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On-line DACC-HPLC analysis of polycyclic aromatic hydrocarbons in edible oils

Sandra Neđeral*, Dragutin Pukec, Dubravka Škevin, Klara Kraljić, Marko Obranović, Petra Zrinjan

Faculty of Food Technology and Biotechnology, University of Zagreb, Pierottijeva 6, 10000 Zagreb, Croatia

Summary

In this work an HPLC method for determination of polycyclic aromatic hydrocarbons (PAH) in edible oils on a DACC (donor-acceptor complex chromatography) column coupled with an on-line HPLC system with fluorescent detection. Method was used to determine the content of individual PAHs in the refined sunflower oil, virgin olive oil, cold pressed pumpkin seed oil, dark sesame oil and pumpkin seed oil produced with roasting obtained from domestic market. Calibration and validation were conducted for 13 individual PAHs among which are all 8 PAHs that are listed as priorities for determination in food and environment by both European Commission and US Environmental Protection Agency (EPA). Correlation coefficients of calibration curves for all standards were above 0.999 in selected range and all validation parameters (limit of detection, limit of quantification, accuracy and precision) were within limits set by European regulation. Content of PAHs in cold pressed pumpkin seed oil, roasted pumpkin seed oil and especially dark sesame oil was several-fold higher than the recommendations set by different European oil research and production organizations even though for both pumpkin seed oils it was lower than limits set by official European legislation. High content of cancerogenous heavy fraction PAHs were found in dark sesame oil that advises to limitation of its consumption in human diet..

Keywords: *polycyclic aromatic hydrocarbons, DACC-HPLC analysis, method development, method validation, edible oils*

Sažetak

U ovom radu proveden je razvoj i validacija HPLC metode za određivanje policikličkih aromatskih ugljikovodika (PAH) u jestivim uljima na DACC (eng. donor-acceptor complex chromatography) koloni povezanoj online s HPLC sustavom s fluorescentnim detektorom. Metodom je određen udjel pojedinačnih PAH-ova u rafiniranom ulju suncokreta, djevičanskom maslinovom ulju, hladno prešanom bučinom ulju, tamnom sezamovom ulju i nerafiniranom bučinom ulju proizvedenom uz prženje koja su nabavljena s domaćeg tržišta. Kalibracija i validacija napravljene su za 13 pojedinačnih PAH-ova među kojima je svih 8 PAH-ova koji se nalaze na listama prioriteta Europske zajednice i američke Agencije za zaštitu okoliša vezanim uz praćenje ovih spojeva u hrani i okolišu. Koeficijenti korelacije kalibracijskih pravaca u ispitivanom rasponu za sve spojeve bili su viši od 0,999, a svi parametri validacije (limit detekcije, limit kvantifikacije, točnost i preciznost) bili su unutar granica koje za metode za određivanje PAH-ova propisuje europsko zakonodavstvo. Udjeli PAH-ova u hladno prešanom bučinom ulju, nerafiniranom bučinom ulju proizvedenom uz prženje i posebice tamnom sezamovom ulju višestruko su premašili granice za udjel ukupnih PAH-ova koje preporučuju različite europske uljarske organizacije iako je udjel PAH-ova u bučinim uljima bio unutar limita propisanih službenih europskim propisima. U tamnom sezamovom ulju nađen je visok udjel kancerogenih PAH-ova teške frakcije što poziva na ograničenje primjene ovog ulja u prehrani ljudi.

Ključne riječi: *policiklički aromatski ugljikovodici, DACC-HPLC analiza, razvoj metode, validacija metode, jestiva ulja*

Introduction

Polycyclic aromatic hydrocarbons (PAH) present a class of diverse organic compounds, each of them containing two or more aromatic rings. Some of these compounds are known or suspected to be mutagenic and/or carcinogenic in mammals. PAH containing up to four fused benzene rings are known as light PAH and those containing more than four benzene rings are called heavy PAH. Heavy PAH are more stable and considered to be more toxic than the light ones (Wenzl et al., 2006). Polycyclic aromatic hydrocarbons originate mainly from incomplete combustion and pyrolysis processes of organic compounds at high temperatures. However, aromatization can occur at lower temperatures ranging from 100 to 150 °C (Moret and Conte, 2000). PAH compounds are emitted from processing of coal, crude oil, petroleum, and natural gas, from production of aluminum, iron and steel, from heating in power plants and homes, burning of refuse, wood fires, and from motor vehicle exhausts (SCF, 2002).

In 2002 Scientific Committee on Food of the European Union (SCF, 2002) concluded that 15 PAHs, namely benzo[a] anthracene, benzo[b]fluoranthene, benzo[j]fluoranthene, benzo[k]fluoranthene, benzo[g,h,i]perylene, benzo[a]pyrene, chrysene, cyclopenta[c,d]-pyrene, dibenz[a,h]anthracene, dibenzo[a,e]pyrene, dibenzo[a,h]pyrene, dibenzo[a,i]pyrene, dibenzo[a,l]pyrene, indeno[1,2,3-c,d]pyrene and 5-methylchrysene show clear evidence of mutagenicity/genotoxicity in somatic cells in experimental animals in vivo and with the exception of benzo[g,h.i]perylene also show clear carcinogenic effects in various types of bioassays in experimental animals. Commission Regulation No. 1881/2006 (European Commission, 2006) therefore limited the content of benzo[a]pyrene in oils and fats (excluding cocoa butter) intended for direct human consumption or use as an ingredient in foods to 2 µg/kg. In 2011 new Commission Regulation No. 835/2011 (European Commission, 2011a) amending the one from 2006 (European Commission, 2006) was passed stating that benzo[a]pyrene is not a suitable marker for the occurrence of the other PAHs in food and hence a system of four specific PAHs (benzo[a]pyr-

Corresponding author: snedjer@pbf.hr

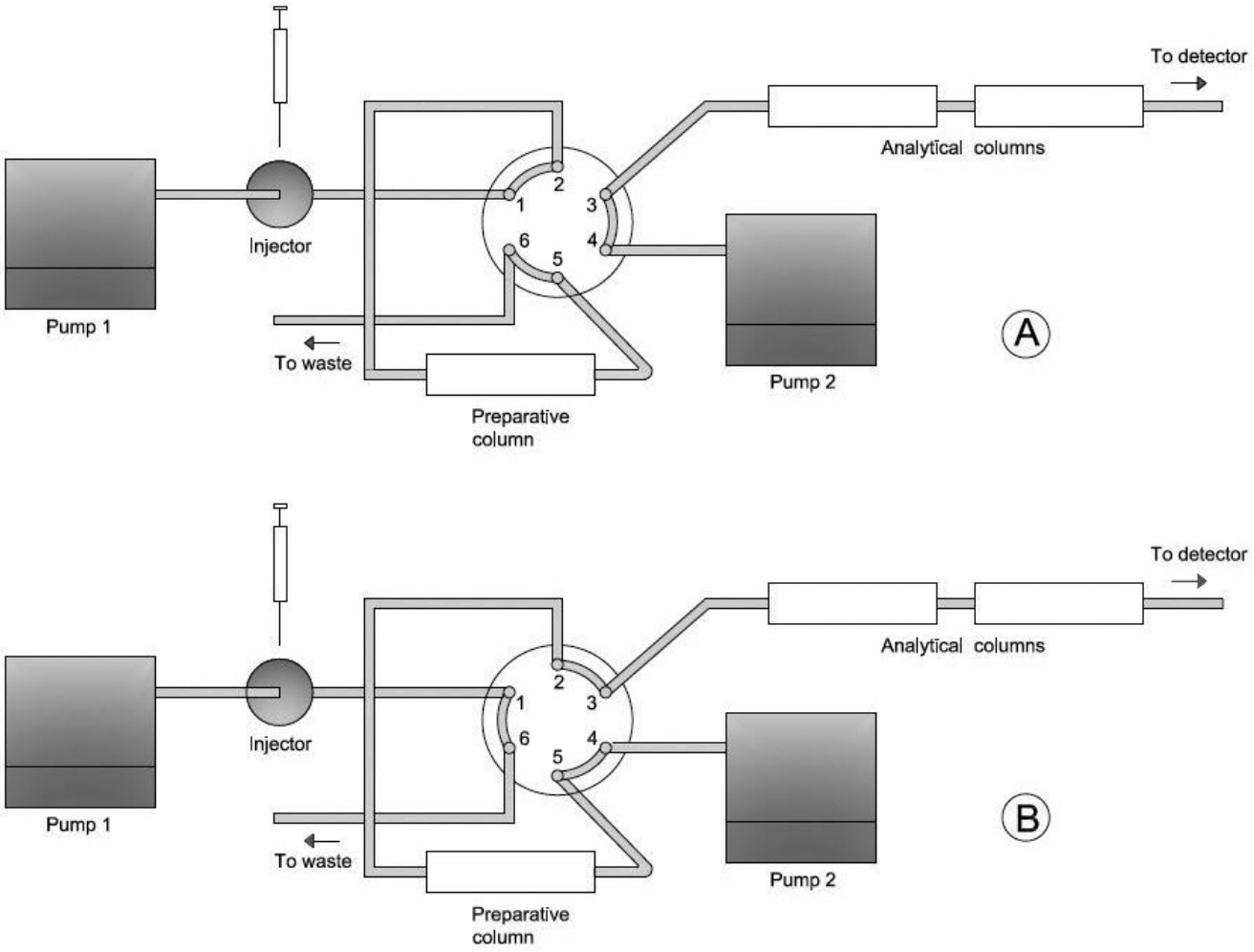

Figure 1. *HPLC system used for PAH sample and determination with loading (A) and injecting (B) positions of 6-port valve.*

ene, benzo[a]anthracene, benzo[b]fluoranthene and chrysene) would be more suitable. In addition to the limit set for benzo[a]pyrene it also limited the sum of these four PAHs to 10 µg/kg. United States Environmental Protection Agency (EPA, 1994) also issued a list of 16 PAH with environmental priority. The compounds on this list are somewhat different than the European priority ones however there are 8 PAH compounds that are shared by the two lists, namely benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenz[a,h]anthracene, benzo[g,h,i]perylene and indeno[1,2,3-c,d]pirene.

Because of their increased presence in environment together with their lipophilic character PAH they are frequent contaminants of oils and fats due to absorption from polluted soil to the seeds. Another potential source for PAH in fats and oils is the production process of an oil mainly if it includes processing at high temperatures e.g. roasting step in the production of pumpkin seed oil (Fruhwirth and Hermetter, 2007). Therefore the monitoring of PAH in various oils and fats is of highest importance. Other European organizations established their own recommendations such as the German Society for Fat Science (DGF) that suggests that total PAHs in edible oils should not be over 25 µg/kg and heavy PAHs should be below 5 µg/kg (Cejpek et al., 1998). Also, FEDIOL (Féderation de l'Industrie d'Huilerie de la Communauté Européenne) has accepted a limit of 25 µg/kg for total PAH, 5 µg/kg for heavy PAH, and 1 µg/kg for benzo[a]pyrene in edible fats and oils. In addition, other European countries (Spain, Italy, Portugal and Greece) have set limits for 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[g,h,i]perylene, indeno[1,2,3-c,d]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 (Spanish Official Bulletin, 2001; Gazzetta Ufficiale, 2001).

Traditionally used methods (Moret and Conte, 2002; Wenzel et al., 1998) for PAH determination in oils and fats are extremely time taking and expensive due to the nature of lipophilic matrices which possess the physicochemical properties that are similar to those of PAH, making them therefore very complex to isolate one from another. The isolation before the actual determination by HPLC with fluorescence detection or GC–MS is necessary because of the coeluting compounds contained in oils and fats that would interfere with PAH during analysis. In recent years, donor-acceptor complex chromatography has become a popular method for the determination of PAH. DACC stationary phases can be used for solid phase extraction (SPE), retaining PAHs while matrix components are flushed to waste. After elution of the analytes, solvent exchange is used to prepare the sample for HPLC analysis. Stjin et al.

(1996) developed an automated process for sample preparation and analysis consisting of an LC-LC coupling of a clean-up DACC column and analytical column. Since then various completely automated methods for the on-line determination of PAH in oils and fats were developed (Dionex, 2008; ISO, 2009). Even though very simple and fast to perform this methods require very expensive HPLC configuration consisting of an autosampler and special SPE unit.

In this paper we report an HPLC method, with fluorescence detection, for analysis of PAH in edible oils and solid food products using a simple HPLC gradient pump for the sample loading to DACC column and clean-up that was connected on-line to an HPLC with fluorescence detector. Second HPLC pump was used for PAH elution and analysis. Method was validated using (ICH, 2005) and used to determine PAH content in several unrefined and refined vegetable oils.

Materials and Methods

The method used in this work is based on donor-acceptor complex chromatography (DACC) developed by Stijn et al. (1996). The HPLC unit consisted of gradient pump (Pump 1) used for loading of the sample to the DACC purification column and eluting the oil from the column with isopropanol to waste. After the oil has been flushed from the purification column the 6-port valve was switched to backflush the PAHs and load them into analytical columns. In this step a second HPLC pump (Pump 2) was used. The described system with regards to "loading" and "injecting" position of the 6-port valve is shown in Figure 1.

Reagent and materials

Isopropyl alcohol, acetonitrile and ethyl acetate were provided by T.J. Parker (Deventer, Netherlands) and were of HPLC grade. Mixture of PAHs at various concentrations in methylene chloride: methanol (1:1) were purchased from Supelco (Bellefonte, USA). This standard mixture contains the 16 EPA PAHs, however, not all 16 EPA PAHs can be detected with this method.

Samples

The materials investigated were various oil obtained from the market shelves in Croatia. Refined sunflower oil, virgin olive oil, cold-pressed pumpkin seed oil, roasted sesame oil and roasted pumpkin seed oil were used as representatives of oil categories set by national legislation (Regulation 41/12, 2012).

Instrumentation

For sample loading to DACC column a HPLC pump (Pharmacia LKB Biotechnology AB, Uppsala, Sweden) was used equipped with 250 µL sample loop. The DACC column was a Chromspher Pi 80×3mm, from Varian (Sint-Katelijn-Waver, Belgium). Analysis and detection of PAH were performed on a HPLC system composed of Varian 9010 gradient pump and Varian Pro Star 363 fluorescence detector (Varian, Sint-Katelijn-Waver, Belgium). Two analytical columns Pursuit 5 PAH 250×4.6mm×5µmVarian (Sint-Katelijn-Waver, Belgium) were kept at the constant temperature of 30 °C.

Sample and standard solutions preparation

Dilution of oil: 4 g of oil samples were weighted in a 10 mL volumetric flask diluted with cyclohexane and filtered through 0.45 µm PTFE filter. The sample was then ready for DACC.

Blank oil matrix that was used for method calibration and validation was prepared. Due to the fact that the method was principally developed for the determination of PAH in pumpkin seed oils same was used for the preparation of the oil matrix. 200 mL of pumpkin seed oil was placed in a 500 mL round bottom flask and 10 d of activated carbon was used for the absorption of PAH. Mixture was heated for 2 hours at 90°C, left to separate and centrifuged at 4000 rpm. Oil was then filtered through PTFE 0.45 filters and kept at 4°C until analysis. Purchased PAH standards were used to prepare standard stock solutions and working solutions by adding them to the prepared blank oil and thus obtaining the required concentrations.

Table 1. *Gradient program for the separation of PAH on HPLC pump 2*

Time (min)	Flow (mL/min)	%A (Water)	%B (Ethyl acetate)	%C (Acetonitrile)
0	0.4	20	0	80
2.49	0.4	20	0	80
2.50	1	20	0	80
3.90	1	20	0	80
17.90	1	0	0	100
45.99	1	0	0	100
46.00	1	0	30	70
68.00	1	0	30	70
69.00	1	0	0	100
73.00	1	0	0	100
83.00	1	20	0	80
90.00	0.40	20	0	80

HPLC analysis

The DACC column was connected to an HPLC system with a 6-port valve that was manually operated. 250µL of the diluted oil was injected on the column and the matrix components were eluted in the forward direction with isopropanol at 0.35ml/min during11.5 min. After that time 6-port valve was turned into inject position and Pump 2 was switched on. The PAHs were then eluted from the preparation column in the backflush mode using the conditions shown in Table 1. DACC column was cleaned before each run with isopropyl alcohol at 0.35 mL/min for 10 minutes. Detection of the eluted PAHs was carried out by application of a variable wavelength fluorescence detector. Excitation and emission wavelengths are given in Table 2.

Table 2. *Fluorescence detector program for detection of individual PAH compounds*

Time (min)	Excitation (nm)	Emission (nm)	PAH detected
0.0	225	320	-
14.5	256	390	Fluorene, phenantrene, anthracene
16.7	240	460	Fluoranthene
18.0	240	390	Pyrene
19.5	270	385	Benzo[a]anthracene, chrysene
24.0	290	430	Benzo[b]fluorantene, benzo[k]fluorantene, benzo[a]pyrene, dibenzo[a,h]anthracene,
39.0	305	480	Benzo[g,h,i]perylene, indeno[1,2,3,-c,d]pyrene
55.0	305	480	-

Quantification

For the creation of calibration curves a 5-point calibration range was used for all PAHs. Phenantrene, anthracene, pyrene, benzo[a]anthracene, crysene, benzo[k]fluoranthen and benzo[a]pyrene were calibrated at 0.25, 0.5, 2.5, 10 and 17.5 µg/kg of oil. The calibration ranges used for fluoren, fluoranten, benzo[b]fluoranten, benzo[a,h]antracen and benzo[g,h,i]perilen were: 0.5, 1, 5, 20 and 35 µg/kg of oil while indeno[1,2,3-c,d]pyrene first level was set at 1 µg/kg and last at 60 µg/kg due to the lower responses on fluorescence detector.

Validation

Method was validated for linearity, limit of detection (LOD) and limit of quantification (LOQ), accuracy and precision according to ICH procedures for validation of analytical methods (ICH, 2005) that comply with international requirements. For validation purposes a purified pumpkin seed oil was used as blank oil matrix in which the determined concentrations of standards were added.

Linearity was established through correlation coefficients (r2) of the calibration curves for the described ranges obtained by triplicate injections.

The LOD and LOQ were determined by seven independent sample blanks fortified with PAHs at lowest level of calibration for each PAH analyzed and calculated from the slope of calibration curve (S) and standard deviation of the response (σ) as LOD=3.3 σ/S and LOQ=10 σ /S respectively.

Recovery was evaluated by spiking of blank samples with PAHs at 1 and 5µg/kg for phenantrene, anthracene, pyrene, benzo[a]anthracene, crysene, benzo[k]fluoranthen, benzo[a] pyreneandindeno[1,2,3-c,d]pyrene and at 2 and 10µg/kg for fluoren, fluoranten, benzo[b]fluoranten, benzo[a,h]antracen and benzo[g,h,i]perilen.

Repeatability was evaluated as relative standard deviation (RSD%) at 1 and 5µg/kg for phenantrene, anthracene, pyrene, benzo[a]anthracene, crysene, benzo[k]fluoranthen, benzo[a] pyrene and indeno[1,2,3-c,d]pyrene and at 2 and 10µg/kg for fluoren, fluoranten, benzo[b]fluoranten, benzo[a,h]antracen and benzo[g,h,i]perylene measured in seven replicated injections during the same day and then checking the RSD of peak areas.

For statistical analysis of results Statistica10 (Statistica, 2012) software was used.

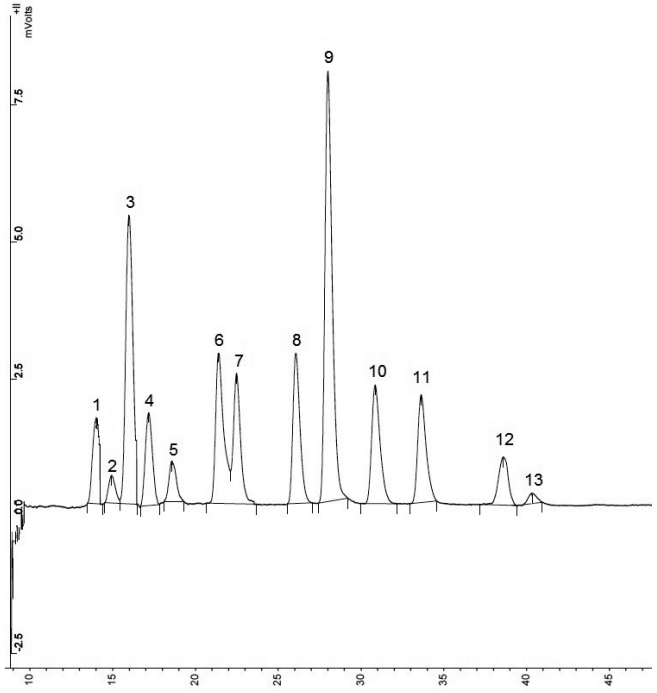

Figure 2. *HPLC chromatogram of 13 PAHs in blank oil spiked at the level of 5-10 µg/kg with the standard mixture (peaks – 1: Fluorene; 2: Phenantrene; 3: Anthracene; 4: Fluoranthene; 5: Pyrene; 6: Benzo[a] anthracene; 7: Crysene; 8: Benzo[b]fluoranthene; 9: Benzo[k]fluoranthene; 10: Benzo[a]pyrene; 11: Dibenzo[a,h]anthracene; 12: Benzo[g,h,i]perylene; 13: Indeno[1,2,3-c,d]pyrene)*

Results and discussion

This method was primarily developed for the determination of PAHs in unrefined pumpkin seed oils obtained from roasted seeds, therefore pumpkin seed oil purified from PAHs by active carbon was used as blank. After oil purification, some impurities persisted which could have affected determination of some PAHs therefore the baseline of the purified pumpkin seed oil blank was subtracted during data processing. In addition, it was determined that analyzed pumpkin seed oil samples show a peak with very large area, eluting between peaks of benzo[a]anthracene and crysene standards and covering their peaks in the HPLC analysis. Concentrations of benzo[a]anthra-

Table 3. *Method calibration data for 13 PAH compounds*

PAH	Method linearity range (μg/kg)	Calibration curve*	Correlation coefficient r2
Fluorene	0.5-35	y=2635.2x+735.07	0.9995
Phenantrene	0.25-17.5	y=1876.5x-65.047	0.9996
Anthracene	0.25-17.5	y=16083x+1234.5	0.9997
Fluoranthene	0.5-35	y=2592.6x+177.98	0.9998
Pyrene	0.5-35	y=3101.4x+1288.8	0.9995
Benzo[a]anthracene	0.25-17.5	y=7374.9x+9397.7	0.9994
Crysene	0.25-17.5	y=6322.8x+5884.8	0.9994
Benzo[b]fluoranthene	0.5-35	y=4430.4x-55.422	0.9996
Benzo[k]fluoranthene	0.25-17.5	y=24847x+3326.8	0.9999
Benzo[a]pyrene	0.25-17.5	y=7556.4x+177	0.9994
Dibenzo[a,h]anthracene	0.5-35	y=3633.4x-47.933	0.9998
Benzo[g,h,i]perylene	0.5-35	y=2067.1x+487.18	0.9994
Indeno[1,2,3-c,d]pyrene	1-60	y=1216x-904.21	0.9993

*y – peak area, x – concentration (μg/kg)

cene and crysene therefore couldn't be determined in pumpkin seed oils. It is supposed that mentioned peak belongs to some other, non-PAH compound coeluting with the compounds of interest. In addition, roasted sesame seed oil showed large peak eluting at retention time of fluorene standard. Calculated based on calibration curve for fluorene concentration of that peak would measure around 2800 μg/kg exceeding greatly linearity range. Moreover, such high levels of PAH in roasted sesame seed oil weren't reported in literature therefore the peak was not quantified.

For quantification purposes external standard method was used through diluting of standard PAH mix with purified blank oil. Calibration linearity was investigated by making three replicate injections of a standard PAH mix prepared at five different concentrations, described in the previous section, to ensure a well fitted calibration curve. Even though the standard mixure contained 16 PAHs not all of them could be determined by this method. Peaks of naphthalene, acenaphtene and acenaphtylene were not separated due to the bad resolution in the beginning of the chromatogram. Calibration curves for the rest 13 PAH are shown in Table 3.

For the curve fit function, a linear regression line, i.e. a function ($Y= \alpha X + \beta$) was chosen with Y as the peak area and X as the concentration of analyte. Calibration curves for all standards had correlation coefficients above 0.999 for the linear regression in the range studied. Due to the lower response on fluorescence detector indeno[1,2,3-c,d]pyrene was prepared in higher concentration range (1-60 μg/kg) for which a linearity range was confirmed. Similar ranges of linearity were found in works of other authors (Windal et al., 2008; Ciecierska and Obiedzinski, 2013).

In order to determine the limit of detection (LOD) and limit of quantification (LOQ) seven independent sample blanks fortified with PAHs at lowest level of calibration for each PAH.

LOD and LOQ were calculated using three and ten times the standard deviation on the calculated amount in the spiked blank samples, respectively and dividing it by the slope of regression line. Results presented in Table 4 as a part of methods sensitivity study show that limit of detection ranged from 0.04oil for benzo[k]fluoranthene to 0.61 μg/kg oil for indeno[1,2,3-c,d]pyrene. The values for limits of quantification ranged therefore from 0.13 μg/kg oil for benzo[k]fluoranthene to 1.84 μg/kg oil for indeno[1,2,3-c,d]pyrene. Comparing these results to the Commission Regulation (EU) No. 836/2011 (European Commission, 2011b) that states performance criteria for methods of analysis for benzo[a]pyrene, benzo[a]antrachene, benzo[b] fluoranthene and chrysene being lower than 0.3 μg/kg for LOD value and lower than 0.9 μg/kg for LOQ value it is clear that the described method fulfills these parameters for all analyzed compounds except indeno[1,2,3-c,d]pyrene that was found to have lower response on FD.

For testing of the methods accuracy, blank samples were spiked with 1-2 μg/kg as well as 5-10 μg/kg standard and each solution was injected seven times. Mean results (Table 4) show satisfactory mean accuracy of the proposed method with results varying from 94.1% for pyrene to 109.4% for anthracene compared to recovery from 50-120% proposed by Commission Regulation (EU) No. 836/2011 (European Commission, 2011b).

Precision was tested as repeatability with spiked blank samples of concentrations 1-2 and 5-10 μg/kg analyzed seven times during the same day and is shown as RSD (%) in Table 4. The obtained results generally showed good method precision and the highest RSD% was 4.7% for benzo[a]pyrene.

It was therefore proven that proposed method satisfied validation parameters for the determination of 13 PAH determined providing a relatively inexpensive and time sav-

ing procedure for assessment of their content in various oils and fats. Among analyzed PAHs are all compounds set by the EFSA (2008) as the most suitable indicators of PAHs in food (benzo[a]pyrene, benzo[a]anthracene, benzo[b]fluoranthene and chrysene) known as PAH4, as well as eight PAHs (PAH8) with high carcinogenic potential (benzo[a]anthracene, chrysene, benzo[b]fluoran-thene, benzo[k]fluoranthene, benzo[a]pyrene, indeno[1,2,3-c,d]pyrene, di-benzo[a,h]anthracene and benzo[g,h,i]perylene).

Table 4. *Validation parameters for 13 PAH compounds (LOD – limit of detection; LOQ – limit of quantification, RSD – relative standard deviation)*

PAH	LOD (μg/kg)	LOQ (μg/kg)	Recovery* (%)	RSD* (%)
Fluorene	0.29	0.87	105.7	4.2
Phenantrene	0.30	0.92	106.7	3.0
Anthracene	0.05	0.15	109.4	4.2
Fluoranthene	0.05	0.17	108.8	2.7
Pyrene	0.16	0.49	94.1	2.7
Benzo[a]anthracene	0.20	0.61	102.3	2.3
Crysene	0.09	0.27	102.1	2.7
Benzo[b]fluoranthene	0.07	0.22	99.8	2.4
Benzo[k]fluoranthene	0.04	0.13	96.4	3.6
Benzo[a]pyrene	0.18	0.56	101.7	4.7
Dibenzo[a,h]anthracene	0.23	0.70	100.7	2.7
Benzo[g,h,i]perylene	0.14	0.43	96.7	4.3
Indeno[1,2,3-c,d]pyrene	0.61	1.84	109.3	3.7

**Recovery and relative standard deviation (RSD) measured at 1-2 and 5-10 μg/kg expressed as mean values of n=7 injections*

As it was mentioned before, due to their toxic potential and high occurrence in oils and fats, determination of PAH in this type of products is of great importance. Furthermore, data from various authors show that maximum tolerable limits for their contents set by different authorities are often exceeded. In a recent research of (Ciecierska and Obiedzinski, 2013) PAH contents in various vegetable oils from unconventional sources including sesame seed oil, pumpkin seed oil and flax seed oil was determined. Pumpkin seed oil was found to have the highest content of PAHs reaching 234.3 μg/kg. Relatively high levels were also found in common flax seed oil and sesame seed oil of 170.89 μg/kg and 30.09 μg/kg respectively. In addition, in the case of pumpkin seed oil the mean level of B[a]P content was about 8 times higher than the maximum tolerable limit stated in the Commission Regulation (EU) No. 835/2011 (European Commission, 2011a), being equal to 2 g/kg. The mean sum of EFSA (2008) 4 PAHs (benzo[a]pyrene, chrysene, benzo[a]anthracene and benzo[b]fluorantene) was 3.5 times higher than the maximum tolerable limit set at the level of 10 μg/kg by the Commission Regulation (EU) No. 835/2011 (European Commission, 2011a).

In the work of Węgrzyn et al. (2006) PAH content in various foodstuff was investigated. Concentrations of benzo[a] pyrene (and of the other PAHs) in various vegetable oils were below the maximum value for edible oils (2 μg/kg) except for cold pressed rapeseed oil, pumpkin and sesame seed oils.

Teixeira et al. (2007) were determining the content of PAHs in extra virgin olive oil, as well as sunflower oil and soybean oil during refining. They state that the total PAHs content in the studied samples was generally low as well as that the light PAHs were predominant. Highest values (up to 26 μg/kg) were measured in virgin olive oils. Alkaline refining caused a decrease of PAHs contents especially of light PAHs with the neutralization and deodorization being the most effective refining steps. The same effect of refining was also stated in the work of Cejpek et al. (1998). Neutralization and deodorization were involved in the reduction of the 5-6 rings PAHs but only with statistical significance for benzo[a]pyrene.

Unrefined oils are therefore known to contain higher share of light PAHs. Moret et al. (1997) in their research of PAH concentrations in virgin olive oils found more than 25 μg/kg in most of the analyzed samples. Only one sample had more than 5 μg/kg of the heavy PAH fraction and more than 1 μg/kg of benzo[a]pyrene.Analysis of olive oil (virgin + refined blends) showed lower quantities of total PAHs than those measured in virgin olive oils. However four out of five analyzed samples had more than 5 μg/kg of the heavy PAH fraction.

In this work, developed method was used to determine the PAH content in vegetable oils obtained from the local market. Among them, sunflower oil was used as the representative of refined oils group, virgin olive oil and cold pressed pumpkin seed oil were used from the cold pressed oils group. Roasted sesame oil and pumpkin seed oils were used as oils that underwent thermal treatment during the production process. Namely in the production of such oils, integral or milled seeds with or without the addition of water are roasted at high temperatures. In the case of pumpkin seed oil temperatures used are in the range 110-130 °C (Neđeral et al., 2012), while in the production of sesame oil even higher roasting temperatures reaching 250 °C are used (Lee et al., 2010). Even though it is known that temperatures of 500-700 °C are optimal for the PAH creation it is well recognized that some of this compounds are created in various sources at lower temperatures and even at room temperatures (Guillén et al., 2008)

Formation of phenanthrene, anthracene, and benzo[a] anthracene during roasting of coffee beans was observed at temperatures above 220 °C, whereas formation of pyrene and chrysene required 260 °C. Low levels of benzo[g,h,i]perylene were also noted in dark roasting under 260 °C (Houessouetal., 2007). It is therefore probable that at roasting temperatures required for the production of sesame and pumpkin seed oil different PAHs would be created.

Lowest values of PAHs in oils analyzed in this work were found in refined sunflower oil. Namely, none of the PAH species were found in measurable quantities and levels of registered peaks of fluoranthene and pyrene were below their quantification limits proving that the refining process was well conducted.

Virgin olive oil also showed low levels of PAHs with 3.44 μg/kg of total PAH determined. Those values belong to the low

***Table 5**. PAH compounds in various refined, unrefined and cold pressed vegetable oils*

PAH* (µg/kg)	Refined sunflower oil	Virgin olive oil	Cold pressed pumpkin seed oil	Roasted sesame oil	Roasted pumpkin seed oil
Fluorene	n.d.	<LOQ	6.30	n.d.	11.75
Phenantrene	n.d.	2.38	12.80	29.67	10.67
Anthracene	n.d.	0.16	1.87	n.d.	2.16
Fluoranthene	<LOQ	0.76	4.94	7.44	5.51
Pyrene	<LOQ	0.13	3.25	n.d.	2.37
Benzo[a]anthracene	n.d.	<LOQ	n.d.	0.81	n.d.
Crysene	n.d.	<LOQ	n.d.	3.18	n.d.
Benzo[b]fluoranthene	n.d.	n.d.	n.d.	n.d.	0.60
Benzo[k]fluoranthene	n.d.	<LOQ	n.d.	<LOQ	<LOQ
Benzo[a]pyrene	n.d.	n.d.	n.d.	n.d.	0.70
Dibenzo[a,h]anthracene	n.d.	n.d.	n.d.	13.47	1.02
Benzo[g,h,i]perylene	n.d.	n.d.	n.d.	8.09	n.d.
Indeno[1,2,3-c,d]pyrene	n.d.	n.d.	n.d.	40.89	n.d.
TOTAL	0	3.44	29.16	103.56	34.79

n.d. - *not determined;* <LOQ - *below limit of quantification*
** Results shown are mean of 3 parallel determination*

end of range previously reported for virgin olive oil (Moret et al., 1997) and also comply with the legally set PAH limits.

PAH levels in cold pressed pumpkin seed oil and roasted pumpkin seed oil were 29.16 and 34.79 µg/kg respectively. While, in cold pressed pumpkin seed oil only the PAHs from the light fraction were registered, roasted pumpkin seed oil contained certain amounts of benzo[b]fluoranthene, benzo[a] pyrene and dibenzo[a,h]anthracene. Compared to the limits set by European Commission (2011a) it is obvious that both these oils meet determined limits that require the level of benzo[a] pyrene lower than 2 µg/kg and sum of 4 priority PAH lower than 10 µg/kg. However compared to German and French recommendations (Cejpek et al., 1998) content of total PAH for both pumpkin seed oil exceeds the value of 25 µg/kg.

Gfrerer et al. (2004) developed a method for determination of PAH content in pumpkin seed oils. They found differences in the content which they attributed to the roasting step of pumpkin seed oil production. As a consequence, the authors recommend monitoring the PAH content of Styrian pumpkin seed oils produced for the consumer market.

PAH content in dark sesame oil was the highest among all analyzed samples reaching 103.56 µg/kg. This oil also contained up to 62.44 µg/kg of heavy PAH fraction and it was the only oil in which peaks of 6-ring PAH benzo[g,h,i]perylene and indeno[1,2,3-c,d]pyrene were determined. Furthermore 6-ring indeno[1,2,3-c,d]pyrene was the predominat PAH registered in dark sesame oil with 40.89 µg/kg. Considering the fact that this oil is produced at very high temperatures of up to 250 °C such high amounts of PAHs are expected and have been reported in previously published works (Ciecierska and Obiedzinski, 2013). It is therefore important to advise reconsideration of this traditional sesame oil production process and, in addition, to limit its consumption before its effect on health is investigated in more detail.

Conclusions

Method developed in this work meets all the requirements set by Commission Regulation (EU) No. 836/2011 (European Commission, 2011b) in terms of method validation parameters (limit of detection, limit of quantification, precision and accuracy) and is therefore suitable for determination of 13 polycyclic aromatic hydrocarbons in edible oils. Total content of PAHs was the lowest in sunflower oil in which two PAH species were identified but their share was below limit of quantification. Olive oil contained total of 3.44 µg/kg PAHs that belongs to the low range of previously reported data and also below the legal limits. Both pumpkin seed oils contained relatively high levels of PAH with roasted pumpkin seed oil containing certain amount of PAHs from heavy fraction. Highest level of total PAHs was registered in dark sesame oil reaching 103.56 µg/kg out of which as high as 62.44 µg/kg were of the heavy PAH fraction making this oil inedible according to the valid European regulation ….

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