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Measurement of polycyclic aromatic hydrocarbons (PAHs) in edible mushrooms (raw, grilled and fried) using MSPE-GC/MS method: a risk assessment study

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Abstract

The purpose of this study was to evaluate the concentration of PAHs and health risk in edible mushrooms using magnetic solid phase extraction (MSPE) and gas chromatography/mass spectrometry (GC/MS) method. Based on the results, the concentration of total PAHs was in the range from 0.82 to 6.25 $\mu\text{g}/\text{kg}$ (with the means of $3.85 \pm 0.24 \mu\text{g}/\text{kg}$). In this study, acenaphthylene (Ace) had the maximum mean level in mushroom samples ($0.84 \pm 0.19 \mu\text{g}/\text{kg}$) and benzo(a)pyrene (BaP), acenaphthene (Ac), phenanthrene (Pa), dibenzo[a,h]anthracene (DahA), benzo(a)anthracene (BaA), fluoranthene (Fl), indeno[1,2,3-cd]pyrene (IP) and benzo[g,h,i]perylene (BgP) had minimum mean level (non-detected). The highest mean values of total PAHs ($4.88 \pm 0.30 \mu\text{g}/\text{kg}$) was detected in grilled mushroom samples and lowest mean of total PAHs ($2.77 \pm 0.04 \mu\text{g}/\text{kg}$) was detected in raw mushroom samples. The concentration of contamination in edible mushrooms was raw < fried > grilled, respectively. Furthermore, heat map visualization was applied to evaluate the relationship between the quantity and type of 16 PAHs in edible mushroom samples. Ultimately, the incremental lifetime cancer risk in edible mushrooms for adults and children was $6.85\text{E}-08$ and $3.47\text{E}-07$, respectively, which was lower than the acceptable risk limit (10^{-6}) and all of the edible mushrooms sold in Tehran were considered safe for consumers.

Keywords: Polycyclic aromatic hydrocarbons, Edible mushrooms, MSPE, GC-MS, Health risk assessment

Introduction

Considering the high content of protein and essential elements of edible mushrooms (cultivated and wild), its daily consumption in the world is increasing. Furthermore, wild and farmed mushrooms have been partially substituted for animal proteins in many countries, especially in Europe and Asia, due to their nutrients, health benefits, and pleasant taste [11, 57]. The number of cultivated mushroom species has reached about 30 species

(*Agaricus bisporus* and *Pleurotus ostreatus* are two edible mushrooms that are cultivated in most parts of the world) and due to the approach of people to use more plant proteins, the process of mushroom production is expected in the future will increase [21].

Studies have shown that mushrooms are low in fat but high in protein, carbohydrates and fiber. They also contain a variety of trace elements and minerals such as copper and potassium and vitamins such as folate, riboflavin and niacin [10, 45, 59]. Consumption of edible mushrooms and mushrooms products in the daily diet can be beneficial for human health (this product has anti-tumor, antioxidant, anti-viral, reduction of diabetes, cholesterol, asthma, allergies, stress and insomnia). Edible

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mushrooms commonly contain 10% dry matter and 90% water. Protein is an important component of the dry matter of the mushrooms [9, 49].

PAHs chemically include 2 or more rings of benzene attached at the levels of angular, cluster or linear. PAHs are environmental pollutants that are mainly caused by incomplete combustion of organic matter (oil, coal, wood, gasoline, etc.). These compounds are mostly spread through anthropological activities. However, some PAHs in the environment are caused by natural resources like fires, natural disasters, or oil and coal disposal and volcanic activity, so they are existing in food, soil, water and air [19, 20, 55].

PAHs can be transported and accumulated in plant tissues through soil, air and water. The surface of vegetable wax can also absorb PAHs from the air. The levels of PAHs determined in vegetables are usually in concentrations of 0.01–0.5 µg/kg, however, reliant on the environment, higher concentrations are observed and in special cases, it may exceed 5 µg/kg. On the other hands, several factors are involved in the formation of PAHs during the preparation of food. Some of these factors include fuel (gas, wood, charcoal and electric power supplies), cooking method (roasting, frying and baking), temperature, cooking time, food fat, oil drips on the flame, and proximity to or direct contact between the food and flame [19, 20].

Receipt rate PAHs in various parts of the world depends on their diet. According to classification of the WHO (World Health Organization), humans are exposed to PAHs through respiratory, gastrointestinal, and dermal modes of absorption. The absorption of contaminants from the gastrointestinal tract increases when it is soluble in a high-fat varied diet. Experiments revealed that in animals, 30–50% of PAHs are absorbed through the gastrointestinal tract, and it is mainly metabolized in the liver [1, 6, 15].

The USEPA (United State Environmental Protection Agency) has mentioned sixteen PAH analytes including; chrysene (Ch), IP, fluorene (F), benzo(b)fluoranthene (BbF), naphthalene (NA), Ace, BaP, Ac, BgP, anthracene (A), benzo(k)fluoranthene (BkF), Pa, pyrene (P), Fl, BaA and DahA. In terms of food safety, BaP can be deliberated as a marker for carcinogenicity in foodstuff [8, 12, 23].

There are several techniques to extract PAHs from food samples [e.g., liquid liquid extraction (LLE), Soxhlet apparatus, solid-phase extraction (SPE) and pressurized liquid extraction (PLE)]. The most ordinarily used plans to extract PAHs compounds from an intricate solution matrix include silica cartridges, straight immersion solid-phase micro-extraction (SPME), LLE after saponification with caustic soda, columns with gel beads and two phase

HF-LPME [hollow fiber liquid phase micro-extraction (LPME)] [2, 43, 51, 52]. Fatty components and material with alcoholic caustic solutions, are saponified, to avoid difficulties from lipids [13, 52, 53]. Next to the saponification phase, SPE or LLE can be used for extraction [23, 26, 27, 32, 34, 35]. To identify PAHs in food products, little research has ever been done using GC/FID (flame ionization detector) or GC/MS, HPLC (high performance liquid chromatography) equipped with detection of UV/FL (ultraviolet and fluorescence) and FLD [2, 24, 25, 36, 37, 39, 40, 42].

For the first time in Iran, this research was conducted by an effective, simple and reliable technique for determine of 16 PAHs in raw, fried, grilled edible mushrooms. For this purpose, the sample was prepared and clean-up based on the use of MWCNT (multi-walled carbon nanotubes) as MSPE adsorbent. The prepared sample was injected into GC–MS and the PAHs level were measured and in the second stage, the carcinogenic and non-carcinogenic health risk was assessed.

Materials and methods

Reagents

PAHs mix standards containing sixteen mentioned PAHs were obtained from Supelco Company (Bellefonte, PA, USA). In dichloromethane, the standard solutions were ready, with all of 16 PAHs (0.1 mg/mL). After that, these stock standard solutions with dichloromethane-methanol (50:50, v/v) were diluted every week to make a solution of working mixed (1 mg/mL for each PAH) that it was applied to determine extraction function with variant situations. For internal standard (I.S.) biphenyl, was used (0.05 µg/mL in methanol). Working and stock solutions were preserved at four centigrade degree. MWCNT were bought from Hanwha nanotech Company (MWCNT CM-95, Korea). Similar to the past research, MWCNT-MNP (as adsorbent) was ready [41]. Finally, all extra solvents and chemicals were of grade of analytical-reagent.

Preparation and sampling of mushrooms

One hundred and twenty samples of packaged mushrooms and bulk (3 branded packaging, and 1 bulk brand) were purchased in December 2020 from the main centers of supply of fruits and vegetables. The fresh mushroom samples with deionized water were washed and then dried with flow of air and cut into pieces in the shade, then some of them were fried and grilled. The samples grilled on coal at 150 °C for 5 min and fried with a fryer at a temperature of 120 °C for 5 min. After preparing samples, homogenized, in nylon vacuum-packed and kept at – 18 °C in the place of dark until analysis (for no longer than 4 weeks). The samples were evaluated in duplicate and the mean values were applied for quantification.

Sample preparation

Five grams of each frozen and crushed samples, weighed and transferred to 50 mL conical tube. Then, added 1 mL of the internal standard to the samples. Afterward, the sample was homogeneous, and after adding 7.5 mL of 1 M potassium hydroxide and 7.5 mL of 30%v/v methanol–acetonitrile, homogenization was performed on a shaker with 300 rounds for 5 min. The samples were placed in ultrasonic baths of German model Alma, at a temperature of 40–45 °C for 15–30 min at a frequency of 130 Hz. After removing the conical tubes from the ultrasonic bath and their temperature reaches the ambient temperature, for separation of the fat phase, the samples for half an hour were centrifuged at 4500 rpm. The supernatant was then slowly moved to a 100 mL Erlenmeyer flask. The pH was measured by a pH meter. To adjust the pH to 6.5–7, because the initial pH of the samples was 13, a few drops of concentrated hydrochloric acid (about 6 drops) were added to the samples to reduce pH 9–10, then with hydrochloric acid (1 M), slowly adjusted to pH 6.5–7.

After primary clean up, for adsorption of analytes, 10 mg of magnetic nanocarbon adsorbent and 500 mg of NaCl were added to the samples and then homogenized with a shaker for 10 min. The samples were then transferred to the incubator at 40–45 °C for 12–24 h. The last step involves separating the analytes from the absorbent, which, after the relative drying of the samples, add 5 mL of dichloromethane as solvent to the specimens. It was then homogenized with a shaker at 300 rpm for 10 min. Then, by a magnet (external), the non-impure fluid was slowly moved to the vials. The samples are then sealed, covered with aluminum, and stored at – 18 °C until they are sent to the laboratory and injected into the GC–MC device to prevent light from entering.

Analytical conditions and instrumental

The examination was done on a device of Agilent GC 6890 with a detector of MS 5973 quadrupole (Technologies of Agilent, Palo Alto, CA, USA). It was regulated with a column of capillary of DB-5 ms (30 m, 0.25 mm i.d., 0.25 μm thickness of film). For inlet, the mode was splitless. The temperatures were regulated as follows: the primary temperature of oven 70° (on the Celsius scale), the temperature of injector 290° (on the Celsius scale); retained for 1 min, at a rate of 10° (on the Celsius scale) /min raised temperature to 295° (on the Celsius scale), retained for 7 minutes. The temperature of transfer line was retained at 300° (on the Celsius scale). For carrier gas, helium (He) with constant flow was used (1 mL/min). The temperature of quadrupole was retained at 150° (on the Celsius scale) and temperatures of source were kept

at 230° (on the Celsius scale). At 70 eV, the energy of electronic beam of the MS was regulated. The qualification was done by comparing the achieved mass spectra and times of retention to reference spectra. By injection calibration standards in same GC/MS conditions, the times of retention were obtained. With using SIM mode, the PAH analytes were quantified.

Health risk assessment

In this research, daily dietary (DD) PAHs contact levels (μg.kg-1 body weight) and BaP daily intake equivalents [$\sum(\text{BaPeqi})$] (μg/day) through consuming mushroom was conducted based on Eq. 1 and 2:

$$DD = \frac{C \times IR}{BW} \quad (1)$$

$$DI_{\text{BaPeq}} = \frac{(\sum_{i=1}^n C_i \times \text{TEF}) \times IR_i}{BW} \quad (2)$$

where IR is the weight of mushroom consumed (3.56 g day⁻¹) [22]; C is the level of the PAHs congener i in mushroom,, the toxicity equivalency factor (TEF) was adopted to calculate BaP equivalent concentration (Table 1), BW, the average Iranian weight of body (for adults 70 and children 15 kg).

Besides, the ILCR (incremental lifetime cancer risk) of mushroom due to PAHs dietary exposure was evaluated according to Eq. (2).[5, 56]:

$$ILCR = DI_{\text{BaPeq}} \times EF \times E_D \times CSF/AT \quad (3)$$

where DI_{BaPeq} , EF, AT, and CSF denote the daily intake of BaP equivalents, exposure duration (year), lifespan for carcinogens (25,550 days)[54] and the frequency of exposure (365 days/year) and slope factor of oral cancer for BaP which is 7.3 (mg/kg-day)(USEPA. Risk assessment guidance for Superfund: volume III part A and risk assessment. Washington), respectively.

Table 1 PAHs and their toxic equivalent factors (TEFs)

PAHs	TEF	PAHs	TEF
Benzo(a)pyrene (BaP)	1	Anthracene (A)	0.01
Dibenz(a,h)anthracene (DahA)	1	Naphthalene (NA)	0.001
Benzo(k)fluoranthene (BkF)	0.1	Acenaphthylene (Ac)	0.001
Indeno(1,2,3-cd)pyrene (IP)	0.1	Acenaphthene (Ace)	0.001
Benz(a)anthracene (BaA)	0.1	Phenanthrene (Pa)	0.001
Benzo(b)fluoranthene (BbF)	0.1	Fluorine (F)	0.001
Chrysene (Ch)	0.01	Pyrene (P)	0.001
Benzo(g,h,i)perylene (BgP)	0.01	Fluoranthene (Fl)	0.001

Table 2 Comparison of current research with other studies with different adsorbents and in different foods

Sample	Adsorbent	Analysis method	Instrumental analysis	LOD	Linear range	Recovery %	Reference
Beef, lamb, chicken meats	MWCNT-Fe ₃ O ₄	Magnetic-SPE	GC-MS	0.075–0.200 (µg/kg)	0.1–250 (µg/kg)	81.3–96.7	[41]
Tea and coffee	MWCNT-Fe ₃ O ₄	Magnetic-SPE	GC-MS	0.040–0.084 (µg/kg)	0.050–10.000 (µg/kg)	91.1–102.3	[52]
Milk and milk powder	MWCNT-Fe ₃ O ₄	Magnetic-SPE	GC-MS	0.040–0.075 (µg/kg)	0.050–1.000 (µg/kg)	86.1–100.3	[53]
Water sample	MWCNT	µ-SPE ^a	GC-MS	0.0042–0.0465 (ng/mL)	0.1–50.0 (ng/mL)	72.3–99.2	[16]
Water sample	MWCNT	HS-µ-SPE ^b	GC-FID	0.03–1.0 (ng/mL)	0.1–200 (ng/mL)	88–105	[31]
Environmental water, vegetable, and fruit juice samples	IL-MIL-100(Fe) ^c	VA-d-µ-SPE ^d	GC-FID	0.002–0.005 (ng/mL)	0.02–200 (ng/mL)	97.0–103.5	[46]
Environmental water samples	MWCNT	SPE	GC-MS	0.002–0.0085 (ng/mL)	0.02–5.00 (ng/mL)	76.0–125.5	[30]
Environmental water samples	CTAB/TiO ₂ ^e	µ-SPE	HPLC-UV	0.026–0.82 (ng/mL)	0.2–100.0 (ng/mL)	75.0–114	[17]
Water samples	Nylon 6 composite	µ-SPE	UPLC-DAD	0.050–0.580 (ng/mL)	–	80–111	[50]
Environmental water samples	ZIF-8 ^f	µ-SPE	GC-MS	0.002–0.012 (ng/mL)	0.1–50 (ng/mL)	96.9–106.5	[14]
Water samples	MCFG ^g	µ-SPE	GC-MS	0.2–1.8 (ng/mL)	0.01–100 (ng/mL)	67.5–106.9	[44]
Real water and urine samples	PFu/Fe ₃ O ₄ ^h	µ-SPE	GC-FID	0.005–0.02 (ng/mL)	0.02–200 (ng/mL)	93.2–99.2	[4]
Mushroom samples	MWCNT-Fe ₃ O ₄	Magnetic-SPE	GC-MS	0.020–0.080 (µg/kg)	0.1–250 (µg/kg)	91.2–101.7	Current study

^a µ-SPE solid-phase micro-extraction

^b Headspace solid phase microextraction

^c Liquid-modified metal-organic frameworks

^d Vortex-assisted dispersive solid-phase microextraction

^e Cetyltrimethylammonium bromide modified ordered TiO₂

^f Zeolite imidazolate framework 8

^g Magnetic chitosan functionalized graphene oxide

^h Magnetite nanoparticles modified with polyfuran

Statistical analyses

The results were analyzed using SPSS version 25 software. At first, descriptive indexes of variables such as variance, deviation of variance, mean, maximum and minimum were obtained. To compare other PAHs values (means), analysis of variance (one-way) was applied. A heat map clustering was performed to establish a more precise distinction among the PAHs congener in mushrooms [26–28]. Multivariate analysis of statistical was used to assess the correlation among NA, Ace, F, A, B(k) F, Ch, B(b)F and P concentration. Analysis of heat map (variable scaling mode: z-score; method of clustering: average linkage; distance measurement method: Pearson was performed to evaluate the correlation between samples online at <http://www.heatmapper.ca/>.

Results and discussion

Performance assessment of the analytical method

In the 1st step, the analytical technique involved liquid extraction of PAHs and in the 2nd phase, SPE technique using a composite of MNP-sized. The extracted polycyclic aromatic hydrocarbons were then evaluated using a technique of GC-MS (sensitive instrument). The mass spectrum (full scan), toward the recognition goal, the ratios of four characteristic ions and the ± 0.5 percent relative retention time (RRT) tolerance criteria compared to the standard was applied. For purpose of quantification, the most intense ion was applied of each compound. The compounds were quantified by SIM mode (selected ion monitoring). The dwell time (for each ion) was regulated at 100 min. To reduce the time of examination, the conditions of GC were selected while allowing all

Table 3 Statistics analysis of PAHs levels in mushroom samples ($\mu\text{g}/\text{kg}$)

	Min.	Max.	Mean	SD
NA	0.1	1.03	0.67	0.15
Ace	0.24	1.38	0.84	0.19
Ac	nd	nd	nd	nd
F	0.09	0.43	0.28	0.07
Pa	nd	nd	nd	nd
A	0.14	0.61	0.37	0.1
Fl	nd	nd	nd	nd
P	0.06	0.63	0.44	0.12
BaA	nd	nd	nd	nd
Ch	0.09	0.81	0.34	0.09
BbF	nd	0.57	0.39	0.08
BkF	0.1	0.79	0.52	0.1
BaP	nd	nd	nd	–
IP	nd	nd	nd	–
DahA	nd	nd	nd	–
BgP	nd	nd	nd	–
Total	0.82	6.25	3.85	0.24
PAH4	0.19	1.38	0.73	0.05

compounds (PAHs) to elute in acquisition collections include a proper number of ions for checking. For each compound were controlled two qualifier ions and one quantitation [41]. For analysis, the conditions of optimum

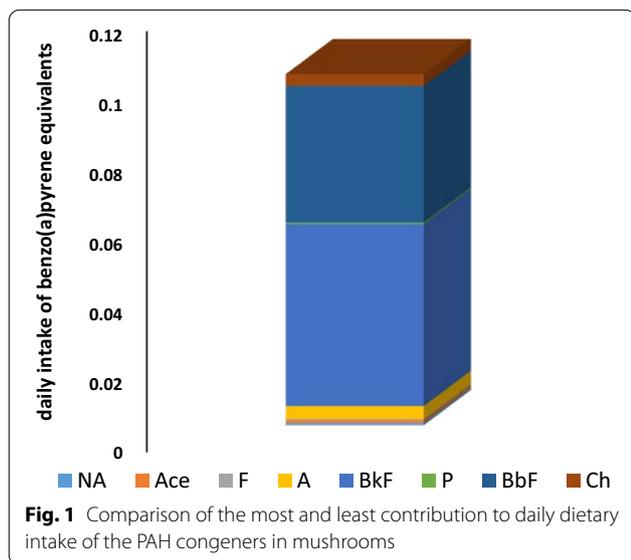
were supplied and the calibration curves were made (0.050–5.000 $\mu\text{g}/\text{kg}$) with a coefficient of correlation was 0.979–0.994. Based on the guideline of International Council for Harmonization (ICH), the limit of quantification (LOQ) for each compound, was determined [41]. The conclusion of validation tests designated that limit of detection (LODs) and LOQs for the PAHs analytes altered 0.020–0.080 and 0.063–0.242 $\mu\text{g}/\text{kg}$, respectively. According to the precision of interday via analysis QC (quality control) samples, ready on three repeated days at four levels, the accuracy of the method was assessed. Other hands, for all of the PAH compounds, the precision of interday values were fewer than 8.9%. For repeatability, the estimated values were between 4.1 and 10.6 percent and the recoveries estimated between 91.2 and 101.7 percent. By all these results to measurement the PAHs in kinds of edible mushroom samples, the reliability and feasibility of the established technique were confirmed. By examining 120 edible mushroom samples, the selectivity of the technique was confirmed. At the end, no interfering peak was seen, in the area of the analytes and internal standard. A comparison of the present study with other studies with adsorbents and in different foods can be seen in Table 2. The findings show acceptable levels for different adsorbents in different foods.

Table 4 Concentration of PAEs in raw samples and different process of mushroom samples ($\mu\text{g}/\text{kg}$)

Process	Raw			Fried			Grilled			P-Value
	n	Mean	Std. deviation	n	Mean	Std. deviation	n	Mean	Std. deviation	
NA	20	0.45	0.01	20	0.66	0.01	20	0.89	0.02	0
Ace	20	0.69	0.02	20	0.85	0.02	20	0.98	0.03	0
Ac	20	nd	–	20	nd	–	20	nd	–	–
F	20	0.21	0.01	20	0.28	0.01	20	0.35	0.02	0
Pa	20	nd	–	20	nd	–	20	nd	–	–
A	20	0.23	0.01	20	0.38	0.01	20	0.49	0.03	0
Fl	20	nd	–	20	nd	nd	20	nd	–	–
P	20	0.31	0.01	20	0.47	0.012	20	0.54	0.01	0
BaA	20	nd	nd	20	nd	–	20	nd	–	–
Ch	20	0.18	0.01	20	0.34	0.01	20	0.52	0.02	0
B(b)F	20	0.33	0.01	20	0.36	0.02	20	0.49	0.01	0
B(k)F	20	0.38	0.02	20	0.52	0.03	20	0.65	0.03	0
BaP	20	nd	–	20	nd	–	20	nd	–	–
IP	20	nd	–	20	nd	–	20	nd	–	–
DhA	20	nd	–	20	nd	–	20	nd	–	–
BgP	20	nd	–	20	nd	–	20	nd	–	–
Total	20	2.77	0.04	20	3.88	0.12	20	4.88	0.3	0
PAH4	20	0.5	0.01	20	0.88	0.03	20	0.98	0.05	0

Table 5 Uncertainty analysis for the daily intake ($\mu\text{g}/\text{kg}$ bw/day) of PAHs in mushroom

	Compound name	Percentiles			
		5%	50%	75%	95%
Adults	NA	2.55E-5	3.35E-5	3.74E-5	4.48E-5
	Ace	3.22E-5	4.28E-5	4.74E-5	5.72E-5
	F	1.05E-5	1.40E-5	1.56E-5	1.89E-5
	A	1.39E-5	1.83E-5	2.08E-5	2.44E-5
	B(k)F	1.68E-5	2.23E-5	2.47E-5	2.90E-5
	P	1.28E-5	1.73E-5	1.96E-5	2.25E-5
	B(b)F	1.50E-5	2.01E-5	2.26E-5	2.65E-5
	Ch	1.99E-5	2.64E-5	2.94E-5	3.41E-5
Children	NA	1.18E-4	1.58E-4	1.76E-4	2.06E-4
	Ace	1.48E-4	1.97E-4	2.22E-4	2.62E-4
	F	5.11E-5	6.63E-5	7.41E-5	8.67E-5
	A	6.44E-5	8.63E-5	9.70E-5	1.12E-4
	B(k)F	7.80E-5	1.04E-4	1.16E-4	1.36E-4
	P	6.12E-5	8.01E-5	8.96E-5	1.07E-4
	B(b)F	7.03E-5	9.21E-5	1.04E-4	1.21E-4
	Ch	9.43E-5	1.22E-4	1.37E-4	1.65E-4

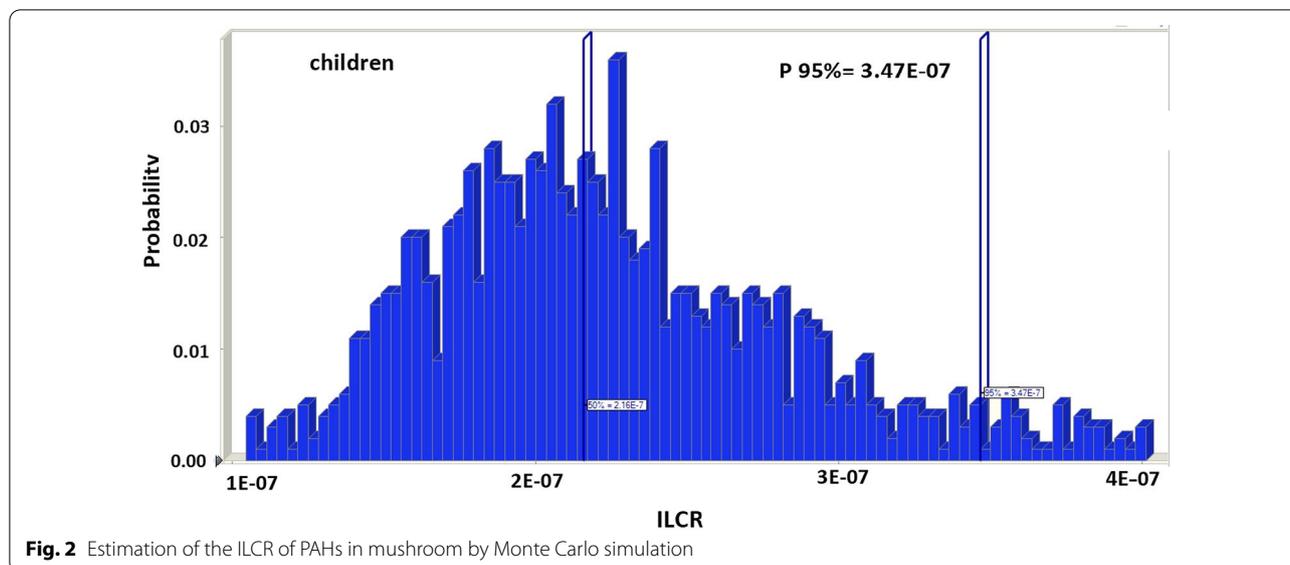


Evaluation of PAHs levels in mushroom samples

In Table 3, the mean values of PAHs compounds in mushroom samples are existing. The recorded means of total PAHs in edible mushroom was $3.85 \pm 0.24 \mu\text{g}/\text{kg}$ (Table 3). The European Commission (EC) established maximum level of BaP in various foods (for dried herbs $10 \mu\text{g}/\text{kg}$). According to existing regulations, concentration of BaP in fruit and vegetable shouldn't be more than $1 \mu\text{g}/\text{kg}$ [47]. The present outcomes exhibited that the

BaP concentration was below the standard levels (ND or non-detected) in all samples. In this research, Ace had the maximum mean level in edible mushroom samples ($0.84 \pm 0.19 \mu\text{g}/\text{kg}$) and Ac, Pa, Fl, BaA, BaP, IP, DhA and BgP had minimum mean level (ND). Among the groups recommended in EC Regulation Number 835/2011 (2011), for PAH4 maximum value of $1 \mu\text{g}/\text{kg}$ was regulated in processed cereal-based foods and $50 \mu\text{g}/\text{kg}$ in dried herbs. The mean levels of PAH4 detected in the current research were below the standard limits since the detected values were $0.73 \pm 0.05 \mu\text{g}/\text{kg}$ in edible mushroom samples.

Igbiri et al. [18] by measuring PAHs in mushroom showed that the ΣPAHs varied from ND to $8320 \mu\text{g}/\text{kg}$, and BaP ranged ND- $2460 \mu\text{g}/\text{kg}$, which in some samples were lower and in others samples higher than the our study. Abou-Arab et al. [1] by measuring PAHs in fruits and vegetables showed that the maximum concentration of ΣPAHs was $8.977 \mu\text{g}/\text{kg}$ in Spinach samples, $6.196 \mu\text{g}/\text{kg}$ in potato samples, $2.867 \mu\text{g}/\text{kg}$ in apple samples and $2.334 \mu\text{g}/\text{kg}$ in guava samples, which were higher than the our study (except apple and guava). Ashraf et al. by measuring PAHs in fruits and vegetables showed that anthracene level was higher in all vegetables, among all PAH congeners. The highest concentration of BaP was in potatoes ($2.90 \pm 1.10 \mu\text{g}/\text{kg}$) and in turnip ($2.10 \pm 1.09 \mu\text{g}/\text{kg}$), respectively. The levels of ΣPAH of the root vegetables like carrot and potato were presented higher values ($11 \mu\text{g}/\text{kg}$), however levels of ΣPAH in turnip showed a lower ratio ($9.26 \mu\text{g}/\text{kg}$). In tuberous vegetable cores, the cores were less contaminated than the peels. For leafy vegetables, highest concentration of PAHs were presented in cabbage ($8.34 \mu\text{g}/\text{kg}$), which were higher than our results [6]. Camargo et al. [8] by measuring PAHs in fruits and vegetables showed that the mean concentrations of ΣPAHs were 3.77 in grape, 3.87 in pear, 4.05 in apple, 8.86 in cabbage, 9.50 in tomato and $13.53 \mu\text{g}/\text{kg}$ in lettuce, and Ch was not detected in any sample and total PAHs were more than our study (except in apple) and Ch was less than our study. Bishnoi et al. [7] by examining PAHs in fruits and vegetables showed that the ΣPAHs concentration in different fruit and vegetable samples ranged from 25.5 to 51.7 and 59.6 to $194.3 \mu\text{g}/\text{kg}$, respectively that were higher than our study. Lee et al. [28] by measuring PAHs in fruits and vegetables showed that the mean level of BaP in fruits was not detected and in vegetables was $0.05 \mu\text{g}/\text{kg}$ that was more than our study. Zhu et al. [62] by measuring PAHs in vegetables showed that the estimated levels of total 15 PAHs were $79 \mu\text{g}/\text{kg}$ in cabbage shoots and $83 \mu\text{g}/\text{kg}$ in carrot roots that were higher than our results. Lei et al. [29] by measuring PAHs in vegetables showed that the ΣPAHs



concentrations in these vegetables were from 3.91 to 96.98 $\mu\text{g}/\text{kg}$ that were higher than our results. Waqas et al. [61] by measuring PAHs in fruits and vegetables reported that the level of total PAHs in vegetables were from 51.6 to 402 $\mu\text{g}/\text{kg}$ on dry weight bases, which were higher than this research. Wan et al. [60] by measuring PAHs in vegetables reported that the PAHs levels in the vegetable samples were ranged from 26.35 to 3748 $\mu\text{g}/\text{kg}$ with the mean of 656.3 $\mu\text{g}/\text{kg}$ that were higher than our results. In a research published by Paris et al. [48] reported that in several studies, the level of some PAHs in various vegetables and fruits can exceed 0.5 $\mu\text{g}/\text{kg}$ and even reach 5 $\mu\text{g}/\text{kg}$.

The existence of PAHs in food products relies on the time of growing, the type of plants and plants growth environment (e.g., water, air and soil) as well as the proximity of farms of plants to high-traffic highways and industrial centers.

The results of this study show a lower level of pollution compared to other studies, which could be due to reasons such as distance from contaminated areas, use of water, soil and compost free of pollution, careful and continuous monitoring of agricultural products especially edible mushrooms.

Comparison of the PAHs concentration between raw samples and various process (fried and grilled)

In Table 4, the means of the level of PAHs among raw mushroom samples and process mushroom samples (grilled and fried) were demonstrated. The uppermost mean values of total sixteen PAHs and PAH4 were detected in grilled mushroom samples 4.88 ± 0.30 and 0.98 ± 0.05 $\mu\text{g}/\text{kg}$, respectively, and raw mushroom

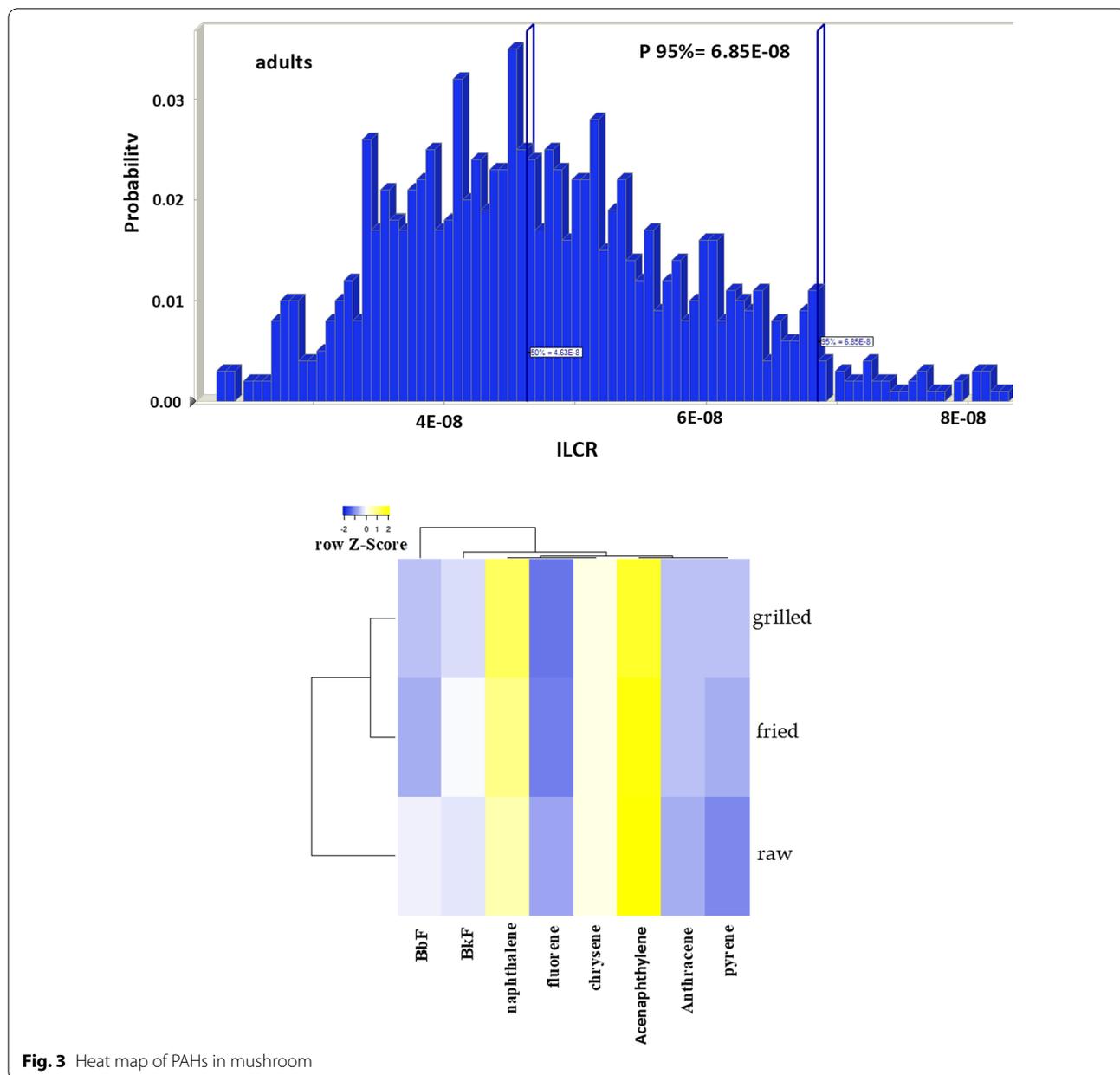
samples had minimum mean values of them (2.77 ± 0.04 and 0.5 ± 0.01 $\mu\text{g}/\text{kg}$, respectively). BaP in all samples was not detected. The amount of contaminant in the mushroom was raw < fried < grilled, which may be due to the use of coal or the use of direct heat in grilled process. Other studies were shown analogous results [3, 15, 41].

Human Risk assessment and exposure to toxic elements

The daily dietary intake of the PAHs congener through edible mushrooms consumption for children and adults in Iran are listed in Table 5. For adults, the rank order of PAHs congener based on DD (P95) was Ace ($5.72\text{E-}05$) > NA ($4.48\text{E-}05$) > Ch ($3.41\text{E-}05$) > B(k)F ($2.90\text{E-}05$) > B(b)F [$2.65\text{E-}05$ > A ($2.44\text{E-}05$) > P ($2.25\text{E-}05$)] > F ($1.89\text{E-}05$). The rank order of PAHs congener based on DD (P95) for children was Ace ($2.62\text{E-}04$) > NA ($2.06\text{E-}04$) > Ch ($1.65\text{E-}04$) > B(k)F ($1.36\text{E-}04$) > B(b)F ($1.21\text{E-}04$) > A ($1.12\text{E-}04$) > P ($1.07\text{E-}04$) > F ($8.67\text{E-}05$). Furthermore, the higher and lower contribution to overall, DD in mushrooms was related to BkF and F, respectively (Fig. 1).

Further, the outcomes of the Monte Carlo simulation revealed that the mean of ILCR for adults and children based on P95 was $6.85\text{E-}08$ and $3.47\text{E-}07$. In Fig. 2, the carcinogenic risk for PAHs congener was clearly lower than the acceptable risk level (10^{-6}) by USEPA. In a research conducted in Nigeria, the ILCR ranged from 1.56×10^{-8} to 1.73×10^{-6} with the uppermost calculated risk found for edible mushrooms [18].

The contamination profiles, human exposure risks of PAHs and corresponding knowledge obtained are crucial for contamination mitigation strategies and human



health risk development on the PAHs study. The biological monitoring of human exposure about the dangers and potential side effects of PAHs depend on various parameters, including the route and concentration of exposure, as well as the toxicity of the PAHs. It is important to demonstrate linkages between outcomes of risk patterns and similar risk behavior of PAHs and may thus give the best possible risk assessment in realistic, intermediate and complex contaminant distribution. The two main parameters which contribute to general PAHs persistence in the environment depends on molecule stability and hydrophobicity. However, the percentages of PAH

compounds change with food consumption, water, sediment, soil, sites and seasons, air–soil exchange and associated carcinogenic risks of PAH compounds [33, 38]. Physical, chemical, and biological methods are the most preferable and cost-effective remediation methods for contamination degradation or transformation until safe levels in food, air, water, and soil [35–37, 39].

Multivariate analysis of PAH congeners

Heat map was used to understanding the relationships among the PAHs congener in different raw, fried and grilled samples. A basic heat map, in addition to grouping

columns and rows of comparable variables, gives a very general impression of the largest and smallest variables in the generating matrix. Furthermore, heat maps revealed that the different samples (raw, fried and grilled) is considered as independent factors in the PAHs congener clustering. The heat map clustered samples of edible mushrooms into 2 major clusters and three sub clusters (Fig. 3). The first cluster such as A, Ace, B(k)E, Ch, F and P contains two sub-groups, The second cluster only includes B(b)F. ClustVis was used to visualizing clustering of similarity and variability data. The NA, Ace, Ch, F and P concentrations were the closer, indicating that the concentration changes of these PAHs congener had similar trend in different samples (raw, fried and grilled).

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Authors' contributions

NS: supervision, methodology, writing—reviewing and editing. GJK: data curation, conceptualization, writing—reviewing and editing. MA: visualization, investigation, software, methodology, validation. MM: methodology, software, validation, MM: methodology, visualization, investigation, PS: validation, writing—reviewing and editing. All authors read and approved the final manuscript.

Availability of data and materials

The data set used will be available upon reasonable request from the corresponding author.

Declarations

Ethics approval and consent to participate

This research does not include any human or animal experiments and is approved by Tehran University of Medical Sciences. The authors were fully involved in this study.

Consent for publication

The authors agree with the publication.

Competing interests

Authors have no competing interests.

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