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Effect of solvents on extraction of polycyclic aromatic hydrocarbons from Coffee, Chicken and Kitchen exhaust depositions

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Abstract: *Polycyclic aromatic hydrocarbons are carcinogenic compounds which are formed and accumulated in different food products at pre- and post-harvest stages. Extraction is an important step performed prior to PAHs determination and is governed by various factors. Type of solvent used for extraction is one such factor affecting the quantification and profiling of PAHs based on its interaction with food matrix. The present study focused on selection of solvent for the higher extraction efficiency of PAHs from matrices such as chicken, coffee and kitchen exhaust depositions. The different solvents such as cyclohexane, hexane, toluene, acetonitrile and DCM:Hexane were targeted. The highest concentration of total PAHs extracted from food matrix varied greatly with solvent used. Highest PAHs concentration (36.30 mg/kg) was obtained in kitchen exhaust depositions followed by coffee (28.96mg/kg) and chicken (19.39mg/kg) when the solvents of extraction were in cyclohexane, acetonitrile and toluene, respectively.*

Keywords: *PAHs, Kitchen exhaust, Coffee, Chicken, Solvents*

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I. INTRODUCTION

Incomplete combustion or pyrolysis of organic material produces carcinogenic compounds called as polycyclic aromatic hydrocarbons [1]. Most of these aromatic ringed compounds are known for their possible and probable genotoxic and carcinogenic nature. Benzo(a)pyrene(BaP) is the most studied PAH belonging to human carcinogen Group 1 category [2]. Environment Protection Agency enlisted 16 PAHs pollutants with toxic potential as food and environmental contaminants, while four PAHs namely B(a)P, Benzo(a)anthracene [B(a)A], Benzo(b)Fluoranthene [B(b)F] and Chrysene [Chry] have been recommended as suitable indicator for PAHs in food [3]. Dietary PAHs exposure to humans comes from different food categories such as dairy, fruits and vegetables, cereal foods, confections, beverages, oils, medicines and decoctions, infant based formulations, nuts, eggs, meat and related animal foods.

In animal food categories, substantial amount of PAHs have been reported for fried pork loin (21.45μg/kg), lamb (16.91μg/kg), chicken (14.96-457.6μg/kg), Kabab (17.94μg/kg), smoked meat and fish (2-73.01mg/kg), peanut (28.21μg/kg) etc.[4, 5, 6, 7, 8, 9]. However, chicken is the most commonly available and consumed meat product worldwide and thus PAHs presence and formation in this food product has achieved immense importance. The product is cooked by various methods such as grilling, roasting, barbecuing, on-fire toasting, frying [10, 11], boiling and smoking [12, 13]. During these processes, chicken is exposed directly to heat source which contributes in pyrolysis and burning followed by the dripping of fat on heat source. This generates PAHs and facilitates its accumulation in the food product [14]. Among different beverages, the higher prominence and concentration of PAHs up to 3091.1µg/kg is also observed in coffee [14] owing to its toasting, roasting and brewing at higher temperatures. Roasting at and above 260°C leads to degradation of 3-ringed PAHs, transforms light PAHs to heavy PAHs and generates Chry, pyrene (Pyr) and benzo(ghi)perylene [15]. The reaction is accompanied by caramelization, fat and sugar degradation, maillard reaction, moisture and weight loss. The increasing consumption of this beverage makes it important to evaluate PAHs profile and identify the means to cut down PAHs formation in this beverage. As observed, the PAHs contamination in these two products needs to be addressed to determine their contributory role in exposing humans to ingestion based cancer risk.

Humans' exposure to PAHs also occurs from smoke released during thermal and combustion processes [15]. Cooking fumes and emissions have been identified as the major sources responsible for indoor air pollution [16]. PAHs adsorb on smaller and ultrafine particles of particulate matter, which on inhalation may

result in respiratory problems, lung cancer, cardiovascular deaths etc. The third route (besides diet and inhalation) by which humans is exposed to PAHs in a kitchen environment or related cooking processes is dermal exposure. The kitchen dust and soot act as sink to these pollutants and may be inhaled or absorbed through skin after re-suspension in air [17, 18]. Hence, it may be stated that exposure to PAHs through food ingestion, dermal exposure to kitchen dusts and inhalation of cooking fumes or emissions are inevitable. Thus it becomes necessary to monitor the concentrations of PAHs in these sources and take necessary prevention measures.

Monitoring of PAHs in a matrix is dependent on various extrinsic and intrinsic factors such as type of sample, fat composition, method of extraction, instrumentation etc. The effective determination of PAHs also depends upon the solvent used for the extraction which is known to exhibit prominent effect on the method efficiency and overall results. The present study was thus aimed to observe the effect of solvent on PAHs extraction and profiling from chicken, coffee and kitchen exhaust depositions which are the most common sources of PAHs exposure in a kitchen environment.

II. MATERIAL AND METHODS

2.1 Sample

Sample of tandoori chicken, commercial coffee product and kitchen exhaust depositions were used for the study. Sample of tandoori chicken and coffee were collected from a local market in Kundli (Sonipat, Haryana, India). The depositions on kitchen exhaust fan were collected from a local dhaba situated on Grand trunk road near Kundli area (Haryana, India).

2.2 Chemical reagent

Analytical standard mixture for 16 priority PAHs in variable concentrations (500 and 1000µg/kg) was procured from Restek company (Bellefonte, PA). Acetonitrile, n-hexane, toluene and cyclohexane (HPLC grade) was obtained from SRL Pvt Ltd. (Mumbai, India). Hexane, toluene and cyclohexane (HPLC grade) was obtained from Sd-fine chemical Ltd. (Mumbai, India). All the chemicals and reagents used were of analytical grade. Water was obtained from a Millipore Milli-Q water purification system (Model: 2003-D, SG, Germany). Nylon filters of 0.2µm and 25mm size were purchased from Axiva-Sichem Biotech (New Delhi). Silica gel with 70-230 mesh size and 60Å was procured from Merck-Sigma-aldrich (Switzerland). Stock standard solutions were prepared by diluting the PAHs standard in an appropriate volume of acetonitrile and stored in refrigerated conditions till further use.

2.3 Standard curve

Working standard solutions of concentrations 6.25, 12.5, 25, 31.25 and 37.5ppb were used to prepare an external standard plot. Duplicate HPLC injections for above concentrations in acetonitrile were used to construct linear regression lines (peak area v/s PAH concentration) which was further utilized for measuring the PAHs concentration of unknown samples.

2.4 Extraction and clean up

The extraction of PAHs from tandoori chicken and kitchen exhaust deposition was performed in three solvents, namely cyclohexane, hexane and toluene. One gram of the sample was taken and homogenized with 10ml of 1M solution of potassium hydroxide methanolic solution (50:50v/v). The solution was then collected in a glass bottle and heated at 60°C for 5h in a water-bath (Sanco, Canada). After 5h, the sample was taken out and cooled. In one solvent set, water (5mL) and Cyclohexane (5mL) was added. In second and third solvent set, cyclohexane was replaced with hexane and toluene. The solution was then heated at 60°C for 1h, shaken vigorously and centrifuged at 4000g for 15min. The supernatant layer was collected and concentrated to 500-1000µL in a rotary evaporator (Buchi, Switzerland). On other hand, the extraction of PAHs from coffee sample was done in the acetonitrile and dichloromethane: hexane (85:15v/v) solvents. One gram of coffee sample was extracted with 10mL of each solvent using a probe-sonicator (Q sonica, Q500 model, USA). The pulse cycle was '2s off' and '2s on' and the complete time of sonication was 30min. A 40% amplitude level was set for the instrument. The extract was collected and concentrated in a rotary evaporator to a volume of 500-1000µL.

PAHs in extracts were then fractionated by silica gel column (4mm i.d.) using the method described by Agarwal et al., [19] with slight modifications. Five grams of silica gel was activated at 180°C for 24h and kept in dessicator. Before use, the powder was deactivated with 1% water and 40mL of n-hexane was added to make the slurry. A packed column was made and concentrated sample extract was poured on the top of the column. The column was first eluted with 10mL of n-hexane. The eluate was discarded and the PAH fraction was further eluted from packed column with 20mL hexane: tolune (50:50 v/v) solution. The fraction possessing PAH was then concentrated to 0.5mL after solvent exchange to acetonitrile. The final volume for each sample concentrate was then made 1mL and passed through nylon filters before chromatographic analysis.

2.5 PAHs analysis using high performance liquid chromatography

HPLC analysis was performed using a Waters LC system consisting of Waters 515 pump, Waters 2707autosampler, thermostatted column compartment and Waters 2475 fluorescence detector with standard FLD flow cell using Empower-two software. Spherisorb ODS-2 column with pore size 80Å, particle size 5 µm, 250mm ×4.6mm ID and 30°C temperature was used. Separation was carried out at isocratic flow rate of 0.50 with 10% water and 90% acetonitrile condition for 35 minutes. Samples were detected using multiple wavelength fluorescence with following excitation-emission wavelength combinations and timed events: a) time: 0.10-6min, Ex: 260 and Em: 350, b) time: 6-11.50min, Ex: 256 and Em: 370, c) time: 11.50-21min, Ex: 290 and Em: 420 and d) time: 21-35min, Ex: 280 and Em: 500. Peak identification was based on the retention time for peaks obtained for standard. The HPLC elution profile of the PAH compounds analyzed in this investigation are shown in fig. 1.

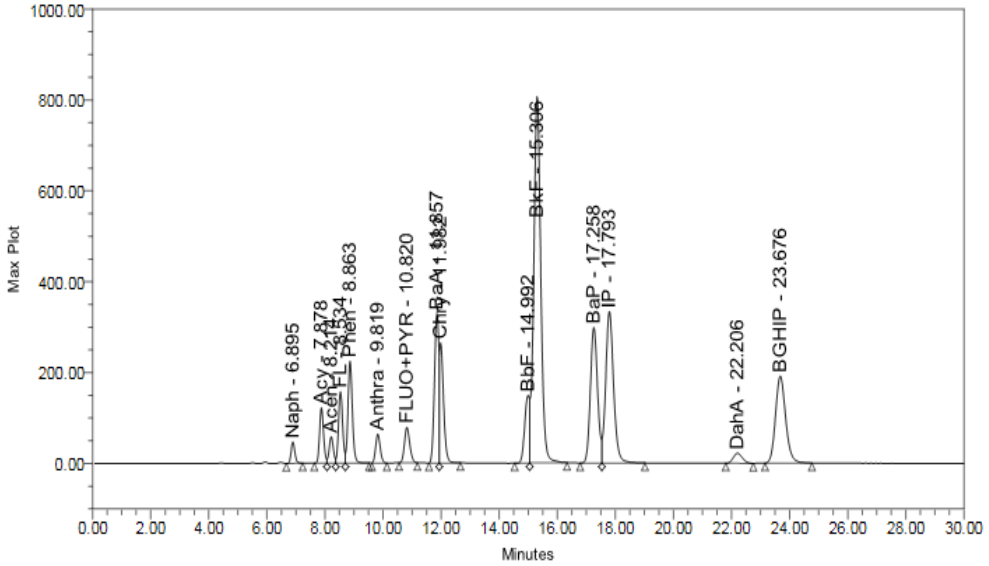

Figure 1: HPLC elution profile of the PAH compounds analyzed in standards.

III. RESULT AND DISCUSSION

The PAHs concentrations were determined in the samples of chicken, kitchen exhaust and coffee samples. The results of PAHs extraction are summarized in the Table 1. Among the three samples, higher threat of PAHs exposure to humans comes from kitchen exhaust. The PAHs released in kitchen atmosphere during cooking process, accumulates on particulate matter and dust before being sucked out by exhaust fan and consequent deposition. The highest total concentration of 16PAHs (36.30mg/kg) was observed in kitchen exhaust sample prepared in cyclohexane followed by hexane (17.15mg/kg) and toluene (16.24mg/kg). The highest concentration of B(a)P (299.30µg/kg) and lowest concentration (120.20µg/kg) was found for the sample extracted in cyclohexane and hexane, respectively. It was also observed that almost all PAHs (light and heavy) were extracted in higher amounts when cyclohexane was used as extraction solvent for kitchen exhaust. These levels are at par with those reported by Iwegbue et al. [17] where the total PAHs in kitchen dusts ranged from 29-479µg/kg. The prominent PAHs reported were phenanthrene, acenaphthylene and flourene. The dust samples did not exhibited presence of 5-6 ring PAHs exhibiting role of fossil fuels in PAHs release. PAHs in another study focusing on household cooking areas were found to be 1067-3624µg/kg. In our study, occurrence of all PAHs took place demonstrating role of both fossil fuel combustion and heat temperature combustion processes i.e. cooking. It may be noted that we have not considered the time taken for these depositions and type of cooking performed in this study which will commensurately contribute towards the observed high PAHs levels.

The concentration of 16PAHs in chicken sample also varied with the solvent used. The highest concentration of 16PAHs and BaP in chicken was found to be 19.39mg/kg and 705.0µg/kg for the extraction in toluene while the lowest concentration of PAHs16 (12.49mg/kg) was found to be for the sample extracted in cyclohexane. Highest naphthalene concentration was found in samples extracted with hexane, but PAHs namely acenaphthylene, fluorene, phenanthrene, anthracene, fluoranthene and pyrene were prominent in extracts made in cyclohexane. On other hand, samples prepared from toluene showed dominancy of heavy PAHs. Jahurul et al. [20] extracted PAHs from chicken through alkaline hydrolysis followed by DCM and hexane solvent treatments. The PAHs in different chicken food products ranged between 17.86-42.31µg/kg along with the prominence of

fluoranthene, B(b)F and B(a)P compounds. Wongmaneepratip et al. [7] also performed the extraction in hexane in grilled chicken and reported 190.1 -457.6 µg/kg PAHs concentrations. The higher levels of PAHs in chicken compared to other studies may be due to the type of cooking practice followed in local dhaba (Traditional Tandoor made of earthen oven) and the chicken breed (variable fat content).

In present study, the coffee sample extract was prepared in acetonitrile and DCM: hexane. The higher value (28.96mg/kg) of 16PAHs was found in the sample extracted using acetonitrile whereas lower amount (21.17mg/kg) was observed for the sample extracted in DCM: hexane. Opposite trend was observed for BaP where lower concentration occurred for sample extracted in acetonitrile (9.4µg/kg) and higher (39.40µg/kg) for DCM: hexane. Naph, Acy and D(ah)A were prominent on acetonitrile based extraction while extracts prepared in DCM:Hexane had higher amounts of Acen, FL, Phen, Anthra, Fluo, Pyr, BaA, Chry, BkF, BbF, BaP and IP. Jimenez, et al. [21] also used acetonitrile for the determination of polycyclic aromatic hydrocarbons in roasted coffee and observed the concentrations ranged from 0 to 561µg/kg for naphthalene, 0 to 512µg/kg for acenaphthylene, 60 to 459µg/kg for pyrene and 56 to 371µg/kg for chrysene. In similar study, Pissinatti et al., [22] used DCM:Hexane for the determination of PAHs in roasted coffee and obtain the contamination in the samples ranged from 1.00 ± 0.35 to 11.29 ± 2.33 mg/kg.

The results of present study are in accordance with other studies and it may thus be concluded that the type of solvent used for PAHs extraction from different food matrix has a pronounce effect on PAHs profile and concentrations. It may also be noted that the high levels of PAHs determined in tandoori chicken, coffee and kitchen depositions will pose threat to human health especially to people of Haryana region of India. It is thus recommended to regulate the consumption dose for these food products and to properly ventilate the cooking area to reduce PAHs exposure and associated cancer risk.

Table 1: Profile of PAHs extracted with different solvents from different matrices.

PAHs Concentration (µg/kg)																
Solvent	Naph	Acy	Acen	FL	Phen	Anthra	Fluo +Pyr	BaA	Chry	BbF	BkF	BaP	IP	DahA	BghiP	Total PAHs (mg/kg)
	Light PAHs						Heavy PAHs									
Chicken																
Cyclohexane	3806.6	4495.3	332.6	676.2	409.2	585.4	1150.1	154.1	605.2	103.5	55.5	61.6	10.1	37.3	10.7	12.49
Hexane	12036.1	450.2	911.02	105.01	101.0	392.3	170.8	5.2	86.6	1.4	3.1	-0.12	1.57	24.5	1	14.29
Toluene	10576.2	2118.0	1719.7	413.3	104.2	393.9	726.2	377.4	730.1	418.4	311.2	705.1	209.9	209.6	379.2	19.39
Kitchen exhaust (diluted ten times)																
Cyclohexane	6472.9	8569.5	1196.1	3471.4	1545.5	2997.0	6533.1	985.8	2908.9	711.7	253.0	299.3	72.8	87.5	193.7	36.30
Hexane	4074.1	2539.9	540.9	1809.6	862.1	1509.2	3290.9	495.7	1537.7	230.4	115.0	120.2	17.6	1.5	8.2	17.15
Toluene	6356.4	2808.9	574.8	1043.4	123.1	1249.6	1002.4	514.9	1137.5	466.4	168.7	276.4	38.8	287.2	189.7	16.24
Coffee																
Acetonitrile	14991.7	12197.6	0.9	409.5	142.8	450.6	333.1	36.9	203.1	101.8	3.9	9.4	7.4	49.9	19.8	28.96
DCM:hexane	6896.1	6453.6	494.2	1340.8	813.2	1219.6	1930.6	201.3	928.9	733.9	56.9	39.4	17.1	1.5	41.3	21.17

IV. CONCLUSION

PAHs concentration and profile is significantly affected with the type of solvent used for extraction. People of Haryana region (India) are exposed to higher PAHs concentrations on consumption of tandoori chicken and dark roasted coffee. On the other hand, the cooks at local dhabas are also highly susceptible to PAHs present in cooking environment as exhibited by high PAHs accumulation in kitchen exhaust depositions. It is thus advisable to use better cooking techniques and ventilation conditions while cooking food in a kitchen.

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