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Monitoring Polycyclic Aromatic Hydrocarbons in Edible Vegetable Oils Consumed in Iran

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ABSTRACT

Concentrations and profiles of 15 Environmental Protection Agency (EPA) priority Polycyclic Aromatic Hydrocarbons (PAH) of six different edible oils consumed in Iran markets (oils of olive, sesame, coconut, sunflower, frying and blend oil) were studied. The evaluated edible oils in the present study have not previously been analyzed concerning their contents of PAH compounds. PAHs of 207 edible oil samples were determined and quantified by High-Performance Liquid Chromatography with Spectrofluorometric Detector (HPLC/FLD). The results revealed that the highest content of total PAHs was in coconut oil group (46.8 µg kg^{-1}), followed by blend oil (22.48 µg kg^{-1}), frying oil (20.67 µg kg^{-1}), sesame oil (19.92 µg kg^{-1}), olive oil (18.4 µg kg^{-1}) and sunflower oil (17.6 µg kg^{-1}). The light PAHs (Naphthalene, Acenaphtene, Phenantherern, Antrathene, and Fluorene) had the highest portion of PAHs concentration. Benzo[a]pyrene and PAH4 contents (Benz[a]anthracene+Chrysene+Benzo[b]fluoranthene+Benzo[a]pyrene) were ND-1.32 µg kg^{-1} and 0.14-9.2 µg kg^{-1}, respectively; coconut oil had the highest content. In general, the highest values of Benzo[a]pyrene and PAH4 were not higher than the maximum allowable values of 2 and 10 in any sample, respectively. However, due to the significant content of total PAHs in some vegetable oils, such as coconut oil, it is necessary to determine the limits and evaluate it in the national standard and regulations of the country.

Keywords: Edible oil, HPLC/FLD method, Iranian oil market, PAH.

INTRODUCTION

As a lipophilic organic compound, PAHs (Polycyclic Aromatic Hydrocarbons) contain several fused aromatic rings. Structurally, there are two types of PAHs: (A) Group of PAHs known as Low Molecular Weight (LMW) PAHs containing 2 to 3 benzene rings. This group include naphthalene, acenaphthalene, acenaphthylene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz[a]anthracene, and chrysene, and (B) There are a number of heavy PAHs containing 3 or more rings, such as benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, benzo[ghi]perylen, indeno[1,2,3-cd]pyrene, and dibenz[a,h]anthracene (Heshmati *et al.,* 2018). While LMW PAHs are very toxic and not carcinogenic, the heavy PAHs are less toxic and highly carcinogenic. Due to their stability, resistance and cumulative properties, PAH compounds remain unchanged in the environment or in the body of living organisms for a very long time and have caused concern in human society. It has been determined by the Environmental Protection Agency (EPA) that naphthalene, acenaphthene, acenaphthylene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene,

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benzo[a]pyrene, benzo[ghi]perylen, indeno[1,2,3-cd]pyrene, and dibenz[a,h]anthracene are listed among the most important carcinogenic compounds of PAH (US EPA, 1984).

Contamination by PAH occurs in three ways: respiration, skin contact, and nutrition. The most important one is through contaminated water and foods. The most vital food sources for the entry of PAHs into the body are oils and vegetable fats followed by dried fruits, meat products and smoked fish (Hao *et al.,* 2016). Environmental pollution that contaminates soil and water as well as technological processing (heating and drying), contact with mineral oils, and contaminated packaging leave PAH compounds in food, particularly vegetable oils and fats. Drying and heating of oily seed and fruits are the most important sources of pollution. Depending on temperature and heating time, distance from heat source, type of processing, fuel type, and fat content in the seed and fruits, the amount of PAH produced in the vegetable oils could vary significantly. They are also contaminated by PAHs during solvent extraction process. Adequate oil refining process (neutralization, neutralization and deodorization) under standard conditions reduce PAHs content to 2 μg kg^{-1} (Sánchez-Arévalo *et al.,* 2020). As noted earlier, PAHs content of fats and oils is carcinogenically important (Bertoz *et al.,* 2021; Iwegbue *et al.,* 2020; Hao *et al.,* 2016). Benzo[a]pyrene and PAH4 (benzo[a]pyrene, benzo[a]anthracene, benzo[b]fluoranthene, and chrysene) are considered as the best indicators of PAHs in food and edible oils by the European Food Safety Authority (EFSA) and the EU Council Regulation (EU) No. 835/2011 of 19 August 2011 (EFSA, 2012; EU, 2011a; Singh and Agarwal, 2018). Furthermore, according to the Institute of Standard and Industrial Research of Iran, the allowable values of B[α]P and PAH4 in edible oils are 2 and 10 μg kg^{-1} , respectively.

In Iran, vegetables, crops and fruits are cultivated in plenty, mainly in the four seasons. In recent years, some oil-related crops, such as colza, sesame, olive etc., are commonly cultivated but not sufficient for our population. In this context, Iran imports more than 80 percent of edible oil and fats from other countries, refining and packing them locally under a different brand. Since, there is no comprehensive research about the content of benzo[a]pyrene and heavy PAH4 (as chemical contaminants) of edible fats and oils in Iran, this study aimed to evaluate these compounds in consumed edible oil and fats by the Iranian population. Notably, a comprehensive research to estimate the total household oil consumption has not been conducted.

MATERIALS AND METHODS

Reagent and Chemicals

Sigma Aldrich (Bellefonte, PA) provided a standard mixture of 16 EPA PAHs (PAH-mix 4S8743), containing naphthalene, acenaphthene, acenaphthylene, anthracene, fluoranthene, fluorine, phenanthrene, pyrene, Benz[a]Anthracene (B[α]A), Benzo[b]Fluoranthene (B[b]F), benzo[k]fluoranthene, benzo[ghi]perylene, Benzo[a]Pyrene (B[α]P), Chrysene (CHR), dibenz[a,h]anthracene, and indeno[1,2,3-cd]pyrene (10 ng μL^{-1} in acetonitrile). Merck (Darmstadt, Germany) provided high-purity acetone, acetonitrile, dichloromethane, hexane, methanol, and toluene for HPLC analysis. Deionized water was purified using the Milli-Q system (Millipore, Billerica, MA, USA). Sep-Pak C_{18} cartridges were provided by Waters, Ireland, while Chromabond cartridges were provided by Machery-Nagel, Germany.

Instruments

A YL 9100 HPLC system was used for the evaluation of samples and standard solutions.

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This system included a vacuum degasser YL 9101, a quaternary pump YL 9110, a column compartment YL 9130, and a fluorescence detector FP-2020 plus running YL clarity software (Young Lin, Korea). Agilent Technologies, USA; 150 mm by 4.6 mm by 5 m ZORBAX Eclipse column and C_{18} guard column (10 mm by 2.1 mm). Additionally, ultrasonic baths (Elma, Germany) and tabletop centrifuges (Dynamca, United Kingdom) were used at the present work (VelpScientifica, Italy, and Dynamca, United Kingdom). Vacuum manifolds were used to prepare, filter, and elute SPE samples (CNW, China). In terms of polymer phase cartridges, Sep-Pak C_{18}, 6 mL, 500 mg LN 034031034B was the one used by Waters, Ireland, and Chromabond, 3 mL, 500 mg from Macherey-Nagel, Germany was used for Florisil-bonded phase cartridges.

Filters made from nylon (0.45 µm), micropipettes (200-1,000 µL), centrifuge tubes made from polypropylene (11 mL), injection vials with screw tops (2.0 mL), and septa and inserts made from butyl rubber with Poly Tetra Fluoro Ethylene (PTFE) coating (200 µL), and 5 mL syringes were used as disposables. A Millipore system was equipped with PTFE filters having a pore size of 1 µm, with an id of 25 mm. These filters were manufactured by Bio-Analytics, Gdansk, Poland.

Calibration Standards

For this study, 200 and 50 µg L^{-1} in acetonitrile stock and standard solutions of PAHs were made. The calibration curve standard solution was used to make eight standard solutions of PAHs in acetonitrile. The solutions were stored in dark place at 4°C. Peaks of calibration curves represent function of standard PAHs concentration.

Extraction and Sampling Procedure

The procedure proposed by ISO 15753 (2016) was followed. The polycyclic aromatic hydrocarbons were extracted with ultrasound-assisted solvent extraction (acetonitrile/acetone mixture); finally, purified by using reverse-phase C_{18} and Florisil-bonded phase cartridges.

HPLC-FLD Analysis

YL Chromatographic analyses of samples and standard solutions were conducted using an HPLC-9100 system equipped with a HPLC-FLD fluorescence detector. Isothermal temperature of the column was 30°C. A volume of 20 µL was injected.

According to gradient method, the conditions were 1.2 mL min^{-1} flow rate, acetonitrile (A) as the mobile phase, and 50/50 acetonitrile/water concentration (B). In order to determine PAHs by Fluorescence Detector (FL), the following Excitation and Emission wavelengths were used (Ex/Em):

270/324 nm (NPH, ACE, FL) at baseline, 248/375 nm (PHE, ANT) at 12.8 minutes, 280/462 nm (FT) at 16.8 minutes, 270/385 nm (PYR, B[a]A, CHR) at 18.1 minutes, 256/446 nm (B[b]F) at 28 minutes, 292/410 nm (B[k]F, B[a]P, D[ah]A, B[ghi]P) at 31.2 minutes, and 270/470 nm (IP) at 38 minutes (ISO 15753 2016).

Technique Validation

The used technique was validated by ISO 15753 (2016). Repeatability and recovery were evaluated by spiking blank oil samples with 15 PAHs (1 µg kg^{-1}) and 15 PAHs (5 µg kg^{-1}). Using the same conditions, five analyses were performed on the same day to evaluate reproducibility. Linearity (R^2) was calculated for all 15 PAHs by eight concentrations of 1-200 µg kg^{-1} PAHs spiked to blank samples. To calculate LOD (Limit of Detection) and LOQ (Limit of Quantification), we multiplied the standard deviation and mean of the fortified blank samples (n= 10) by 3.3 and 10, respectively, as well as the slope of the calibration curve.

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Table 1. Linear range, correlation coefficient (R^2), Limit Of Quantitation (LOQ), Limit Of Detection (LOD) and recoveries of PAHs.

Compound	Linear range (µg kg^{-1})	R^2	LOD (µg kg^{-1})	LOQ (µg kg^{-1})	Recovery (%) (1 µg kg^{-1})	Recovery (%) (5 µg kg^{-1})
Naphthalene	1 - 200	0.9851	0.09	0.27	80	83
Acenaphthene	1 - 200	0.9885	0.11	0.33	84	87
Fluorene	1 - 200	0.9887	0.20	0.60	83	96
Anthracene	1 - 200	0.9954	0.18	0.54	87	92
Phenanthrene	1 - 200	0.9981	0.19	0.57	89	101
Fluoranthene	1 - 200	0.9978	0.10	0.30	90	97
Pyrene	1 - 200	0.9993	0.15	0.45	93	95
Benz[a]anthracene	1 - 200	0.9983	0.20	0.60	89	96
Chrysene	1 - 200	0.9989	0.18	0.54	92	105
Benzo[b]fluoranthene	1 - 200	0.9979	0.18	0.54	93	97
Benzo[k]fluoranthene	1 - 200	0.9984	0.20	0.60	91	94
Benzo[a]pyrene	1 - 200	0.9988	0.16	0.36	95	98
Dibenz[a, h]anthracene	1 - 200	0.9985	0.24	0.72	104	106
Benzo[g,h,I]perylene	1 - 200	0.9978	0.26	0.78	106	110
Indeno[c,d]pyrene	2 - 200	0.9975	0.45	1.35	91	94

Samples

The sampling of edible oils and fats was done according to ISO 5555 (2001) and samples included 51 olive oil samples (virgin and refined), 27 sesame oil samples, 45 frying oil samples, 45 sunflower oil samples, 12 coconut oil samples, and 27 blend oil samples. In order to conduct the analysis, oil samples (207) were purchased from local supermarkets and stored at room temperature for further assessment.

Statistical Analysis

The experiments were randomly designed. Average of three separate measurements was reported as result. Statistical analysis was performed using SPSS software (version 22; SPSS Inc., Chicago, IL, USA). One-way Analysis Of Variance (ANOVA) and Duncan's multiple range test (P-value< 0.05) were used to calculate significance of differences between mean values.

RESULTS AND DISCUSSION

Validation

In the linearity, limits of detection, limits of quantification, and recovery tests were performed to determine whether the technique was analytically controlled or not. External standard calibration was used (HPLC/FLD technique) to determine analytes values by eight calibration solutions containing 1-200 µg kg^{-1} PAHs. According to Table 1, the lowest correlation coefficient (R^2) was related to Naphthalene (0.9851), Acenaphthene (0.9885), and Fluorene (0.9887), belonging to light PAH. Table 1 illustrates the standard linearity supported by regression data. It was observed that there was a linear relationship with a satisfactory linear coefficient in all PAHs (R^2> 0.9851). LOD ranged from 0.09 to 0.45 µg kg^{-1} and LOQ ranged from 0.27 to 1.35 µg kg^{-1}. The recoveries varied from 80 to 110%. According to the results obtained by other authors, the limit of quantitation and limit of detection ranged between 0.6–1 and 0.2–0.3

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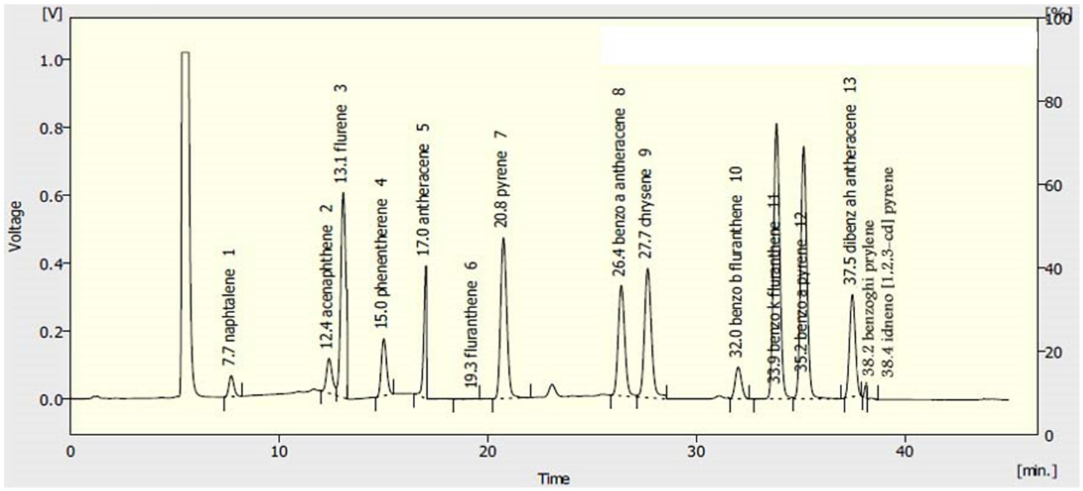
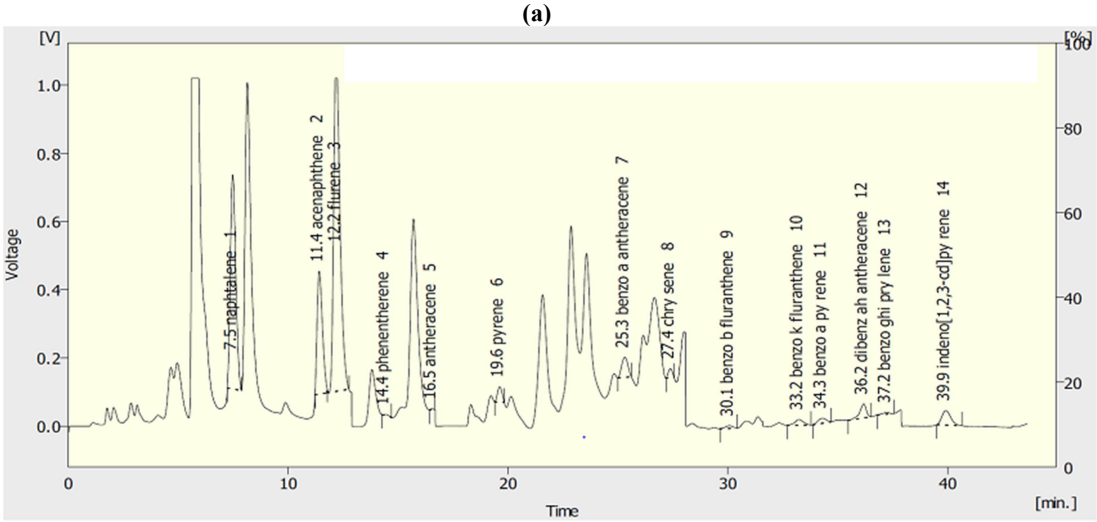
µg kg^{-1}, respectively. The average recovery was 58.6–90.6% (Liu *et al*., 2023). Lee *et al*. (2019) evaluated the occurrence and risk characterization of polycyclic aromatic hydrocarbons of edible oils. They also indicated that the LOQ and LOD ranged from 0.06 to 0.44 µg kg^{-1} and 0.02 to 0.13 µg kg^{-1} in four types of oil samples (Sesame oil, prilla oil, Oliva oil and Pepper seed oil), respectively. The relative recoveries of PAH4 were from 79.9 to 112.6% at 10 µg kg^{-1} and from 70.7 to 110.4% at 2 µg kg^{-1} (Lee *et al*., 2019)

Total PAHs

The values of some PAHs (naphthalene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[k]fluoranthene, dibenz[a,h]anthracene, benzo[g,h,i]perylene, indeno[cd]pyrene) and total PAHs are presented in Table 2. The chromatograms of PAHs along with their retention times in standard and edible oil samples are shown in Figure 1. According to Table 2, the concentration of naphthalene in all different



(a)



(b)

Figure 1. Chromatograms obtained from HPLC/FLD method. (a) Pure standard solution (15 PAHs: 2 µg/kg); (b) An olive sample.

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Table 2. Contents of different contaminants (µg kg^{-1}) in different types of edible oil.[a]

Edible oils	Blend		Coconut		Frying		Sunflower		Sesame		Olive	
PAHs	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Naphthalene	7.83^{b}	1.90	12.97^{c}	5.01	5.95^{ab}	3.72	6.30^{ab}	2.71	4.92^{a}	4.94	5.59^{ab}	4.91
Acenaphthene	5.21^{ab}	3.27	11.17^{c}	3.78	6.02^{ab}	2.57	4.23^{a}	3.53	6.64^{ab}	2.40	5.54^{ab}	4.82
Fluorene	1.60^{a}	1.78	9.61^{b}	3.50	1.73^{a}	2.29	1.36^{a}	1.92	1.87^{a}	1.81	1.92^{a}	2.38
Phenanthrene	2.89^{a}	4.50	0.69^{a}	.58	1.51^{a}	1.43	0.84^{a}	0.90	0.36^{a}	0.40	1.28^{a}	1.47
Anthracene	0.15^{a}	0.15	0.92^{b}	1.43	0.72^{ab}	0.60	0.84^{b}	1.77	0.38^{ab}	0.36	0.69^{ab}	0.82
Fluoranthene	1.12^{a}	0.26	2.31^{b}	0.93	0.84^{a}	0.17	0.69^{a}	0.13	2.00^{b}	0.38	0.45^{a}	0.12
Pyrene	0.51^{a}	0.44	1.64^{b}	1.27	1.25^{ab}	0.73	1.07^{ab}	0.97	0.56^{a}	0.46	0.88^{a}	0.60
Benzo[k]Fluoranthene	0.04^{ab}	0.07	0.23^{c}	0.20	0.14^{abc}	0.40	0.20^{bc}	0.32	0.02^{a}	0.05	0.17^{abc}	0.39
Dibenz[ah]anthracene	0.70^{a}	1.03	0.40^{a}	0.39	0.81^{a}	0.80	0.40^{a}	0.76	1.38^{b}	1.37	0.62^{a}	1.13
Benzo[g,h,I]Perylene	1.14^{b}	1.17	0.22^{a}	0.26	0.35^{a}	0.44	0.34^{a}	0.88	0.09^{a}	0.14	0.05^{a}	0.10
Indeno[cd]pyrene	ND	0.00	2.60^{c}	0.68	0.17^{a}	0.62	0.07^{a}	0.13	ND	0.00	0.52^{b}	0.84
Benzo[a]pyrene	0.29^{a}	0.50	0.31^{a}	0.20	0.25^{a}	0.24	0.18^{a}	0.19	0.30^{a}	0.31	0.16^{a}	0.25
Chrysene	0.08^{a}	0.07	1.24^{c}	0.58	0.23^{b}	0.25	0.21^{ab}	0.16	0.30^{b}	0.22	0.28^{b}	0.23
Benzo[b]fluoranthene	0.54^{ab}	0.98	1.73^{c}	0.61	0.56^{ab}	1.20	0.76^{b}	1.09	0.30^{ab}	0.86	0.17^{a}	0.42
Benz[a]anthracene	0.34^{a}	0.28	1.12^{c}	0.11	0.66^{ab}	0.85	0.20^{a}	0.14	0.88^{bc}	1.68	0.48^{ab}	0.62
PAH4	1.24^{a}	1.62	4.40^{b}	0.81	1.70^{a}	1.61	1.33^{a}	1.09	1.78^{a}	2.70	1.08^{a}	0.68
15PAHs	22.48^{b}	8.84	46.81^{c}	5.67	20.67^{ab}	5.61	17.60^{a}	6.82	19.92^{ab}	6.67	18.40^{ab}	10.62

[a] (a-c): Mean values in row with different superscripts (a, b and c) are significantly different by Duncan's multiple range test at P< 0.05.

edible oils, except for sesame oil and frying oil, was the highest in comparison to other PAHs. Coconut oil had the highest content of naphthalene (12.97±5.01 µg kg^{-1}), followed by Acenaphthene and fluorene. The sum of 15 PAHs and PAH4 were 46.81 and 4.40 µg kg^{-1}, respectively, indicating that such high content of PAHs could be referred to low quality of imported coconut oil in Iran. The German Society for Fat Science (DGF) suggests that PAHs content in edible oils should not exceed 25 µg kg^{-1}. FEDIOL (Féderation de l'Industrie d'Huilerie de la Communauté Européenne) also recommend allowable content of 25 µg kg^{-1} for PAHs in edible fats and oils. Clearly, concentration of coconut oil was higher than the commended values (EUR-Lex 1989). The 15 PAHs for blended oil, frying oil, sunflower oil, sesame oil, and olive oil, were 22.48, 20.19, 17.60, 19.92, and 18.40 µg kg^{-1}, respectively, indicating that other oils had no more than the specified range, except coconut oil. Moreover, other European countries (Spain, Italy, Portugal and Greece) recommend maximum value of 2 µg kg^{-1} for each individual PAH and 5 µg kg^{-1} for sum of heavy PAHs listed as follows: 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. In this regard, which will be discussed below, the concentrations for other PAHs such as benzo[k]fluoranthene, dibenz[a,h]anthracene, benzo[g,h,i]perylenes and indeno[cd]pyrenes were lower than 2 µg kg^{-1}, except for PAH4. Ma *et al*. (2021) assessed the levels of the 15-priority PAHs in the edible vegetable oil (canola oil, olive oil, sunflower oil, and corn oil) collected from Egypt. The authors indicated presence of 15-priority PAHs in all examined oil samples. The highest residual concentrations of PAHs were reported in Canola oil. Heat treatment of canola oil resulted in a drastic increase in the formed B[a]P (316.55%), total 4-PAHs (297.42%), total 2-PAHs (322.47%), total 15-PAHs (443.32%), and total 8-PAHs (285.26%).

According to the result of the total PAHs regarding light PAHs, naphthalene had the highest concentration in all of the different edible oils (especially in coconut oil: 12.97±5.01 µg kg^{-1}), except in sesame oil. Since International Agency for Research on

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Cancer classified naphthalene as one of the most important carcinogenic compounds for humans and animals (Group 2: B), edible oils were required to be monitored (Bempelou *et al.,* 2019).

As mentioned above, since Iran imports most of the vegetable oil for edible purposes, its detailed control and monitoring is very important (particularly in coconut oil). The content of total PAHs in different edible oils varied from 17.6 to 46.81 µg kg^{-1}. The lowest concentration belonged to sunflower oil and the highest was related to coconut oil. The higher concentration of PAH in coconut oil in comparison with other edible oils are also reported by Zachara *et al*., 2017; Silva *et al*., 2017). The authors announced that higher PAH content of coconut oil was related to environmental contaminations (water, air and soil) and contamination during the drying process. Hence, the observed high content of PAHs could be referred to the low quality of the imported coconut oil in Iran. These findings in relation with the minimum concentration of total PAHS are in paralleled with the results reported in a similar study performed in China. Wang *et al.* (2014) developed a method to determine PAHs content of sesame oil, peanut oil, soybean oil, rapeseed oil, and virgin olive oil, and reported that total PAHs content of vegetable oils varied from 18.00 to 639.96 µg/kg.

The PAHs content of different vegetable oils are shown in Table 3. The results of the present study were relatively lower than those reported by other authors (Alomirah *et al.,* 2010; Gharbi *et al.,* 2017; Iwegbue *et al.,* 2020; Ju *et al.,* 2020; Krajian *et al.,* 2016; Lee *et al.,* 2019; Rascón *et al.,* 2018; Stenerson *et al.,* 2015; Taghvaee *et al.,* 2015; Zachara *et al.,* 2017). In particular, Alomirah *et al.* (2010) reported that sum of 16 PAHs content of sunflower oil and olive oil ranged from 0.42 to 41.30 µg/kg and 1.09 to 182.22 µg kg^{-1}, respectively. However, the content of corn and canola oils ranged from 0.30 to 34.49 and 10.29 to 12.23 µg kg^{-1}, respectively, which are in agreement with the present study. On the contrary, Molle *et al.* (2017) reported that PAHs content of 69 samples was up to 13.11 µg kg^{-1}. In particular, they reported 13 PAHs content of canola oil, sunflower oil and corn oil (up to 31.70, 0.65-17.88, and 2.61-38.23 µg kg^{-1}, respectively). Among PAHs analyzed, B[α]P, CHR, B[b]F and B[α]A were the most common PAHs present in the three oils (Canola oil, Sunflower oil and corn oil) studied (99, 97, 97 and 96% of the samples, respectively) (Molle *et al*., 2017). Liu *et al*. (2023) evaluated the levels and health risk of PAHs in frying oils and vegetable oils. The profiles and levels of PAH15 in frying oils (after repeated frying by restaurants) and vegetable oils were analyzed. The authors revealed that vegetable oils are highly contaminated. They also reported that more than 32.4% of the vegetable oils from China exceeded the EU standard limit of the levels of PAHs. Only 6.5% of the oil samples were under allowable level for BaP (2 µg kg^{-1}). The mean concentrations of PAH4 (10.49 µg kg^{-1}) and BaP (2.16 µg kg^{-1}) were marginally above the EU maximum permitted levels in oils (Liu *et al*., 2023).

Effect of PAH Type and Oil Type on Total PAHs in Edible Oils

A factorial experiment was used in a completely randomized design to evaluate the effects of PAH and oil types on PAHs content. After performing the respective variance analysis, the effects of oil type, PAH type and their interactions were significant. The results of the Duncan multi-range comparison test (to determine the effects of oil type) showed that the average total PAHs were categorized into three classes. The first category included coconut oil with the highest amount of these compounds (Figure 2-a), the second group included other oils, and the remaining oils settled in a third group that had no significant difference in terms of total PAHs. In addition, the graph of the effect of PAH type on the total PAHs increasing is shown

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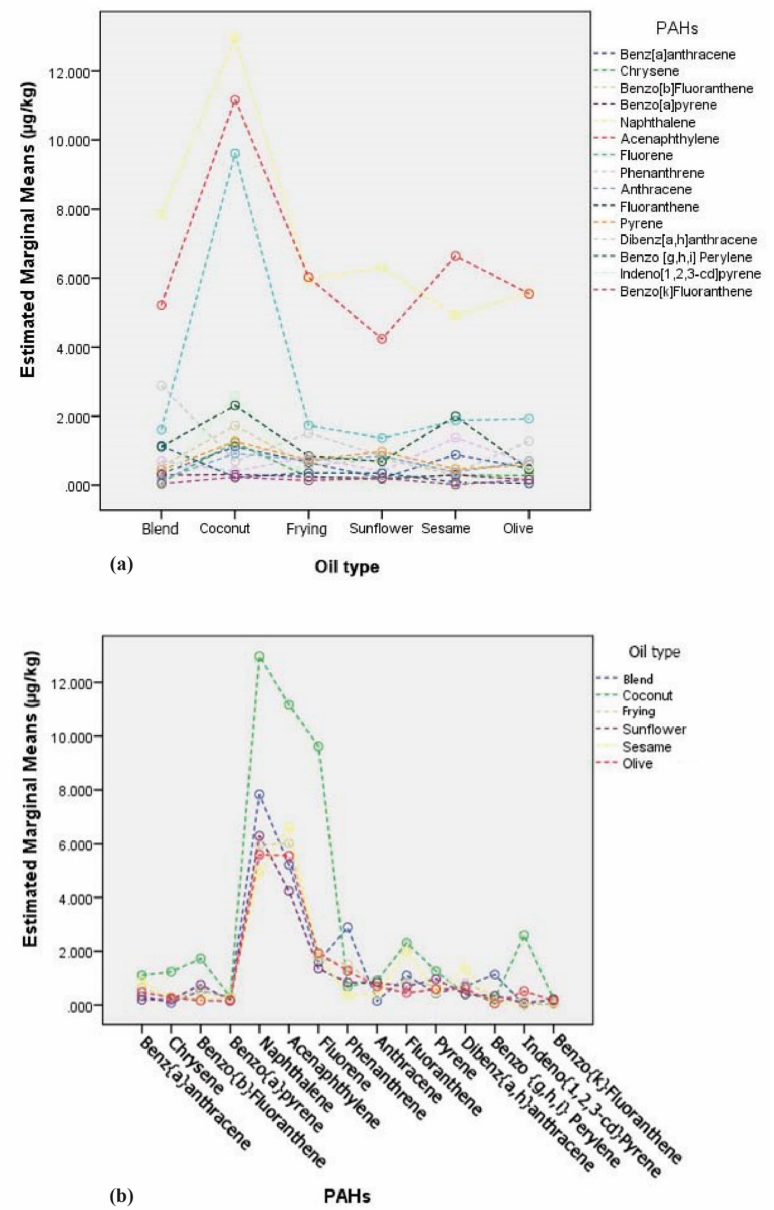

Figure 2. The interaction of (a) Edible oil type and (b) PAHs type on the average estimate of total PAHs.

in Figure 2-b. As can be seen, light PAH compounds, such as naphthalene, acenaphthene, fluorene, etc., had the highest effect on increasing the total PAHs.

PAH4

The content of PAH4, as an important indicator of chemical contaminants, was obtained from total contents of four PAHs, B[α]P, CHR, B[b]F and B[α]A. According to Table 2, no significant difference was observed in B[α]P content between oil samples. B[α]P content ranged from ND to 1.32 µg kg^{-1}. The average B[α]P content in the coconut oil samples was higher than the observed content for other oils. The content of B[α]P in all of the oil samples was less

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than 2 g/kg. It is noteworthy that B[α]P is the most important PAH due to its carcinogenic properties. There was a significant difference between CHR contents of oil samples so that edible oils were classified into three separate groups. The first group consisted of sunflower and blended oils, which had the lowest amount; the second group included olive, sesame and frying oils; and the third group was coconut oil, containing the highest amount of chrysene. The samples were categorized into three groups in terms of the B[b]F amount. Coconut oil and olive oil had the highest and lowest amounts, respectively. The sunflower oil group was placed in the second group and the other groups did not differ from each other significantly. The content of B[α]A was different among edible oil samples. In coconut, the oil group had a much higher

Table 3. PAH contents (μg kg^{-1}) reported in different vegetable oils from a literature review of the last decade.[a]

Oil	Country	Number of samples	EPA 16 PAHs Min–Max (Mean)	PAH4 Min–Max (Mean)	BaP Min–Max (Mean)	References
Olive, sesame, coconut, sunflower, frying blend	IR	207	1.41-52.25 (21.14)	ND-9.20 1.57	ND-1.32 0.23	This study
Olive	TR, SY, IT, ES, PS, TN, LB, CA, SA	21	0.30–182.22 (37.88)	ND	0.06–6.77 (0.53)	Alomirah *et al.* (2010)
Olive	ES, IT, TR, TN, GR, MA, USA	4	9.9–48.3 (22.5)	ND	< LOQ	Stenerson *et al.* (2015)
Olive	IR	5	NS (19.05)	NS (1.28)	< LOQ	Taghvaee *et al.* (2015)
Olive	SY	9	33.4–82.4 (54.8)	0.34–20.2 (7.66)	-	Krajian *et al.* (2016)
Olive	TN	5	11.4–45.8 (33.7)	0.2–0.6 (0.5)	< LOQ	Gharbi *et al.* (2017)
Rapeseed, sunflower, olive, soybean, coconut	PL	3	ND	1.11–3.15 (2.13)	ND–0.25 -	Zachara *et al.* (2017)
Olive	ES	2	4.42–6.36 (5.4)	0.15–0.32 (0.24)	0.045–0.058 (0.051)	Rascón *et al.* (2018)
Olive	KR	53	ND	0.42–4.07 (2.05)	ND–1.15 (0.22)	Lee *et al.* (2019)
Olive	KR	1	ND	2.508	0.481	Ju *et al.* (2020)
Vegetable oil	NI	6	-	3.97-15.1	0.15-0.80	Iwegbue *et al.* (2020)

[a] ND: Not detected, LOD: Limit of detection, LOQ: Limit of quantification, TR: Turkey, SY: Syria, IT: Italy, ES: Spain, PS: Palestine, TN: Tunisia, LB: Lebanon, CA: Canada, SA: Saudi Arabia, GR: Greece, MA: Morocco, USA: United States of America, IR: Iran, KR: Korea, PL: Poland, NI: Nigeria.

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other hand, the collected samples belong to different manufacturers, whose technology and their refining methods are likely to be different. This part of our research are in paralleled with the results of Amzad-Hossain and Salehuddin (2012) who reported different results for different vegetable oils.

These results are in agreement with other studies. Thus, Molle *et al.* (2017) reported that PAH4 content of canola was up to 22.15 μg kg^{-1}, 15.61 μg kg^{-1} in sunflower, and 30.98 μg kg^{-1} in corn; whereas, Ingenbleek *et al.* (2019) reported that PAH4 contained 77% of 13 genotoxic and carcinogenic PAHs. They also reported that PAH4 content of edible oils (including palm oil and peanut oil) was higher than the maximum 10 μg kg^{-1} in 50% of the cases (12.0 μg kg^{-1} on average up to 60.6 μg kg^{-1}).

The maximum mean value of PAH4 was related to the coconut oil group. This issue might be due to the source of oil, coconut as a fruit that has much higher moisture than seed oils. This difference had led to the application of different drying methods involving different duration times, which finally resulted in higher amounts of PAH4 in the coconut oil. The results of this section were in agreement with Moret and Conte (2000) results.

In one sample of sesame oil that was produced by the cold pressing method, the amount of B[α]A (5.60 μg kg^{-1}) and the sum of PAH4 (9.20 μg kg^{-1}) were higher than that observed for other oils. The reason for this result might be related to the production method in which the refining process was not used. This result confirms the finding of Wang *et al.* (2014), who reported high PAHs content of sesame oil because of high temperature of sesame roasting.

It is important to keep in mind that the refining process, especially the bleaching with activated carbon and deodorization, can be an efficient technique in removing PAHs. The results of other researches carried out on virgin and cold-pressed oils also confirm these results (Aliyar-Zanjani *et al.,* 2019). However, the results of this study showed that although Iran imports more than 90% of its crude oil from other countries, owing to the refining processes, the amount of B[α]P is less than 2 μg kg^{-1} and the sum of the four PAHs is less than 10 μg kg^{-1} as well.

It should be noted that the concentrations of PAH4 reported in this study were relatively lower than those reported in previous studies (Niu *et al.,* 2021; Yousefi *et al.,* 2018). The soybean oil purchased from China showed the distribution of B[α]P content ranging from 0.50 to 10.95 μg kg^{-1} (mean 6.26 μg kg^{-1}), from 0.53 to 11.07 μg kg^{-1} (mean 6.96 μg kg^{-1}) in peanut oil, and from 0.53 to 9.88 μg kg^{-1} (mean 5.95 μg kg^{-1}) in colza oil (Niu *et al.,* 2021). Also, Yousefi *et al.,* (2018) analyzed 40 samples of different edible oils available in Iran (frying oil, blended oil, sunflower oil, corn oil and canola oil) and reported 0.90 to 11.33 μg kg^{-1} for B[α]P, 3.51 to 84.03 μg kg^{-1} for PAH4, and 129.28 to 19.54 μg kg^{-1} for PAH13. The sesame oils and perilla oils were highly contaminated with PAHs (Lee *et al*., 2019). A maximum limit value of 2 μg kg^{-1} in the perilla oils and sesame oils were highly contaminated with PAHs. A maximum limit value of 2 μg kg^{-1} for BaP was established in edible oils in Korea and EU. The mean concentration of PAHs in 129 sesame oil samples analyzed was 0.18 μg kg^{-1} for BaP, 0.35 μg/kg for BbF, 0.41 μg kg^{-1} for CHR, 0.41 μg kg^{-1} for BaA, and 1.35 μg kg^{-1} for the sum of 4 PAHs (Lee *et al*., 2019).

Light PAHs account for 65% of all PAHs, while the remaining 35% are heavy PAHs. B[α]P content of 12 samples, according to the Standard Organization of Iran and the European Union, was higher than the standard value (2 μg kg^{-1}), whereas PAH4 content of 15 samples was higher than the maximum 10 μg kg^{-1}.

Effect of PAH Type and Oil Type on PAH4 Content in Edible Oils

A factorial experiment was used by a completely randomized design to evaluate the effects of PAH compounds and oils on PAH4 content. The results of the Duncan multi-range comparison test showed that the

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mean of the PAH4 content in coconut oil differed from other oils (Figure 3-a). Moreover, the diagram of the effects of four PAH compounds on PAH4 content in coconut oil are shown in Figure 3-b. As can be seen, Benzo[b]fluoranthene and Chrysene had a significant effect.

Statistically significant differences were observed between different types of oil as well as different brands. These differences can be attributed to the environmental pollutions; for example, PAHs content may vary in crops of different regions. Moreover, different variables may be involved in the drying process and oil production/refining processes (Molle *et al.,* 2017). Wen-ting Yin *et al*. (2022) evaluated the effects of microwave pretreatment of sunflower kernels on the aroma-active composition, tocopherols, lipid oxidation, sensory quality, heterocyclic amines and polycyclic aromatic hydrocarbons of sunflower oil. The authors reported that the temperature rise by microwaves was responsible for the gradually increased PAHs (7.78–109.76 μg kg^{-1}) and HCAs (7.3–820.6 μg kg^{-1}) in oils.

CONCLUSIONS

In this study, PAHs, as important chemical contaminants, were evaluated in edible oils available in Iran. According to the obtained results, the content of PAH4 was different among edible oils of this study with a mean PAH4 values of 1.57 μg kg^{-1}. The coconut oil group exhibited the highest average content (4.40 μg kg^{-1}) among different kinds of oil and sesame oil presented the highest value of the sum of PAH4 (1.78 μg kg^{-1}). According to the results, none of the oil samples exhibited B[α]P and sum of PAH4 contents higher than 2 μg kg^{-1} and 10 μg kg^{-1}, respectively; whereas, 16PAHs content varied significantly. Therefore, the use of active carbon in purification processes is recommended because it efficiently reduces PAHs content of oils. Moreover, total PAHs factor requires further evaluation and adjustment of allowable limits.

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پایش میزان هیدروکربن های آروماتیک چند حلقه ای در روغن های گیاهی خوراکی مصرفی در ایران

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چکیده

مقادیر ۱۵ ترکیب هیدروکربن آروماتیک چند حلقه ای (PAHs) اولویت دار آژانس حفاظت از محیط زیست امریکا در شش گروه روغن گیاهی مختلف مورد مصرف در ایران از جمله روغن زیتون، کنجد، نارگیل، آفتابگردان، سرخ کردنی و مایع مخلوط مورد بررسی قرار گرفت. روغن‌های گیاهی مورد ارزیابی در این مطالعه، قبلا" از نظر میزان ترکیبات PAH مورد بررسی قرار نگرفته بودند. میزان ترکیبات PAH در ۲۰۷ نمونه روغن گیاهی با استفاده از روش ISO 15753 و توسط دستگاه کروماتوگرافی مایع با کارایی بالا مجهز به آشکارساز فلورسانس (HPLC/FLD) اندازه گیری شدند. نتایج نشان داد که مقادیر مجموع ترکیبات PAH (Total PAHs) به ترتیب در گروه روغن نارگیل (٤٦/٨ میکروگرم بر کیلوگرم)، روغن مخلوط (۲۲/٤۸ میکروگرم بر کیلوگرم)، روغن سرخ کردنی (۲۰/٦۷ میکروگرم بر کیلوگرم)، روغن کنجد (۱۹/۹۲ میکروگرم بر کیلوگرم)، روغن زیتون (۱۸/٤ میکروگرم بر کیلوگرم) و روغن آفتابگردان (۱۷/٦ میکروگرم بر کیلوگرم) بودند. ترکیبات PAH سبک از جمله نفتالین، آسنفتن، فنانترن، آنتراتن و فلورن بیشترین تاثیر را بر افزایش مجموع ترکیبات PAH داشتند. مقدار بنزو[a]پیرن و PAH4 (مجموع چهار ترکیب بنز[a]آنتراسن، کریسن، بنزو[b] فلورانتن و بنزو[a]پیرن) به ترتیبND-۱/۳۲ میکروگرم بر کیلوگرم و ۹/۲-۰/۱٤ میکروگرم بر کیلوگرم بود که روغن نارگیل بیشترین مقدار را داشت. دو ترکیب بنزو[b] فلورانتن و کرایسن اثر معنی داری بر افزایش شاخص PAH4 در روغن‌های مورد بررسی داشتند. با اینکه، مقادیر بنزو[a]پیرن و PAH4 در هیچ کدام از - نمونه‌ها از بیشینه مجاز ۲ و ۱۰ میکروگرم بر کیلوگرم متجاوز نبود، اما با توجه به مقادیر قابل توجه مجموع ترکیبات PAH در برخی از روغن‌های گیاهی مانند روغن نارگیل، تعیین حدود و ارزیابی آن در استاندارد ملی کشور ضروری به نظر می رسد.

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