:25 (v/v) (1 ml) was added three times to the extract tube, shacked 15 s with vortex and transferred again to the cartridge. The tube was also rinsed with 2×1 ml of hexane/dichloromethane 75:25 and these solvents were introduced in the Florisil cartridge. The cartridge was eluted with 4 ml of hexane/dichloromethane 75:25 and submitted to vacuum in order to fully recollect the solvents. The extract was evaporated under a flow of nitrogen to about 1 ml. At this moment, 0.5 ml of toluene

Table 1
Excitation and emission wavelengths program

PAH	Excitation (nm)	Emission (nm)
Naphthalene	275	326
Acenaphthene	270	324
Fluorene	270	324
Phenanthrene	251	358
Anthracene	251	382
Fluoranthene	280	462
Pyrene	270	385
Benz[*a*]anthracene	270	385
Chrysene	270	385
Benzo[*b*]fluoranthene	256	446
Benzo[*k*]fluoranthene	292	410
Benzo[*a*]pyrene	292	410
Dibenz[*a,h*]anthracene	295	416
Benzo[*ghi*]perylene	292	410
Indeno[1,2,3-*cd*]pyrene	274	507

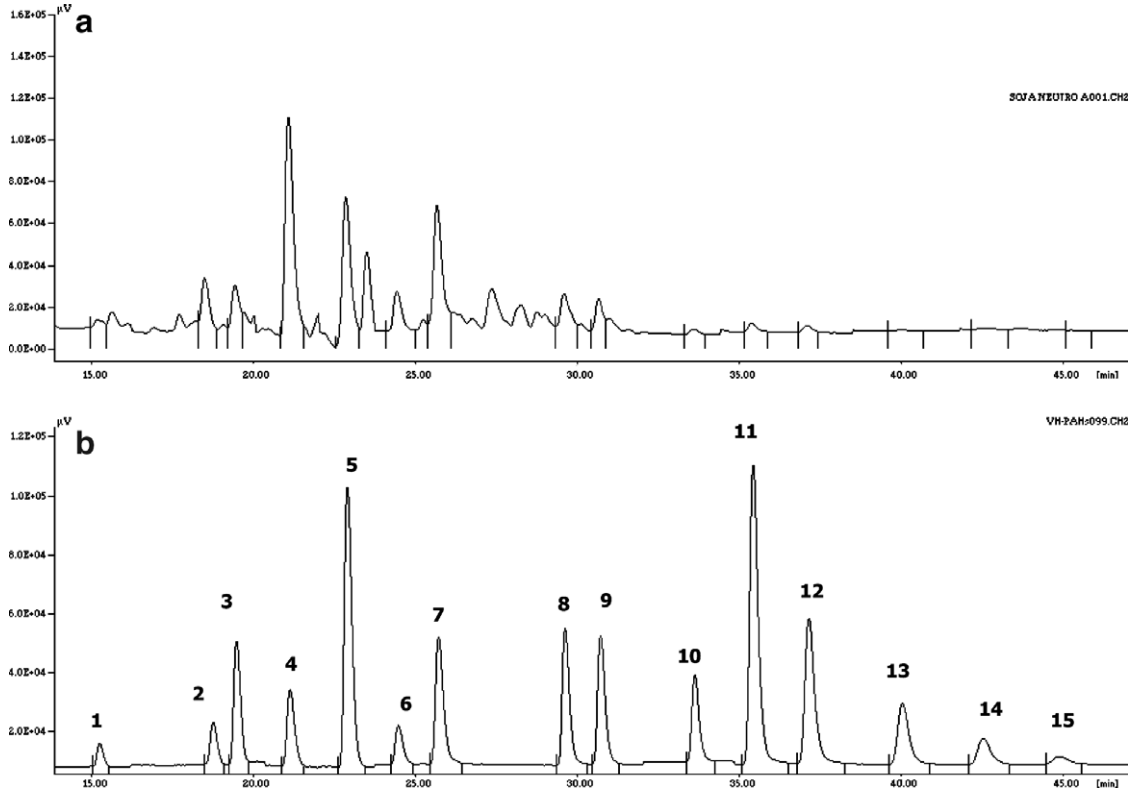

Fig. 1. Chromatograms of a PAHs standard mixture (50 μg/l) (a) and a soybean oil sample (b). 1, naphthalene; 2, acenaphthene; 3, fluorene; 4, phenanthrene; 5, anthracene; 6, fluoranthene; 7, pyrene; 8, benz[*a*]anthracene; 9, chrysene; 10, benzo[*b*]fluoranthene; 11, benzo[*k*]fluoranthene; 12, benzo[*a*]pyrene; 13, dibenz[*a,h*]anthracene; 14, benzo[*ghi*]perylene; 15, indeno[1,2,3-*cd*]pyrene.

was added, and the evaporation continues to about 20 μl. This residue was then taken up with tetrahydrofuran/methanol 50:50 (v/v) until to 250 μl, transferred into a microvial and kept at −20 °C until HPLC analysis. In all evaporation steps the extract cannot be taken to complete dryness, otherwise naphthalene, acenaphthene and fluorene are loss. These compounds are extremely volatile and therefore cannot be accurately quantified by this method.

2.5. HPLC analysis

The analytical determination and quantification of PAHs was performed on a Jasco chromatograph equipped with a Jasco AS-950 auto-sampler, two PU-980 pumps, and a FP-920 programmable fluorimetric detector (Jasco, Japan). The column used was a C_{18} reverse-phase Supelcosil™ LC-PAH column (5 μm, 25 cm × 4.6 mm) from Supelco (USA), thermostated at 20 °C. The mobile phases consisted of a gradient of acetonitrile in water (acetonitrile 40% from 0 to 5 min, acetonitrile 100% from 30 to 45 min and equilibrium from 46 to 50). The flow rate was 1.5 ml/min and the injection volume 20 μl. Excitation and emission wavelengths were programmed as reported in Table 1. Acenaphthylene determination was not performed due to its absence of fluorescence.

The 15 PAHs studied were identified and quantified by chromatographic comparison with external standards based on their retention times. Chromatograms corresponding to a 50 μg/l standard mixture and to an olive oil are represented in Fig. 1.

Each sequence of samples included a blank to control the absence of contamination of solvents and cartridges, and a standard solution extracted in the same conditions as the samples, in order to calculate the recoveries. Two extractions of each sample were done and the extracts were analysed (injected) twice by HPLC.

3. Results and discussion

3.1. Validation study

The calibration curves were obtained by the injection in triplicate of the diluted PAHs standard mixtures (500, 200, 100, 50, 20, 10, 5, 2 and 1 μg/l). A linear response was observed for every compounds analysed, in the range concentration tested (1–500 μg/l), that encompass the observed vegetable oils PAHs content. The detection and quantification limits were calculated as the concentration corresponding to 3 and 10 times, respectively, the standard deviations of the blank noise. Detection limits ranged from 4 ng/kg, for benzo[*k*]fluoranthene, to 92 ng/kg, for indeno-[1,2,3-*cd*]pyrene. The repeatability study revealed a precise method, with coefficients of variation varying between 1.09% and 4.23%. Due to the high volatility of acenaphthene, fluorene and phenanthrene, only their qualitative evaluation was possible. The recovery obtained with the standard solution was satisfactory taking into account the complexity of the extraction procedure, ranging from 76%, for fluoranthene, to 107%, for benzo[*a*]pyrene. The recovery of a vegetable oil spiked with PAHs presented a lower value than expected, varying between 29%, for indeno[1,2,3-*cd*]pyrene, and 65%, for anthracene. Among others, the observed differences may be due to the lipophilic nature of the matrix.

3.2. Commercial brands

The contamination levels determined in the commercial vegetable oils are displayed in Table 2. Sunflower oil samples presented total PAHs contents between 8.78 and 9.72 μg/kg which are within those described by others authors (Hopia, Pyysalo, & Wickström, 1986; Kolarovic & Traitler, 1982; Speer et al., 1990). For this vegetable oil

Table 2
PAHs levels (mean ± sd) determined in commercially available sunflower, soybean and virgin olive oils (μg/kg)

PAH	Sunflower oil			Soybean oil			Virgin olive oil	
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6	Sample 7	Sample 8
Naphthalene	4.36 ± 0.80	3.24 ± 0.61	1.98 ± 0.50	4.97 ± 0.23	2.37 ± 0.20	4.43 ± 1.12	3.69 ± 0.16	6.12 ± 0.21
Acenaphthene	0.05 ± 0.01[a]	0.04 ± 0.02[a]	0.22 ± 0.03	0.07 ± 0.01[a]	n.d.	0.04 ± 0.01[a]	2.28 ± 0.02	0.86 ± 0.01
Fluorene	0.96 ± 0.03	0.62 ± 0.02	0.56 ± 0.07	0.74 ± 0.12	0.51 ± 0.02	0.74 ± 0.15	1.95 ± 0.10	1.32 ± 0.03
Phenanthrene	2.12 ± 0.13	1.70 ± 0.17	1.96 ± 0.39	0.95 ± 0.06	2.42 ± 0.01	1.67 ± 0.31	9.43 ± 0.05	4.72 ± 0.37
Anthracene	0.12 ± 0.01	0.13 ± 0.01	0.11 ± 0.01	0.08 ± 0.01	0.19 ± 0.01	0.08 ± 0.02	0.40 ± 0.02	0.18 ± 0.02
Fluoranthene	0.64 ± 0.05	1.18 ± 0.11	0.97 ± 0.10	0.65 ± 0.02	1.50 ± 0.16	0.62 ± 0.05	2.91 ± 0.05	1.40 ± 0.09
Pyrene	0.62 ± 0.04	1.53 ± 0.10	1.72 ± 0.17	0.80 ± 0.09	1.82 ± 0.33	0.63 ± 0.11	2.74 ± 0.13	1.52 ± 0.06
Benz[*a*]anthracene	0.14 ± 0.01	0.18 ± 0.01	0.12 ± 0.01	0.15 ± 0.03	0.22 ± 0.01	0.14 ± 0.01	0.92 ± 0.01	0.48 ± 0.06
Chrysene	0.19 ± 0.02	0.25 ± 0.04	0.11 ± 0.03	0.08 ± 0.02	0.31 ± 0.03	0.16 ± 0.01	0.59 ± 0.02	0.07 ± 0.01
Benzo[*b*]fluoranthene	0.19 ± 0.03	0.14 ± 0.01	0.19 ± 0.01	0.18 ± 0.03	0.28 ± 0.02	0.22 ± 0.01	0.38 ± 0.04	0.33 ± 0.01
Benzo[*k*]fluoranthene	0.05 ± 0.00	0.04 ± 0.01	0.04 ± 0.00	0.04 ± 0.01	0.10 ± 0.00	0.06 ± 0.00	0.11 ± 0.01	0.07 ± 0.01
Benzo[*a*]pyrene	0.09 ± 0.01	0.08 ± 0.00	0.09 ± 0.00	0.09 ± 0.00	0.17 ± 0.02	0.09 ± 0.01	0.28 ± 0.00	0.07 ± 0.02
Dibenz[*a*,*h*]anthracene	0.03 ± 0.00[a]	0.03 ± 0.00[a]	0.04 ± 0.01[a]	0.04 ± 0.01[a]	0.09 ± 0.02	0.09 ± 0.00	0.07 ± 0.00	0.08 ± 0.02
Benzo[*ghi*]perylene	0.18 ± 0.02	0.24 ± 0.01	0.40 ± 0.06	0.24 ± 0.04	0.49 ± 0.12	0.34 ± 0.02	0.37 ± 0.01	0.35 ± 0.11
Indeno[1,2,3-*cd*]pyrene	n.d.	n.d.	0.29 ± 0.02[a]	0.24 ± 0.03[a]	0.32 ± 0.05	n.d.	0.51 ± 0.03	0.43 ± 0.07
Total	9.72 ± 0.58	9.38 ± 0.24	8.78 ± 1.77	9.33 ± 0.39	10.81 1.06	9.31 ± 1.42	26.35 ± 0.24	18.02 ± 0.54

n.d., not detected.
[a] Below quantification limit.

the highest values (41.4 μg/kg) were found by Kolarovic and Traitler (1982), while Hopia et al. (1986) just observed 2.8 μg/kg. The levels of PAHs in commercial soybean oils stated in other works (Dennis et al., 1991; Hopia et al., 1986; Kolarovic & Traitler, 1982; Larsson et al., 1987; Lawrence & Weber, 1984), ranging from 3.6 μg/kg to 220.2 μg/kg, encompass the values observed in this work (9.31–10.80 μg/kg). The PAHs concentrations observed in virgin olive oil samples can be considered comparatively low and within those described by others authors that range from 1.0 to 142.5 μg/kg (Hopia et al., 1986; Lodovici et al., 1995; Menichini, Bocca, Merli, Ianni, & Monfredini, 1991; Speer et al., 1990; van Stijn, Kerkhoff, & Vandeginste, 1996). Virgin olive oils showed higher values than the other vegetable oils because they were not refined, a process with a recognized lowering effect on PAHs content (Biernoth & Rost, 1967; Cejpek et al., 1998; Dennis et al., 1991; Larsson et al., 1987; Sagredos et al., 1988).

The light PAHs (2–4 rings) were predominant in all samples (87–95%). Naphthalene, phenanthrene, fluoranthene and pyrene were the predominant PAHs, also in accordance with the published results (Dennis et al., 1991; Hopia et al., 1986; Kolarovic & Traitler, 1982; Lodovici et al., 1995; Menichini et al., 1991; Speer et al., 1990; van Stijn et al., 1996). The contribution of the heavy PAHs, which include the majority of the carcinogenic ones, was much less significant (mean = 8%). The same PAHs profile in sunflower oils was also observed in the other works, but in higher concentrations (Hopia et al., 1986; Kolarovic & Traitler, 1982; Speer et al., 1990). The low levels of heavy PAHs in the sunflower oils analyzed in this work could result from the application of activated charcoal in the refining process of these samples. However, in other studies the heavy PAHs prevailed in soybean oils (Kolarovic & Traitler, 1982; Larsson et al., 1987; Lawrence & Weber, 1984).

The maximum level of benzo[*a*]pyrene detected in all the analysed samples (0.28 μg/kg in a virgin olive oil) was much smaller than the limit imposed by the European Union (2 μg/kg). Despite benzo[*e*]pyrene being not determined, the sum of the other seven heavy PAHs was below the recommendation of 5 μg/kg, with a maximum of 2.64 μg/kg (again in a virgin olive oil) and none of them individually reached 2 μg/kg, being the highest content 0.92 μg/kg for benzo[*a*]anthracene in an olive oil sample.

All the vegetable oils analysed accomplished the recommendations for limit total and heavy PAHs content established by the German Society for Fat Science (Cejpek et al., 1998), with highest values of, respectively, 4.54 and 1.04 μg/kg in sunflower oils, 5.73 and 1.46 μg/kg in soybean oils, and 14.53 and 1.73 μg/kg for olive oils. These results are in conformity with published data (Lawrence & Weber, 1984), for soybean oil, and in agreement with the majority (Hopia et al., 1986; Speer et al., 1990) but not all studies (Kolarovic & Traitler, 1982), for sunflower oil. Lodovici et al. (1995) and Hopia et al. (1986) presented similar results in olive oil, while almost all the samples studied by Menichini et al. (1991), Speer et al. (1990), Moret et al. (1997), and van Stijn et al. (1996) had total PAHs concentrations higher than the recommended 25 μg/kg.

3.3. Refining process

A very significant decrease in total PAHs content (72%, 87% and 82% for sunflower, soybean and virgin olive oils, respectively) throughout refining was observed (Tables 3–5). The decrease of PAHs levels during refining described in previous works were 82–84% (Larsson et al., 1987), 27–82% (Cejpek et al., 1998), 50–54% (Dennis et al., 1991) and more than 99% (Biernoth & Rost, 1967; Sagre-

Table 3
PAHs content (mean ± sd) in sunflower oil throughout the refining steps (μg/kg)

	Crude	Neutralized	Bleached	Deodorized
Naphthalene	6.28 ± 0.53	2.12 ± 0.10	2.02 ± 0.51	2.03 ± 0.40
Acenaphthene	0.18 ± 0.01	n.d.	n.d.	0.13 ± 0.00
Fluorene	0.53 ± 0.01	0.54 ± 0.01	0.42 ± 0.03	0.16 ± 0.02
Phenanthrene	5.03 ± 0.32	5.00 ± 0.05	3.58 ± 0.29	1.38 ± 0.16
Anthracene	0.39 ± 0.02	0.47 ± 0.02	0.17 ± 0.01	0.08 ± 0.00
Fluoranthene	1.18 ± 0.13	1.29 ± 0.02	0.70 ± 0.09	0.34 ± 0.08
Pyrene	1.72 ± 0.50	1.37 ± 0.13	0.54 ± 0.04	0.36 ± 0.05
Benz[*a*]anthracene	0.15 ± 0.01	0.16 ± 0.00	0.06 ± 0.00	0.03 ± 0.01[a]
Chrysene	0.12 ± 0.00	0.11 ± 0.02	0.02 ± 0.00[a]	0.03 ± 0.00[a]
Benzo[*b*]fluoranthene	0.51 ± 0.13	0.23 ± 0.00	0.12 ± 0.00	0.08 ± 0.01
Benzo[*k*]fluoranthene	0.06 ± 0.00	0.05 ± 0.01	0.03 ± 0.00	0.03 ± 0.00
Benzo[*a*]pyrene	0.15 ± 0.00	0.11 ± 0.01	0.04 ± 0.00	0.04 ± 0.00
Dibenz[*a*,*h*]anthracene	0.07 ± 0.00	0.05 ± 0.00	0.04 ± 0.00[a]	0.06 ± 0.00
Benzo[*ghi*]perylene	0.98 ± 0.02	0.29 ± 0.02	0.23 ± 0.03	0.17 ± 0.01
Indeno[1,2,3-*cd*]pyrene	n.d.	n.d.	n.d.	n.d.
Light PAHs	15.59	11.06	7.50	4.53
Heavy PAHs	1.77	0.73	0.46	0.37
Total PAHs	17.36	11.80	7.96	4.90

n.d., not detected.
[a] Below quantification limit.

Table 4
PAHs content (mean ± sd) in soybean oil throughout the refining steps (μg/kg)

	Crude	Neutralized	Bleached	Deodorized
Naphthalene	5.46 ± 0.96	2.04 ± 0.29	4.50 ± 1.33	2.41 ± 0.03
Acenaphthene	10.01 ± 0.71	6.28 ± 0.20	6.92 ± 0.44	0.28 ± 0.00
Fluorene	2.26 ± 0.16	1.45 ± 0.04	1.81 ± 0.08	0.11 ± 0.01
Phenanthrene	25.04 ± 1.78	16.91 ± 0.79	16.85 ± 1.45	1.58 ± 0.00
Anthracene	4.32 ± 0.30	2.97 ± 0.16	2.49 ± 0.19	0.08 ± 0.00
Fluoranthene	7.40 ± 0.45	5.73 ± 0.28	4.47 ± 0.23	0.90 ± 0.07
Pyrene	5.67 ± 0.50	4.63 ± 0.28	5.04 ± 0.15	1.70 ± 0.00
Benz[*a*]anthracene	2.17 ± 0.14	1.60 ± 0.11	0.95 ± 0.14	0.40 ± 0.00
Chrysene	1.26 ± 0.09	0.95 ± 0.10	0.43 ± 0.05	0.34 ± 0.01
Benzo[*b*]fluoranthene	0.56 ± 0.05	0.43 ± 0.03	0.23 ± 0.01	0.20 ± 0.00
Benzo[*k*]fluoranthene	0.21 ± 0.03	0.15 ± 0.01	0.11 ± 0.02	0.08 ± 0.00
Benzo[*a*]pyrene	0.30 ± 0.03	0.23 ± 0.03	0.17 ± 0.02	0.09 ± 0.00
Dibenz[*a*,*h*]anthracene	0.19 ± 0.04	0.13 ± 0.03	0.15 ± 0.02	0.04 ± 0.01[a]
Benzo[*ghi*]perylene	0.23 ± 0.05	0.41 ± 0.06	0.34 ± 0.06	0.23 ± 0.04
Indeno[1,2,3-*cd*]pyrene	0.27 ± 0.06[a]	0.26 ± 0.02[a]	0.24 ± 0.01[a]	0.25 ± 0.01[a]
Light PAHs	63.59	42.56	43.45	7.79
Heavy PAHs	1.74	1.60	1.25	0.89
Total PAHs	65.33	44.15	44.71	8.67

[a] Below quantification limit.

Table 5
PAHs content (mean ± sd) in olive oil throughout the refining steps (μg/kg)

	Crude	Neutralized	Bleached	Deodorized
Naphthalene	10.64 ± 0.25	3.75 ± 0.14	4.40 ± 0.34	2.68 ± 1.04
Acenaphthene	3.06 ± 0.27	3.13 ± 0.40	4.34 ± 0.39	0.07 ± 0.01[a]
Fluorene	1.46 ± 0.20	1.24 ± 0.05	1.53 ± 0.05	0.08 ± 0.01
Phenanthrene	13.22 ± 0.88	12.09 ± 1.07	12.26 ± 0.82	1.08 ± 0.03
Anthracene	0.67 ± 0.05	0.61 ± 0.03	0. 66 ± 0.03	0.06 ± 0.00
Fluoranthene	3.79 ± 0.26	3.26 ± 0.34	3.47 ± 0.13	0.50 ± 0.02
Pyrene	2.57 ± 0.10	2.30 ± 0.16	2.89 ± 0.54	0.58 ± 0.02
Benz[*a*]anthracene	0.42 ± 0.01	0.42 ± 0.06	0.47 ± 0.05	0.18 ± 0.02
Chrysene	1.05 ± 0.13	0.74 ± 0.07	0.42 ± 0.08	0.38 ± 0.08
Benzo[*b*]fluoranthene	0.43 ± 0.04	0.39 ± 0.02	0.49 ± 0.02	0.31 ± 0.01
Benzo[*k*]fluoranthene	0.12 ± 0.00	0.13 ± 0.01	0.18 ± 0.03	0.11 ± 0.00
Benzo[*a*]pyrene	0.27 ± 0.00	0.20 ± 0.03	0.19 ± 0.02	0.17 ± 0.01
Dibenz[*a*,*h*]anthracene	0.29 ± 0.04	0.15 ± 0.02	0.10 ± 0.00	0.07 ± 0.00
Benzo[*ghi*]perylene	0.51 ± 0.01	0.41 ± 0.02	0.54 ± 0.19	0.35 ± 0.02
Indeno[1,2,3-*cd*]pyrene	0.45 ± 0.04	0.42 ± 0.00	0.43 ± 0.00	0.36 ± 0.01
Light PAHs	36.89	27.55	30.43	5.62
Heavy PAHs	2.06	1.70	1.94	1.38
Total PAHs	38.95	29.25	32.27	7.00

[a] Below quantification limit.

dos et al., 1988). Probably, two situations contributed to the great decreases observed in the latter two studies: the very high initial contamination level (1923 and 1941 μg/kg) and the application of activated charcoal in the bleaching process. With the exception of benzo[*ghi*]perylene in soybean oil, all the compounds were reduced through refining.

After refining, light PAHs contents decreased 71%, 88% and 85% in sunflower, soybean and olive oils, respectively, while the heavy PAHs content diminished 79%, 49% and 33%, also, respectively. The light PAHs decrease was within the values described in other studies (Cejpek et al., 1998; Dennis et al., 1991; Larsson et al., 1987) that varied between 57% and 92%. The exceptions were the works of Biernoth and Rost (1967) and Sagredos et al. (1988) that showed more than 99% of elimination of light PAHs.

The greater reduction in heavy PAHs content in the soybean oil during bleaching comparing to the other two oils could be explained by the use of activated charcoal in the process of the former, as described in the Samples section. This treatment significantly reduces the level of the higher molecular weight PAHs, as previously observed (Dennis et al., 1991; Kolarovic & Traitler, 1982). Using the same process, Biernoth and Rost (1967) and Sagredos et al. (1988) have decreased the heavy PAHs content to less than 1% of the original value. The results from other researchers showed a more modest reduction in the heavy PAHs content during refining: 11% (Dennis et al., 1991), 22% (Larsson

et al., 1987), and 41% (Cejpek et al., 1998). Nevertheless, activated charcoal was not used in any of these studies, which could help to explain the observed results.

In accordance with the work of Cejpek et al. (1998), neutralization and, chiefly, deodorization were the more effective steps contributing to the PAHs decrease, with the effect of the latter being more pronounced in light PAHs. Indeed, deodorization removes mainly the more volatile compounds, having little effect on the heavy PAHs (Biernoth & Rost, 1967; Cejpek et al., 1998). Neutralization and deodorization were involved in the reduction of the 5–6 rings PAHs, as has been shown before but only with statistical significance for benzo[*a*]pyrene (Cejpek et al., 1998). Bleaching was responsible for a slight increase in the PAHs content of soybean and virgin olive oils, and a decrease in sunflower oil. In the bleaching process of the former two oils activated charcoal was not used but only activated clay, which could be contaminated with PAHs explaining the observed raise in the PAHs levels, while in the latter oil it was used activated charcoal that avowedly reduces PAHs content (Cejpek et al., 1998).

In what concerns total PAHs evolution along the refining steps, deodorized soybean oil (8.67 μg/kg) was very similar to those determined in the commercial samples (9.31–10.81 μg/kg), while in sunflower oil deodorized sample exhibited a smaller concentration (4.90 μg/kg) than that observed in the purchased oils (8.78–9.72 μg/kg). In the non-refined olive oil sample the total PAHs (38.95 μg/kg) was higher than that of the commercial virgin olive oils analysed (26.35 and 18.02 μg/kg), whereas after refining the content was lower (7.00 μg/kg). The PAHs profiles observed in these through-refining samples were very similar to that ones of the commercial vegetable oils.

4. Conclusions

The PAHs contents observed in the analysed vegetable oils can be considered comparatively low, within those described by other authors. None of the samples analysed surpassed the limits recommended by GSFS, neither for total or heavy PAHs. An evident decrease of PAHs content during refining was observed, mainly for light PAHs. Neutralization and, particularly, deodorization were the more effective steps. In terms of food safety, the refining process of vegetable oils decrease these type of environmental contaminants.

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