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ORIGINAL ARTICLE

Polycyclic aromatic hydrocarbons (PAHs) in edible oils by gas chromatography coupled with mass spectroscopy

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Abstract The occurrence of polycyclic aromatic hydrocarbons (PAHs) in nine edible oils of three categories of oil samples, such as soy bean oil, mustard oil and coconut oil, has been studied to determine the contamination degree of this type of oil samples. Eight major carcinogenic polycyclic aromatic hydrocarbons (PAHs), such as naphthalene, anthracene, phenanthrene, fluorene, pyrene, crysene, benzo(a)pyrene and benzo(a)anthracene, were identified and quantified in the extract of edible oils collected from Bangladeshi Markets by gas chromatography and mass spectroscopy. All of the carcinogenic PAHs are not present in the edible oils. A few of the carcinogenic PAHs are present in the oils but it is within the permissible limit. The results for the recoveries of naphthalene, fluorene, phenanthrene, anthracene, pyrene, crysene, benzo(a)anthracene and benzo(a)pyrene were in the range of 56–84%. The limit of detection (LOD) of the GC–MS method, established at signals three times that of the noise for naphthalene, fluorene, phenanthrene, anthracene, pyrene, crysene, benzo(a)anthracene and benzo(a)pyrene, was 2.0–2.5 ng, respectively.

1. Introduction

PAHs are a diversified family of more than 100 lipophilic organic contaminants composed of two or more fused aromatic rings (Guillen, 1994). These contaminants compounds are formed organic matter as a consequence of a series of natural processes by incomplete combustion (Moret and Conte, 2002). The potential sources of PAHs contamination are ubiquitously distributed in nature (Baan et al., 1994). However, due to the recognized PAHs' carcinogenic activity virtually human exposure is unavoidable, which raises now an important public health concern. PAHs and its derivatives exposure were epidemiologically associated with an increased risk of skin and lung cancer (Baan et al., 1994). According to the priority pollutants by Environmental Protection Agency (EPA) sixteen PAHs are actually classified on the basis of their occurrence and carcinogenic activity, six being with 4–6 member rings and classified as heavy PAHs (Lodovici et al., 1995).

Diet is the major source of non-occupational of PAHs for non-smokers (Lodovici et al., 1995); meat and meat products, cereals, and oils and fats are also the principal sources (Dennis

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et al., 1991; Ibanez et al., 2005). A significant dietary source in oils and fats contaminate by PAHs that are due to their lipophilic nature and their incorporation into other foods such as cereal-based products either directly or indirectly (Dennis et al., 1991).

Another significance aspect that emphasizes of oils and fats contaminated by PAHs' vehicle is the major source that lipids could raise their intestinal absorption (Starvic and Klassen, 1994). Vegetable oils mainly free from PAHs but they are contaminated by environmental pollution such as vegetable raw material, and also by contamination from seed drying. They are also contaminated by solvent extraction, soil burn, package material, mineral oil residues and migration from contaminated water or soils (Larsson et al., 1987; Speer et al., 1990). A large number of PAHs of a wide range of molecular weights are present in vegetable oils, which many are alkylated compounds, although they are ignored by legal regulations (Guillen and Sopelana, 2004).

Recently has set maximum levels of 2 ppb for benzo[a]pyrene by the European Union (Commission Regulation No. 208/2005) in oils and fats for direct consumption or use as an ingredient in foods (Moret and Conte, 2002). Several countries like Spain, Italy, Portugal and Greece have established their own limits for the concentration of the following toxic and carcinogenic PAHs such as benzo[a]anthracene, benzo[e]pyrene, benzo[b]fluoranthene, benzo[k] fluoranthene, benzo[a]pyrene, dibenz[a,h]anthracene, benzo[ghi]perylene and indeno[1,2,3-cd]pyrene. Maximum limit for each individual single PAH is 2 ppb and 5 ppb for the sum of the eight heavy PAHs was established by Moret et al., 2005. Some organizations establish their own recommendations, such as the German Society for Fat Science (GSFS) that suggests that the total PAHs in edible oils should not exceed 25 μg/kg and heavy PAHs should be below 5 μg/kg (Cejpek et al., 1998).

The contamination in crude edible oils by PAHs or other organic and inorganic contaminates varies widely. Generally, the refined vegetable oils a low level contaminates present than the crude ones, which can be omitted through refining (Cejpek et al., 1998). Up to four aromatic rings of PAHs strongly reduce light by the deodorization but bleaching with activated charcoal is an effective strategy to reduce the higher condensed five and six member of PAHs (Dennis et al., 1991; Guillen and Sopelana, 2004). It is very important to know that the extent of the contamination of vegetable oils with PAHs, as well as the influence that it has been refine well. In this context, only few papers have measured the content contamination of PAHs' after refining of oils (Moret et al., 2005, 2008; Barranco et al., 2004), and the majority papers focuses only on raw and refined samples (Guillen and Sopelana, 2004; Biernoth and Rost, 1967; Sagredos et al., 1988; Zougag et al., 2009).

The aim of this study was to determine the content of PAHs in commercial edible oil samples collected from the Bangladeshi local markets by gas chromatography and mass spectroscopy.

2. Experimental

2.1. Chemicals and reagents

Naphthalene, anthracene, phenanthrene, fluorene, pyrene, crysene, benzo(a)pyrene and benzo(a)anthracene standards were purchased from Sigma–Aldrich Company, UK. The purity of all pure PAH standards ranged from 97% to 99.5%. All solvents, such as acetonitrile, cyclohexane (BDH, UK), dichloromethane, methanol, dimethyl sulfoxide (DMSO) and acetone (Merck, Germany), were of HPLC grade. Anhydrous sodium sulfate (Merck, Germany) was cleaned by heating at 200 °C before use. Silica gel (60–120 mesh, Loba, India) was activated at 400 °C for 12 h prior to use. The other chemicals were from BDH, UK.

2.2. Edible oil samples

There are different brands of edible oils available in Bangladeshi Markets which are imported from oil producing countries in all over the world. For our present experiment, we used nine different varieties and brands of edible oils from three different categories, such as soy bean oils (e.g., Teer, Rupchanda and Muskan), mustard oils (e.g., Teer, Shuresh and Radhuni) and coconut oils (e.g., Swan, Parasut and Jui), collected from the local market of Dhaka Metropolitan City (DMC) in July 2009.

2.3. Preparation of standard

Calibration curves for the samples, treated according to the described analytical procedure, were made using the SIM mode. Different concentrations of the mixture of the 8 priority pollutant PAHs of naphthalene, anthracene, phenanthrene, fluorene, pyrene, crysene, benzo(a)pyrene and benzo(a)anthracene (0.5, 1, 5, 10 and 20 μg/mL) were used to establish the calibration curves. This standard mixture was stored at 4 °C in darkness to avoid volatilization and photo degradation. Stock solutions were prepared by dilution of this standard mixture in tetrahydrofuran and methanol and stored at 4 °C in darkness.

2.4. Methodology

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

2.5. 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), shaken for 10 min by hand, sonicated over 30 min and centrifuged for 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 K-D (Kuderna-Danish) 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 top layer was then transferred to the silica gel column. This operation was repeated twice. Five milliliters of acetonitrile/acetone 60:40 were eluted through the column 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

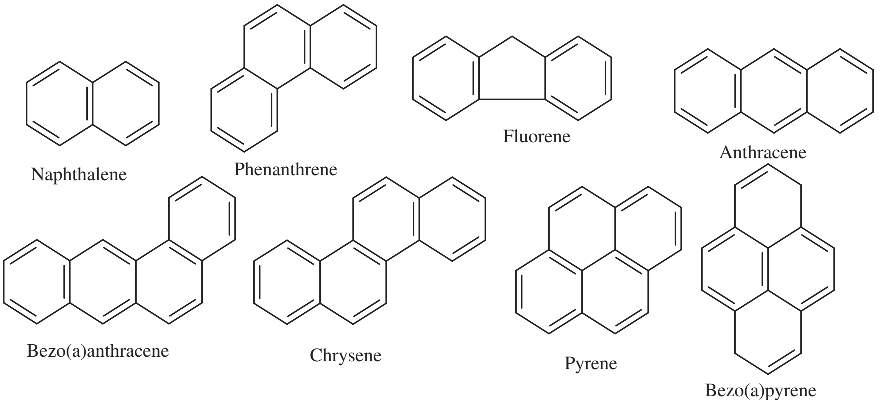


Figure 1 Chemical structures of markers.

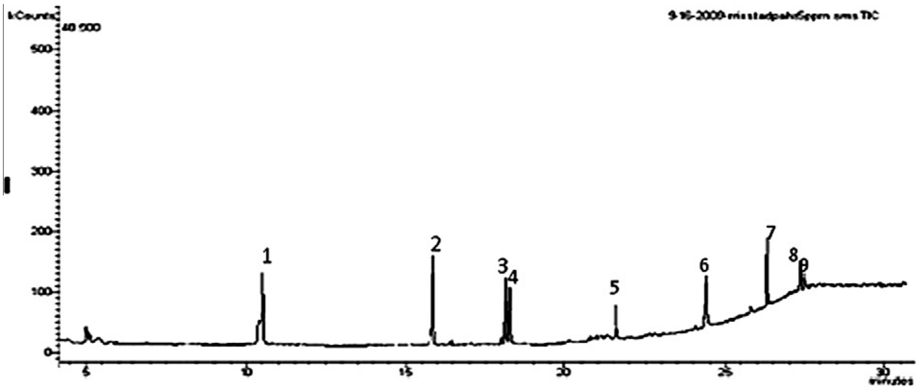
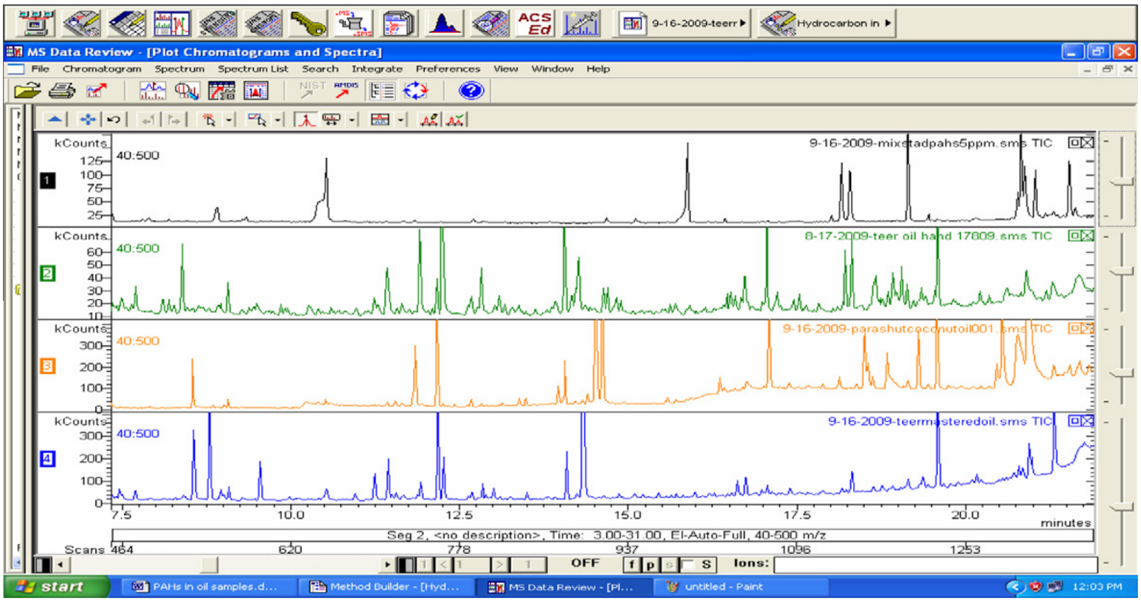


Figure 2 Chromatogram of the mixture of reference compounds, 1, naphthalene; 2, anthracene; 3, phenanthrene; 4, fluorene; 5, pyrene; 6, crysene; 7, benzo(a)pyrene; 8, benzo(e)pyrene; 9, benzo(a)anthracene.



Seg 2, <no description>, Time: 3.00-31.00, EI-Auto-Full, 40-500 m/z

Figure 3 An overlaid chromatogram of the edible oil extract and PAHs markets.

Table 1 Analytical characteristics of calibration curves for the marker PAHs.

Constituent	Ret. time (min)	Range (μg/ml)	Regression equation	Detection limit (μg)	R^2	Precision (%) (SD^2) ($n = 5$, 10 μg/ml)	
						Inter-day	Intra-day
Naphthalene	10.527	2–1000	$3.7926^{e+4x} + 5599.4043$	2.00	0.9987	0.63	0.34
Fluorene	15.885	2.3–1000	$2.3703^{e+4x} + 0.0000$	2.3	0.9999	0.78	0.42
Phenanthrene	18.167	2–1000	$1.8583^{e+4x} + 3456.1672$	2.0	0.9873	0.81	0.29
Anthracene	18.274	2.5–1000	$2.0366^{e+4x} + 921.7867$	2.5	0.9961	1.43	0.67
Pyrene	21.546	3.1–1000	$3.1723^{e+4x} + 103.734$	3.1	0.9981	2.34	0.74
Crysene	23.334	2.3–1000	$1.9783^{e+4x} + 784.9982$	2.3	0.9879	1.93	0.49
Benzo(a)anthracene	24.405	2.0–1000	$2.7742^{e+4x} + 987.3261$	1.9	0.9997	0.89	0.29
Benzo(a)pyrene	27.391	2.7–1000	$3.4947^{e+4x} + 821.9287$	2.5	0.9977	0.97	0.32

SD, standard deviation.

1 mL of hexane and transferred to the previously conditioned silica gel column. The eluted extract from the column was evaporated under a flow of nitrogen to about 1 mL.

2.6. Cleanup procedure

The cleanup column (i.d. = 1 cm) was filled with cotton at the bottom. An activated silica gel (17 g) was soaked with dichloromethane, loaded into the cleanup column (5 cm), which was then topped with 1.5 cm of anhydrous sodium sulfate. Five milliliters of dichloromethane were added to wash the sodium sulfate and the silica gel. The dried 1 mL sample was then transferred into the column and the vessel was rinsed twice with a mixture of acetonitrile/acetone, which was also added to the column. Sixty milliliters of acetonitrile/acetone were added to the column and allowed to flow through the column at a rate of 3–5 mL/min, and the eluent was collected. The collected eluent from the cleanup procedure was reconcentrated to 0.5 mL with K-D concentrator.

2.7. GC–MS operating conditions and analysis

GC–MS was carried out using total ion monitoring mode on a Varian 3800 gas chromatograph interfaced to a Varian Saturn ion trap 2200 GC–MS. The temperatures of transfer line and ion source were 300 and 275 °C, respectively. Ions were obtained by electron ionization mode. The VF-5 capillary column (30 m length, 0.25 mm i.d., 0.25 μm film thickness) was used. A 20% split injection mode was selected with a solvent delay time of 5 min with injection volume 1 μL. The initial column temperature was started at 50 °C for 1 min, programmed at 8 °C min^{-1} to 200 °C and heated until 300 °C at 10 °C min^{-1}. The injection port was set at 250 °C. Helium was used as carrier gas with a flow-rate of 1.0 mL/min^{-1}. Molecular ions were monitored for identification. Mass range: 40–500 m/z. Each sequence of samples included a blank to control the absence of contamination of solvents and silica gel, and a standard solution was 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 analyzed (injected) twice by GC–MS.

2.8. Statistical analyses

Data are expressed as means of triplicate measurements. Correlations were obtained by Pearson correlation coefficient in bivariate correlations. Means were compared by Tukey-HSD and LSD (least significant differences). Differences at $P < 0.05$ were considered to be significant.

3. Results and discussion

Bangladesh is an agricultural country. Vegetables, crops and fruits are grown here in plenty, mainly in the winter season. Some oil related crops are cultivated in our country. Lots of amounts of edible oil are consumed by the population. So, it is not enough for our country, it may be 2–5% of the total consumed edible oils. In this context, Bangladesh imports a lot of edible oils from the oil producing countries. After that it is refined in our country and packed locally under different brand

Table 2 PAHs levels (mean ± SD) determined in commercially available soybean, mustard and coconut oils (μg/kg).

PAH	Soy bean oil			Mustard oil			Coconut oil		
	Rupchada	Teer	Muskan	Teer	Radguni	Shuresh	Parasut	Swan	Jui
Naphthalene	–	–	0.212	0.614	–	0.142	–	0.375	–
Fluorene	–	–	–	0.071	0.83	–	–	–	–
Phenanthrene	–	–	–	0.323	0.116	0.098	0.110	–	-
Anthracene	0.038		0.035	0.419	0.230	0.214	0.115	0.089	0.325
Pyrene	–	–	–	0.098	–	0.116	0.078	0.063	0.178
Crysene	–	–	–	–	–	–	–	–	–
Benzo(a)pyrene	0.045	0.021	–	0.203	0.076	0.0167	0.029	0.041	0.063
Benzo(a)anthracene	0.011	0.009	0.0267	0.054	0.023	0.015	0.0087	0.077	0.017

names. All Bangladeshis consume edible oil for all cooking purposes. Edible oils are contaminated by various ways, such as during the production of crude oil and by refining system. So considering this fact, the need for checks on any toxic compound contaminating it or not cannot be overlooked. Considering its health impact, the objective of this work is to check the highly carcinogenic polycyclic aromatic hydrocarbons in the edible oils that are imported from oil producing countries by GC–MS.

3.1. Collection of the samples

There are different brands of edible oils available in Bangladeshi Markets which are imported from oil producing countries all over the world. For our experiment we used nine different varieties of edible oil samples. Some edible oils are imported and some are local brands.

3.2. Concentrations of the markers in edible oils by GC–MS

The GC–MS analysis of the crude extract of edible oil samples was performed using a Varian GC–MS (Model Varian CP 3800, USA) equipped with a VF-5 fused silica capillary column (30 m × 0.25 i.d. mm film thickness 0.25 μm, Varian, USA). For GC–MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium gas was used as a carrier gas at a constant flow rate of 1 mL/min. Injector and mass transfer line temperatures were set at 275 and 300 °C, respectively. The oven temperature was programmed from 50 to 200 at 8 °C/min, and then held isothermal for 20 min and finally raised to 300 °C at 10 °C/min. Diluted samples of 0.2 μL were manually injected in the split-less mode. Identification of compounds of the crude extract was based on GC retention time on VF-5 capillary column, computer matching of mass spectra with standards (Mainlab, Replib and Tutorial data of GC–MS systems).

The GC–MS method applied is a modification of that reported by Larsson et al. (1987) for the analysis of PAHs present in edible oils. In the present study, a programmed method was used for simultaneous assay of the authentic markers for which chemical structures are shown in Fig. 1. All standards were determined in a single GC–MS run. The standards were resolved and eluted at 10.527, 15.885, 18.167, 18.274, 21.546, 23.334, 24.405 and 27.391 min, with respect to naphthalene, fluorene, phenanthrene, anthracene, pyrene, crysene, benzo(a)anthracene and benzo(a)pyrene (Fig. 2). The markers (5, 50, 500, 750 and 1000 ng on column for naphthalene, fluorene, anthracene, crysene and benzo(a)anthracene and 10, 100, 500, 750 and 1000 ng for phenanthrene, pyrene and benzo(a)-pyrene) showed a good linearity in the range from 2.0 to 1000 ng in the calibration curves that were obtained by GC–MS analysis. All reference markers were not present in the chromatographic profiles of the samples from various locations when the sample solution was analyzed by GC–MS (Fig. 3). The peaks of naphthalene, fluorene, phenanthrene, anthracene, pyrene, crysene, benzo(a)anthracene and benzo(a)pyrene were confirmed by comparison of their retention times with reference standards.

To assess the precision of these methods, standard solutions of naphthalene, fluorene, phenanthrene, anthracene, pyrene, crysene, benzo(a)anthracene and benzo(a)pyrene were determined six times on the same day and over a 6-day period. The results showed a very good precision, ranging from 2 to 1000 μg/ml (Table 1). The accuracy of the method was evaluated through recovery studies. The recovery experiments were performed at three concentrations (5, 50 and 100 ng) of the standard added to sample solutions, in which the marker content had been determined, using a soy bean oil teer brand. The results for the recoveries of naphthalene, fluorene, phenanthrene, anthracene, pyrene, crysene, benzo(a)anthracene and benzo(a)pyrene were in the range of 56–84%. The limit of detection (LOD) of the GC–MS method, established at signals three times that of the noise for naphthalene, fluorene, phenanthrene, anthracene, pyrene, crysene, benzo(a)anthracene and benzo(a)pyrene, was 2.0, 2.3, 2.0, 2.5, 3.1, 2.3, 1.9 and 2.5 ng, respectively.

The GC–MS procedure was applied to the determination of the markers in the edible oil samples from different markets. As shown in Table 2, all the analyzed samples showed a significant range in the concentrations of the markers, in oil samples. The variation may be ascribed to environmental conditions and variation in sample sourcing and refining.

The carcinogenic PAHs' values obtained for the markers appear to fall within the range reported for the markers in oils (Guillen and Sopelana, 2004; Biernoth and Rost, 1967; Sagredos et al., 1988; Zougag et al., 2009). However, the overall levels of the marker concentration were considerably higher in edible mustard oil samples. Anthracene was present almost in all brands of edible oils. The concentration of anthracene is slightly high in all brands of mustard oils, but in the other brands of edible oils it is within the permissible limit. Concentrations of napthalene, fluorene, phenanthrene, anthracene, pyrene, benzo(a)pyrene and benzo(a)anthracene ranged from 0.142% to 0.614%, 0.071% to 0.43%, 0.098% to 0.323%, 0.035% to 0.419%, 0.063% to 0.178%, 0.0167% to 0.203% and 0.0087% to 0.054%, respectively (Table 2). The PAH, crysene, was not present in the edible oil samples. The GC–MS results showed that the relative concentrations of the markers varied considerably.

4. Conclusions

The data available from our studies show that the mustard edible oils have relatively high level of PAHs exceeding the WHO recommended maximum value for safety. This suggests significant risk of cancer to the people of this environment. The PAHs' contents observed in the analyzed other edible oils can be considered comparatively low, within those described by other authors. 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 decreases these types of environmental contaminants.

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