ترجمه فا

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Analytical Methods

Determination of polycyclic aromatic hydrocarbons in edible oils and barbecued food by HPLC/UV–Vis detection

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A B S T R A C T

Determination of nine polycyclic aromatic hydrocarbons in corn, sunflower, olive oils and barbecued meat and fish by HPLC/UV–Vis method is described. The extraction procedure included a saponification, liquid–liquid extraction and finally purification of PAHs through a house-made silica–alumina column. Chromatographic determination was based on separation of PAHs on ODS column and measurement at 254 nm. All polycyclic aromatic hydrocarbons were separated and analyzed in 12 min on reversed phase ODS column with acetonitrile/water mobile phase at 1.5 mL min\(^{-1}\) flow rate. The detection limits of nine polycyclic aromatic hydrocarbons ranged from 0.26 to 1.15 \(\mu\)g L\(^{-1}\) at a signal/noise ratio of 3. The linearity of the method was between 0.9951 and 0.9996. Oil samples contain different PAHs ranging from 0.44 to 98.92 \(\mu\)g L\(^{-1}\). Barbecuing process increased the concentration (in the range of 2- to 8-fold) and caused the formation of PAHs in food samples.

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1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) constitute a large class of organic compounds that are composed of two or more fused aromatic rings. They are primarily formed through incomplete combustion or pyrolysis of organic matter and during various industrial processes. At high temperature, organic compounds are partially cracked to smaller unstable fragments (pyrolysis), mostly radicals, which recombine to give relatively stable PAHs (Lee, Novotny, & Bartle, 1981). PAHs are also formed as a result of certain food preparation methods, for instance grilling, roasting and smoking. The highest PAH concentrations are usually found in charcoal grilled/barbecued foods (especially meat and meat products grilled under prolonged and severe conditions), foods smoked by traditional techniques (fish in particular), mussels and other seafood from polluted waters (Farhadian, Jinap, Abas, & Sakar, 2010; Guillon, Sopelana, & Partearroyo, 1997; Phillips, 1999; Stolyhwo & Sikorski, 2005; Stumpe-Viksna, Bartkevics, Kukare, & Morozovs, 2008).

Different routes of PAHs contamination in edible oils and food have been suggested. Seed drying processes, environmental contamination, such as deposition of airborne particulates on crops or growth in contaminated soil, technological processing or home-cooking, such as grilling and smoking can be responsible for major PAHs contamination of some edible oils and food (Dennis et al., 1991; Farhadian et al., 2010; Larsson, Eriksson, & Cervenka, 1987; Moret & Conte, 2000; Purcaro, Morrison, Moret, Conte, & Marriott, 2007). Smoked and grilled food may contribute significantly to the intake of PAHs, if such foods are a large part of the usual diet. For example, grilled/barbecued meat was the second highest contributor, after the "bread, cereal and grain" group, in a U.S. study (Butler, Post, Lioy, Waldman, & Greenberg, 1993; Rey-Salgueiro, Garcia-Falcon, Martinez-Carball, & Simal-Gandara, 2008).

A number of PAHs have been shown to be genotoxic carcinogens. In 2002, the Scientific Committee on Food (SCF) reviewed PAH toxicity (SCF, 2002). For 15 compounds it concluded that there was clear evidence for their toxicity. In 2005, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) performed a risk assessment on PAHs, basically agreed with the SCF selection, downgraded one substance from the SCF list and nominated one further compound for observation in food (JECFA, 2005). The combined list nominated by either SCF or JECFA would thus comprise of 16 substances as priority PAHs: benz[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,l]pyrene, dibenzo[a,l]pyrene, dibenzo[a,i,]pyrene, dibenzo[a,l]pyrene, indeno[1,2,3-cd]pyrene, 5-methylchrysene and benzo[c]fluoranthene (EFSA, 2008a; IRAC, 2009). There will also be brief mention of other nine compounds tested by Member States, acenaphthene, acenaphthylene, anthracene, benzo[bk]fluoranthene, fluoranthene, fluorene, naphthalene, phenanthrene and pyrene (EFSA, 2008b).
Benzo[a]pyrene may be used as a marker of occurrence and effect of the carcinogenic PAHs in food, based on examinations of PAH profiles in food and on evaluation of a recent carcinogenicity study of coal tars in mice (EFSA, 2008a, 2008b; SCF, 2002; WHO, 2005). A conservative assessment would imply that the carcinogenic potency of total PAHs content in foods products would be 10 times of that contributed by benzo[a]pyrene alone. The Committee however stressed that though, it considers benzo[a]pyrene as a marker of carcinogenic PAH in food, chemical analyses should continue to collect data on the whole PAHs profile in order to be able to evaluate the contamination of food commodities and any future change in the PAHs profile.

In view of disparities caused by different maximum levels for PAHs in food in several Member States, the European Commission set harmonized maximum level of 2.0 µg kg⁻¹ of benzo[a]pyrene in foodstuffs containing fats and oils (EFSA, 2008a). The level is set for food for infants and young children. (EFSA, 2008a, 2008b; SCF, 2002; Zougagh, Redigolo, Rios, & Valcarcel, 2004).

Gas chromatography and high performance liquid chromatography are the most used chromatographic techniques for separation and quantification of PAHs in different kinds of foodstuffs. HPLC with fluorometric detection in mussels (Serpe, Esposito, Gallo, & Serpe, 2010), in propolis (Moret, Purcaro, & Conte, 2010), in grilled meat (Farhadian et al., 2010), in infant foods and toasted bread (Rey-Salguiero et al., 2008; Rey-Salguiero, Martinez-Carballo, Garcia-Falcon, Gonzalez-Barreiro, & Simal-Gandara, 2009), in distilled alcoholic beverage (Touni et al., 2007), in milk (Kishikawa, Wada, Kuroda, Akiyama, & Nakashima, 2003), in grape seed oil (Moret, Dudine, & Conte, 2000), in edible oil (Barranco et al., 2003), in vegetable oil (Zougagh et al., 2004), with UV detection in industrial waste oil (Domeno & Nerin, 2003) and gas chromatography with mass spectroscopic detection in smoked fish (Stumpf-Viksa et al., 2008), in olive oil (Purcaro et al., 2007), in fishing settlement (Anyakora, Ogbeche, Palmer, & Coker, 2005), in electrical insulating oils (Kim et al., 2001), in transformer oil (Pillai et al., 2005) have been successfully applied for the determination of PAHs.

PAHs have attracted most attention because of their carcinogenic potential. Since, edible oils and barbecued food are the daily consumed foodstuff, the level of PAHs should be determined. The aim of the work was to optimize an analytical method that could be used in most analytical laboratories for the determination and investigation the levels of the PAHs in oils and barbecued food and studying the effect of barbecuing on increasing and forming of PAHs during cooking process. We developed a HPLC method with an UV–Vis detection for the determination of nine PAHs (three have been remarked as genotoxic carcinogens by both SCF and JECFA and six by other Member States (EFSA, 2008b); fluorene, phenanthrene, anthracene, benzo[k]fluoranthene, pyrene, benzo[b]fluorene, benzo[a]anthracene, benzo[k]fluoranthene and benzo[a]pyrene) standards were of analytical grade and purchased from Merck (Merck KGaA, Germany). Ultra pure deionised water was purified by Millipore ultra pure system to a specific resistance of 18 mΩ cm or greater (Synergy Water Purification System, Millipore). The stock standard solution containing 1 g L⁻¹ PAHs was prepared separately in acetonitrile and the interval stock standard solutions of 1 mg L⁻¹ were then prepared from the stock standard solutions by dilution in acetonitrile. Working standard solutions of PAHs were prepared from the interval stock solutions. Oil and food samples were bought from local super markets and food samples were barbecued in a local restaurant. All stock and standard solutions and samples were stored at 4°C.

2.2. Instrumentation and chromatographic conditions

High performance liquid chromatography analysis was performed on a Perkin Elmer Series reversed phase HPLC system that consists of a microprocessor controlled Perkin Elmer model 200 eluent delivery pump and a fixed wavelength Perkin Elmer model 200 UV–Vis spectrophotometer detection system. Samples were injected via a Rheodyne Model 7725i injector valve fitted with a 50 µL volume injector loop. All separation was achieved on a 250 mm long × 4.6 mm id reversed phase ODS column with a mobile phase of water 80/20 (v/v) mobile phase at a flow rate of 1.5 mL min⁻¹ under isocratic conditions. All the system was maintained at room temperature. Date collection and handling were carried out by Perkin Elmer software (TotalChrom v6.2.1).

Chromatographic determination was based on separation of PAHs on ODS column and measurement of absorption at 254 nm. PAH standards were injected onto the chromatographic system to have retention times of each PAH and identification of PAHs in samples was made by comparing with the retention times of standards.

2.3. Preparation of calibration standards

The concentration of the PAHs in the working solutions was differed from each other in order to have similar peaks height, since the coefficient of molar absorption of the nine PAHs is different. The concentration of the working standard solutions were 5, 25, 100, 200 and 400 µg L⁻¹ for fluorene, phenanthrene, anthracene, benzo[k]fluoranthene and benzo[a]pyrene; 10, 50, 200, 400 and 800 µg L⁻¹ for fluoranthene, pyrene and benzo[a]anthracene and 5, 12.5, 50, 100 and 200 µg L⁻¹ for benzo[b]fluorene. Calibration curves were prepared by using the peak areas as a function of concentration of PAHs standards.

2.4. Extraction procedure

The extraction procedure included a saponification and following toluene extraction from the alcoholic solution for oil samples and direct toluene extraction for food samples and finally purification of PAHs through a house-made silica-alumina column.

A 50 mL oil sample was put in a 250 mL beaker covered with a watch glass and saponified with 1 M NaOH in MeOH-toluene 2/1 (v/v) mixture at 60°C for 90 min. The mixture was transferred into a separation funnel and left to cool down to the room temperature. The unsaponifiable alcoholic phase was separated and extracted for three times with 20 mL of toluene. These extracts were combined and washed twice with ultra pure deionised water. The organic phase was dried with anhydrous Na₂SO₄ and evaporated by a rotary evaporator. The residue was dissolved in 2 mL of acetonitrile. Whatever solvent is used for the extraction, the extract from such matrices as oil contains not only PAHs, but also numerous...
other polar and non-polar compounds. These components must be removed in further step of analysis in order to facilitate the separation and quantification of individual PAH. For that reason, the resulted extract was cleaned-up with a house-made column containing silicon oxide and alumina oxide before injection into HPLC system.

Equal amount of raw, barbecued meat, fish samples and charcoal (10 g) were weighted with baker and extracted with 50 mL of toluene for 45 min with an ultrasonic bath. The extracts were then filtered on a filter paper to remove the solid particles and then for cleaning-up the same procedure given for oils samples were applied for purification.

2.5. Recovery study

Recovery studies were carried out by using oil and food samples spiked with 100 and 200 µg L⁻¹ of PAHs standard. The spiked samples were prepared in duplicate and each was analyzed in triplicates. Recoveries were calculated from the differences, in total amounts of each PAH, between the spiked and analyzed oil samples after extraction procedure given above. The reproducibility of the extraction and the extractability of PAHs were evaluated through the relative standard deviations and percentages of recovery, respectively.

3. Results and discussions

3.1. Optimization of chromatographic determination

Prior to the analytical determination of PAHs with reversed phase HPLC/UV–Vis method, the UV region was scanned to obtain a shared absorption wavelength for the nine PAHs. As a result, 254 nm showed the best linearity for all nine PAH and it was chosen as the working wavelength for the analytical determination. The chromatographic conditions were then optimized and consisted of 80% acetonitrile and 20% water at 1.5 mL min⁻¹ flow rate was chosen as mobile phase composition and flow rate for the separation of PAHs. The chromatogram A in Fig. 1 shows the HPLC/UV–Vis chromatogram obtained for PAHs standards. The identification of the compounds was based on the retention times. The chromatogram demonstrates Gaussian shape and well separated peaks with an analysis time of 12 min.

3.2. Validation of the method

Calibration curves were obtained using a series of standard solutions containing the nine PAHs at five different concentrations. Five replicated injections for each concentration were made to ensure accurate and reproducible responses have been generated. Linearity was evaluated by using least square method. All nine calibration curves were linear over a wide concentration range in respect to the coefficient of determination ranging from 0.9951 to 0.9996. The theoretical limits of detection at a signal/noise ratio of 3 and theoretical limits of quantification at a signal/noise ratio of 10 for nine PAHs were in the range of 0.26–1.15 and 0.87–3.84 µg L⁻¹, respectively. Table 1 shows the data related to the validation of the method.

Reproducibility and accuracy of the method were checked by injection the standard solutions of PAHs given in Table 2 and measured from five replicated injection for within-day reproducibility and after 5 months. The RSD, that is used to represent the reproducibility of the method, was between 0.35% and 1.60% for within-day and between 0.16% and 0.95% after 5 months. Relative errors, that are used to represent the accuracy, were between 0.17% and 7.40% for within-day and between 0.24% and 7.27% after 5 months. Both reproducibility and accuracy of the method for determination of PAHs were satisfactory. Table 2 shows the data obtained for reproducibility and accuracy of the method for the experimental studies on nine PAHs. The reproducibility of the method was also checked by injection the samples solutions onto the chromatographic system. The RSD calculated from the sample injections was lower than the RSD calculated from the injection of standard solutions of PAHs.

3.3. Optimization of extraction procedure

The efficiency of extraction method and the extractability of PAHs through the saponification, toluene extraction and clean-up procedure were determined using spiked samples. The spiked samples were prepared in duplicate, extracted and purified as the procedure given above. In a preliminary study, the extraction procedure was optimized for the concentration of NaOH, MeOH/toluene ratio, extraction temperature and time to gain maximum and constant extractability. Subsequently, 1 M NaOH in MeOH/toluene of 2/1 (v/v) ratio at 60 °C for 90 min were found out to be the optimum conditions of extraction in saponification step. The extract obtained from the saponification contains some amount of aliphatic and high polar materials other than PAHs, which may interfere the analytical determination (Moret & Conte, 2000; Pillai et al., 2005; Stolyhwo & Sikorski, 2005). Therefore a clean-up step was applied to purify the PAHs from the co-extracted materials. For that reason, a mixture of silica and alumina was experimented and consequently, a house-made column enclosed with a mix of 8 g silica and alumina in 1/1 ratio (w/w) provided good purification. The column was conditioned with 20 mL of hexane and the residual dissolved in 2 mL of acetonitrile was loaded onto the column. After loading the residual, the column was eluted with 30 mL of hexane and the first fraction containing the aliphatic hydrocarbons was collected for further studies. The second fraction containing the PAHs was eluted with 40 mL of hexane/DCM 80/20 (v/v) and collected in a round bottom flask. Finally the column was eluted with 40 mL of DCM/MeOH 95/5 (v/v) to collect the third fraction containing the most polar compounds. The extract (second fraction) was evaporated on rotary evaporator and the residual was dissolved in 2 mL of acetonitrile. For each spiked sample, three injections on HPLC/UV–Vis system were made. The recovery of PAHs was between 80% and 104% with an exception for fluoranthene (127%). The reproducibility of the extraction procedure for the duplicated spiked samples is represented by the respective RSDs, which were between 0.17% and 23.63%. These results were well satisfactory for determination of PAHs at sub µg L⁻¹ levels in oil and food samples. Table 2 shows the data related to the recovery and reproducibility of the extraction method for the nine PAHs.

The first and the third fractions obtained from the purification step were also collected and analyzed in the same way. The aim of the study on the first and third fractions was to detect PAHs possibly extracted with these fractions and to measure the degree of the associated error. The maximum error rises from the first and third fractions was lower than 5%, with an exception for pyrene which was approximately 8%. This study could give an opportunity to improve the results obtained.

3.4. Samples analysis

Corn, sunflower and olive oils were analyzed for the nine PAHs with a reversed phase HPLC/UV–Vis method. After purification, the final residual dissolved in 2 mL acetonitrile for each oil samples was studied. A 50 µL portions were injected for five times and separated on reversed phase ODS column with acetonitrile/water 80/20 (v/v) mobile phase. UV–Vis detector was set at 254 nm. Fig. 1
shows the overlaid chromatogram of the standards of PAHs (chromatogram A) and corn oil (chromatogram B). As seen in chromatogram B, the peaks which belong to the studied PAHs are very clear and come up at right retention times. Therefore, identification of PAHs was simple. The peaks over the range of 2 and 4 min retention times were not obtained on the chromatogram of the PAHs standards (chromatogram A), only on the samples chromatograms. The three oil samples show similar chromatogram for the retention time between 2 and 4 min. The two highest peaks belong to the toluene which was used in extraction procedure and fully removal of toluene was not possible. The other peaks are belonging to the co-extracted materials, but, since they are out range of the retention times of the PAHs, they do not interfere the analytical determination of PAHs.

The nine studied PAHs were determined in corn oil within the range of 6.40–76.08 μg L\(^{-1}\). Fluorene is the most abundant PAH, followed by benzo[k]fluorene; benzo[a]pyrene, that it is used as a marker of occurrence and effect of the carcinogenicity, presents in corn oil at 19.20 μg L\(^{-1}\) level, nearly 10 times higher than the legal value. According to this result, it can be said that the corn oil analyzed was contaminated by PAHs and represents an important health risk for consumers, even if the others PAHs are present in lower levels than fluorene, benzo[k]fluoranthene and benzo[a]pyrene.

Sunflower oil contained six of the PAHs studied, with an exception of fluoranthene, benzo[a]anthracene and benzo[a]pyrene. The PAHs detected in sunflower oil are at low levels. Olive oil contained only three of the PAHs studied, phenanthrene, fluoranthene and pyrene, and it was very reach in phenanthrene (98.92 μg L\(^{-1}\)). Both sunflower and olive oil do not contain benzo[a]pyrene. Consequently, it can be concluded that these two oils do not represent health risk for consumers. But in total PAHs content, these two oils have also been contaminated. Table 3 shows the levels of PAHs determined in corn, sunflower and olive oils. Table 3 shows also the amount of the PAHs detected in first and third fractions. Their concentrations are at very low levels. Therefore no significant error rises from the first and third fractions.

It has been mentioned in literature that, refining process, like deodorisation or charcoal treatment can reduce the amount of these kinds of contaminants (Larsson et al., 1987; Moret & Conte, 2000). But the effectiveness of this kind of treatments to reduce these contaminants down to the legal limits has not been discussed. Therefore, it could be conclude that, the reasons of the high content of the PAHs in edible oils (especially in corn oil) could be

![Fig. 1. Overlaid HPLC/UV–Vis chromatogram of the PAHs standards (A) and corn oil (B) at 1.5 mL min\(^{-1}\) acetonitrile/water 80/20 (v/v) mobile phase flow rate on reversed phase ODS column.](image-url)
Table 2
Accuracy and reproducibility of the method in terms of concentration and retention time for the nine PAHs and the average recovery calculated from the two spiked samples in the optimized conditions.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Accuracy data</th>
<th>Reproducibility in terms of concentration</th>
<th>Reproducibility in terms of retention time</th>
<th>Spiked samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Within-day</td>
<td>After 5 months</td>
<td>Within-day</td>
<td>After 5 months</td>
</tr>
<tr>
<td></td>
<td>Conc µg L⁻¹</td>
<td>RE, %</td>
<td>Conc µg L⁻¹</td>
<td>RSD %</td>
</tr>
<tr>
<td>Fluorene</td>
<td>150</td>
<td>-3.65</td>
<td>200</td>
<td>-1.15</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>150</td>
<td>-4.33</td>
<td>200</td>
<td>0.19</td>
</tr>
<tr>
<td>Anthracene</td>
<td>150</td>
<td>5.67</td>
<td>200</td>
<td>-1.21</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>300</td>
<td>0.17</td>
<td>400</td>
<td>-4.47</td>
</tr>
<tr>
<td>Pyrene</td>
<td>300</td>
<td>-0.95</td>
<td>400</td>
<td>-1.99</td>
</tr>
<tr>
<td>Benzo[a]fluorene</td>
<td>75</td>
<td>-7.40</td>
<td>100</td>
<td>7.29</td>
</tr>
<tr>
<td>Benzo[a]anthracene</td>
<td>300</td>
<td>-0.91</td>
<td>400</td>
<td>0.59</td>
</tr>
<tr>
<td>Benzo[k]fluoranthene</td>
<td>150</td>
<td>2.28</td>
<td>200</td>
<td>0.24</td>
</tr>
<tr>
<td>Benzo[a]pyrene</td>
<td>300</td>
<td>-2.41</td>
<td>200</td>
<td>2.43</td>
</tr>
</tbody>
</table>

RE, relative error, RSD, relative standard deviation, RT, retention time, Rec, recovery.

Table 3
The amount of the PAHs determined in edible oil samples, the last two columns present the PAHs in first and third fraction obtained from the purification step for corn oil.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Corn oil µg L⁻¹ ± SD</th>
<th>Sunflower oil µg L⁻¹ ± SD</th>
<th>Olive oil µg L⁻¹ ± SD</th>
<th>PAHs in F1 for corn oil µg L⁻¹ ± SD</th>
<th>PAHs in F3 for corn oil µg L⁻¹ ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluorene</td>
<td>16.84 ± 0.60</td>
<td>7.44 ± 0.40</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>7.40 ± 1.32</td>
<td>4.96 ± 0.36</td>
<td>98.92 ± 3.08</td>
<td>0.19 ± 0.01</td>
<td>ND</td>
</tr>
<tr>
<td>Anthracene</td>
<td>10.84 ± 1.92</td>
<td>3.36 ± 1.36</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>76.08 ± 3.04</td>
<td>ND</td>
<td>18.36 ± 1.04</td>
<td>0.70 ± 0.18</td>
<td>0.24 ± 0.02</td>
</tr>
<tr>
<td>Pyrene</td>
<td>12.04 ± 0.48</td>
<td>1.44 ± 0.20</td>
<td>3.52 ± 0.28</td>
<td>0.15 ± 0.04</td>
<td>0.86 ± 0.09</td>
</tr>
<tr>
<td>Benzo[b]fluorene</td>
<td>10.08 ± 0.56</td>
<td>0.44 ± 0.04</td>
<td>ND</td>
<td>0.04 ± 0.00</td>
<td>0.47 ± 0.05</td>
</tr>
<tr>
<td>Benzo[a]anthracene</td>
<td>6.40 ± 0.32</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0.21 ± 0.06</td>
</tr>
<tr>
<td>Benzo[k]fluoranthene</td>
<td>37.16 ± 2.00</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>0.27 ± 0.17</td>
</tr>
<tr>
<td>Benzo[a]pyrene</td>
<td>19.20 ± 1.52</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

SD, standard deviation, ND, not detected, F1, first fraction, F3, third fraction.

Table 4
The amount of the PAHs determined in raw and barbecued food samples.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Raw trout µg kg⁻¹ ± SD</th>
<th>Barbecued trout µg kg⁻¹ ± SD</th>
<th>Raw bass µg kg⁻¹ ± SD</th>
<th>Barbecued bass µg kg⁻¹ ± SD</th>
<th>Raw lamb meat µg kg⁻¹ ± SD</th>
<th>Barbecued lamb meat µg kg⁻¹ ± SD</th>
<th>Charcoal µg kg⁻¹ ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluorene</td>
<td>19.72 ± 0.20</td>
<td>30.24 ± 0.92</td>
<td>1.60 ± 0.04</td>
<td>24.72 ± 0.60</td>
<td>7.96 ± 2.32</td>
<td>5.28 ± 0.36</td>
<td>ND</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>12.72 ± 0.16</td>
<td>38.40 ± 0.52</td>
<td>4.44 ± 0.08</td>
<td>34.20 ± 0.72</td>
<td>9.36 ± 0.287</td>
<td>18.56 ± 0.56</td>
<td>7.44 ± 0.52</td>
</tr>
<tr>
<td>Anthracene</td>
<td>12.24 ± 0.48</td>
<td>21.32 ± 0.36</td>
<td>24.84 ± 0.96</td>
<td>198.8 ± 2.28</td>
<td>ND</td>
<td>67.28 ± 3.00</td>
<td>ND</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>ND</td>
<td>18.68 ± 0.60</td>
<td>6.76 ± 0.16</td>
<td>15.32 ± 0.52</td>
<td>ND</td>
<td>15.88 ± 1.40</td>
<td>ND</td>
</tr>
<tr>
<td>Pyrene</td>
<td>ND</td>
<td>10.00 ± 0.72</td>
<td>1.24 ± 0.04</td>
<td>5.44 ± 0.12</td>
<td>ND</td>
<td>3.28 ± 0.20</td>
<td>ND</td>
</tr>
<tr>
<td>Benzo[b]fluorene</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Benzo[a]anthracene</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Benzo[k]fluoranthene</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Benzo[a]pyrene</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>
4. Conclusions

A HPLC/UV–Vis method for the determination of toxic polycyclic aromatic hydrocarbons (PAHs) in edible oils and food samples (raw and barbecued) has been discussed. Recovery, accuracy and reproducibility of the method ranged from 80% to 104%, from 0.17% to 7.40% and from 0.35% to 1.60%, respectively. All PAHs were separated and analyzed in 12 min. The detection limits of the nine PAHs ranged from 0.26 to 1.15 μg L\(^{-1}\) in oils at a signal/noise ratio of 3. The linearity of the method was between 0.9951 and 0.9996. The method is sensitive, reproducible, accurate, selective and was successfully applied for the determination of PAHs in food samples.

Corn oil contains all the nine PAHs (including benzo[a]pyrene). Sunflower contains six PAHs examined, the other three were not detected. Olive oil contains just three PAHs. Sunflower and olive oil do not contain benzo[a]pyrene. Since Benzo[a]pyrene may be used as a marker of occurrence and effect of the carcinogenic for PAHs in food, it can be said that sunflower and olive oils are not contaminated and do not represent health risks associated to PAHs exposition, even if three oils are contaminated, especially corn oil.

In case of raw and barbecued foods examined, they do not contain benzo[a]pyrene, and therefore not represent a health risks associated to benzo[a]pyrene. In conclusion, during barbecuing processes certainly total PAHs concentrations increase and some of PAHs form. For that reason an alternative cooking process should be introduced. Taking into consideration the carcinogenic potential of PAHs, any measurements directed to the reduction of these contaminants in the diet is highly desirable and should be strongly stimulated.

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References


Fig. 2. Overlaid HPLC/UV–Vis chromatogram of the raw trout (B) and barbecued trout (A) at 1.5 mL min\(^{-1}\) acetonitrile/water 80/20 (v/v) mobile phase flow rate on reversed phase ODS column.


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