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Hong Pan [a] & Yuhua Cao [a]
[a] School of Chemical and Material Engineering, Jiangnan University , Wuxi , China
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Extraction

OPTIMIZATION OF PRETREATMENT PROCEDURES FOR ANALYSIS OF POLYCYCLIC AROMATIC HYDROCARBONS IN CHARCOAL-GRILLED PORK

Hong Pan and Yuhua Cao
School of Chemical and Material Engineering, Jiangnan University, Wuxi, China

A method for analysis of 13 polycyclic aromatic hydrocarbons in charcoal-grilled pork was established by high performance liquid chromatography with ultraviolet detection. The cleanup and preconcentration steps include ultrasonic extraction, saponification, liquid-liquid extraction, and solid-phase extraction. Under the optimization experimental conditions, polycyclic aromatic hydrocarbons would be determined at trace level with recoveries between 68.5% and 102.8%. Additionally, the influence of grilling time on polycyclic aromatic hydrocarbons content was investigated. It was testified that when the roasting time reached 4 minutes under the experimental conditions, the content of carcinogen benzo(a)pyrene had exceeded the limit of the European Union.

Keywords: Charcoal-grilled pork; HPLC-UV; Polycyclic aromatic hydrocarbons; Solid phase extraction

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) represent a group of organic compounds consisting of two or more condensed aromatic rings that are widely geographically distributed in the environment (Djinovic, Popovic and Jira 2008). They have been included in the American Environmental Protection Agency (EPA) priority pollutant list, due to their mutagenic and carcinogenic properties (U.S. EPA, Vol. 49). Also, benzo(a)pyrene (BaP) can be used as a marker for the occurrence and the effect of PAHs (Opinion of the Scientific Committee 2002). Traces of PAHs can be found throughout the environment in water, atmosphere, and sediment (Anyakora et al. 2005; Lizhong and Jing 2003; Chung-Yih et al. 2008; Ray et al. 2008; Manoli et al. 2000; Navasumrit et al. 2008; Williamson et al. 2002). Foods may be contaminated through deferent routes, which include direct deposition of PAHs from the atmosphere, contamination from packaging materials, and productions

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Address correspondence to Yuhua Cao, School of Chemical and Material Engineering, Jiangnan University, 1800 Lihu Road, Wuxi 214122, PR China. E-mail: yuhuacao@yahoo.com.cn

of PAHs during the thermal processing of foods from animals. Charcoal grilling is one of the oldest methods of food preservation and is still widely used in fish and meat processing. Hundreds of individual PAHs may be formed and released during the incomplete combustion or thermal decomposition of the organic materials (Lage Yusty and Cortizo Daviña 2005). When meat contacts the flame directly, pyrolysis of the fats in the meat generates PAHs that become deposited on the meat. On the other hand, the wood smoke resulting from grilling meat also contains a large number of PAHs (Stumpe-Vīksna et al. 2008). As early as 1964, PAHs, such as BaP, were detected in broiled meat, and PAHs in smoked meat, due to a high level through intake, draw special attention (Gomaa et al. 1993; Karl and Leinemann 1996; Larsson, Pyysal, and Sauri 1988). Recently, different food categories (Commission Regulation (EC) 208/2005) have introduced a maximum BaP level of 5 μg/kg wet weight for smoked meat and smoked meat products, although different European countries (Germany, Austria, Czech Republic, and the Slovak Republic) had previously adopted a legal limit of 1 μg/kg.

Analysis of PAHs in charcoal-grilled pork is a challenging task owing to the extremely low levels (ng/g) and diversity of potential interferents, mainly fat and protein. It is crucial to isolate PAHs from the complex matrix, which could obtain an even and stable baseline in chromatogram and low detection limits as a consequence. So, an optimization cleanup procedure is more potent for higher sensitivity. The most widely used method for extracting PAHs from smoked meat consisted of soxhlet, saponification, liquid-liquid partition, followed by clean-up steps, using solid phase column packed silica or alumina. However, these methods were unsatisfactory owing to the irregular recoveries and many interfering peaks in the chromatograms. In order to improve purification and overcome the shortcomings of time consuming and tedious labor, many methods have been developed. A novel diethoxydiphenylsilane solid-phase microextraction fiber was used that detected PHAs in milk (Bianchi et al. 2008); the limits of detection (LOD) and limit of quantification (LOQ) values in the low μg/kg magnitude proved the suitability of the innovative coating for selective detection and quantification of PAHs in milk. A hollow-fiber liquid-phase microextraction was applied to determine PAHs in pine needles, resulting in very cleanly separated and readily evaluable PAH peaks in chromatograms (Ratola et al. 2008). An extraction cleanup procedure by supercritical fluid extraction (SFE) was developed in order to isolate PHAs from oil vegetable samples (Lage Yusty and Cortizo Daviña 2005). The SFE could drastically reduce analysis time, volume of solvents consumed, and sample manipulation. Although these methods were novel and of selectivity, but it was not suitable for determination of PAHs in extremely complex matrix, such as meat or fish. Purcaro, Moret, and Conte optimized microwave assisted extraction (MAE) for PAHs determination in smoked meat to decrease the time of pretreatment (2009). However, choose 115°C as the extraction temperature for sample extraction was not suitable for volatile components such as naphthalene, acenaphthene, and fluorene. The determination of PAHs in foodstuffs has been carried out by different chromatographic techniques, including HPLC with fluorescence and/or UV detection, and GC with flame ionization detection or mass spectrometry (Lage Yusty and Cortizo Daviña 2005; Yiwen et al. 2008; Martin and Ruiz 2007; Pena et al. 2006; Veyrand et al. 2007; Dobrinas, Birghila, and Coatu 2008; Chinnici et al. 2007; Ruchirawat et al. 2007; Yurchenko

and Molder 2005; Pyle et al. 1997). Recommended analytical procedures for the determination of PAHs are documented or proposed in several European and US guidelines including the EPA, ISO, and German Standard (DIN) method. All of these methods specify reversed-phase HPLC using octadecyl (C-18) bonded phases in combination with either fixed or wavelength-programmed UV and fluorescent detection techniques (Rivera et al. 1996).

The aim of the present work was to develop a reliable method to analyze PAHs in charcoal-grilled pork. Pretreatment optimization was emphasized on cleanup and preconcentration procedures. The effect of grilling time on content of PAHs in the charcoal-grilled pork was also investigated.

EXPERIMENTAL

Reagents and Materials

The PAHs standard of acenaphthylene, anthracene, benz[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[g,h,i]perylene, benzo[a]pyrene, dibenz[a,h]anthracene, fluoranthene, fluorene, naphthalene, phenanthrene, and pyrene were obtained from DR (Germany). Chromatographic grade methanol was obtained from Hanbang (Jiangsu, China). Acetone, n-hexane, cyclohexane, dichloromethane, potassium hydroxide, sodium chloride, sulphuric acid, and neutral alumina (200–300 mesh) were purchased from Chemical Reagents (Shanghai, China), and all were of analytical grade. Reagent grade silica gel (100–200 mesh) was provided by Marine Chemicals (Qingdao, China). The C-18 solid phase extraction cartridges (500 mg, 6 ml) were purchased from Jin Yang (Hebei, China). Samples of the charcoal-grilled pork were collected from local street vendors (Wuxi, China) and stored at -18°C.

Instruments

The Waters 515 series HPLC system was equipped with a Waters 2487 ultraviolet detector. The HPLC system was controlled and the data were processed by Waters Empower software. A C-18 column (250 mm $\times$ 4.6 mm I.D., 5 μm, nucleodur, USA) was used. A food processor was obtained from Shuaijia Electronic Technology Company (Shanghai, China).

Preparation of PAHs Multicomponent Standard Solution

A 50 mg of acenaphthylene, naphthalene; 10 mg of benzo[b]fluoranthene, benzo[k]fluoranthene, fluoranthene, fluorene and 5 mg of anthracene, benz[a]anthracene, pyrene, benzo[g,h,i]perylene, benzo[a]pyrene, dibenz[a,h]anthracene, phenanthrene were weighed and dissolved together with dichloromethane, diluted to 50 ml in volumetric flask, and stored at 4°C.

Sample Pretreatment

Ultrasonic Extraction. Samples of 60.0 grams grilled for 4 min were minced with a food processor, proceeded by an ultrasonically assisted extraction of 100 ml

hexane-acetone mixed solvent (1:1, V/V) for 30 minutes. The extract solution was transferred to a 500 ml round bottomed flask, and the remaining residues were ultrasonically extracted again at the same condition. The extract solution was then combined.

Saponification. A 150 ml sample mixture of methanol and water (9:1, V/V) containing 2 mol/l potassium hydroxide was added to the 500 ml flask loaded extract solution, then saponification was performed with reflux in a water bath at 80°C for 6 h.

Liquid-Liquid Extraction. After cooling, the saponification solution was transferred from the flask to a separatory funnel, subsequent washing the flask twice with 200 ml cyclohexane. Then, all washing solvents were added to the separatory funnel to extract PAHs from the saponification solution. After shaking sufficiently, a saturated solution of sodium chloride to help break up emulsification between two phases was added and let stand for a while; the two phases were separated clearly. The upper layer of cyclohexane was removed and the remaining solution was extracted again with another 100 ml cyclohexane. Then, the lower layer of methanol and water was discarded and merged with the upper phases of cyclohexane in the separation funnel. The organic phase was washed with 100 ml of a 1:1 mixed solvent of methanol and water twice, and 200 ml of water twice, and then concentrated to 40 ml by rotary vaporization. The concentrated solution was washed again with 50 ml of 60% sulfuric acid twice and, subsequently, washed with deionized water up to neutral. Concentration was carried on further by rotation evaporation to 2 ml, following evaporation under a gentle flow of nitrogen to dryness. Finally, the residues were redissolved in 2 ml methanol as the test solution for further treatment.

Solid Phase Extraction. A cartridge packed C-18 bonded phase was pretreated by rinsing with 5 ml of dichloromethane, 5 ml of methanol, and 5 ml of water, successively. A sample of 2 ml test solution was loaded and flowed through the C-18 cartridge at a flow rate of 1 ml/min. Next, the cartridge was washed with 5 ml of water at a rate of 5 ml/min and then vaporized to dryness. Finally, the PAHs retained in the C-18 cartridge were eluted using 6 ml of dichloromethane at a flow rate of 1 ml/min. The dichloromethane fraction eluted, which contained PAHs, was evaporated to 0.5 ml under a gentle flow of nitrogen for further HPLC analysis.

HPLC-UV Determination

An aliquot (20 μl) of the testing solution was injected into HPLC system and eluted with the mobile phase consisting of methanol-water (93.2:6.8, V/V) at the flow rate of 0.5 ml/min in ambient temperature. Detection wavelength was set at 254 nm.

RESULTS AND DISCUSSION

Optimization of Pretreatment Procedure

Parameters affecting the extraction efficiency and purification performance were investigated to optimize the experimental procedure.

Saponification. Meat contains plenty of lipids, which has similar polarity to PAHs. So, PAHs and lipids separate poorly by conventional separation procedures such as liquid-liquid extraction, solid phase extraction, or liquid column chromatography; whereas, it is easy to remove these impurities by saponification, in which triglycerides react with potassium hydroxide to produce water soluble glycerol and a fatty acid salt. As a result, saponification is the simple, but essential, step for pretreatment of samples rich in fats for the determination of PAHs. Though some works (Welbhaar 2002; Purcaro, Moret, and Conte 2009) have reported rapid determination of PAHs without saponification, it was found that the step of saponification in pretreatment of meat sample was indispensable in the present work. In order to verify this viewpoint, the samples, whether pretreated by saponification or not, were determined with HPLC. As shown in Fig. 1b, there were some large and broad peaks in the chromatogram that covered up the small peaks of analytes, which was obtained by injection of the extract solution into the HPLC system and that were produced by only two steps, ultrasonic extraction and concentration. Once the sample solution was treated by saponification, the peaks of impurities were reduced and the peaks of target analytes emerged as shown in Fig. 1a. The experimental results showed that the pretreatment by saponification could remove a majority of matrices and greatly contribute to cleanup of the complex matrix sample.

During liquid-liquid extraction of saponification solution, emulsification occurred extremely easily. This phenomenon resulted in disparting two phases with difficulty with even more of a loss of PAHs. As a result, demulsification was very important to improve the recoveries of PAHs and save time. Although there are many ways to demulsify, adding a saturated solution of sodium chloride was the most convenient. In this experiment, when the saponification solution formed emulsion between methanol-water and cyclohexane phases, 20 ml of saturated solution of sodium chloride was added to the separatory funnel to demulsify.

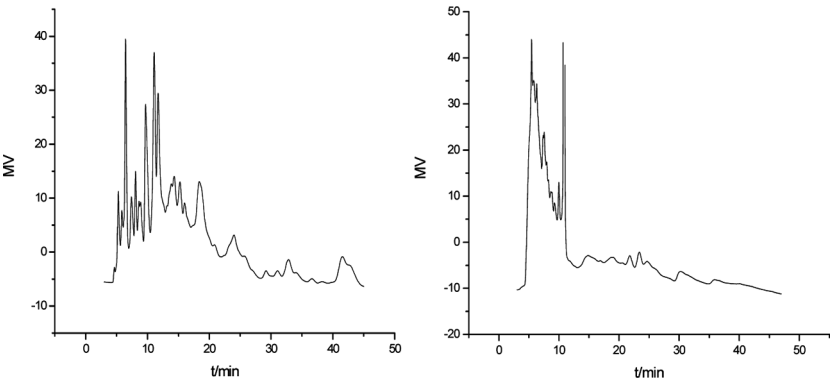

Figure 1. HPLC chromatogram of samples grilled for 4 minutes pretreated using the optimal conditions achieved in this work with saponification procedure (a) and without (b). Reversed-phase C-18 column (250 mm × 4.6 mm I.D., 5 μm, nucleodur, USA); mobile phase: methanol-water (93.2:6.8, V/V); flow-rate: 0.5 ml/min; detection wavelength was set at 254 nm.

Solid Phase Extraction. Throughout the saponification and liquid-liquid extraction, the test solution still contained some impurities that would interfere with the analysis of PAHs. Isolatation and quantification of PAHs at the ng level required further enrichment and purification. Solid-phase extraction (SPE) is the most commonly used in the pretreatment of PAHs in food samples. In this paper, three kinds of column packing (silica gel, neutral alumina, and C-18) were investigated.

First, A column (300 mm × 10 mm) was packed with 10 g of activated silica gel (activated at 250°C for 6 h before use), topped with 2 g of anhydrous sodium sulfate (activated at 120°C for 4 h before use), and washed with cyclohexane in advance. An aliquot of the test solution was loaded onto the column. They were eluted with 50 ml of cyclohexane. The eluent was concentrated to 2 ml using a rotary evaporator, and then further concentrated to 0.5 ml with a gentle flow of nitrogen for HPLC analysis. As shown in Fig. 2a, both the number and height of peaks in the chromatogram were greatly reduced, even more, almost three-quarters of PAHs peaks disappeared. Though the impurities were retained in the column, the target analytes were also bound tightly with the absorbents. In order to improve the recovery of PAHs, many modifications, including increasing the volume of eluent, decreasing the dosage of silica gel and utilizing moderate polarity solvent such as benzene to replace cyclohexane had been tried. However, nothing can be done. It was testified that adsorbability of silica gel was too strong; it was difficult to elute PAHs completely.

Second, alumina was also used as a packing material to cleanup the sample for analysis of PAHs. An aliquot of the test solution was loaded onto the column packed with 1 g of activated neutral alumina (activated at 600°C for 6 h before use), then topped with 0.5 g of anhydrous sodium sulfate; all were washed with cyclohexane in advance. An amount of 10 ml of cyclohexane was used in an attempt to elute PAHs from the column. As shown in Fig. 2b, although the number of peaks was more than those shown in Fig. 2a, target compounds peaks were even fewer. In order to improve the recovery of PAHs, many modifications, including increasing the volume of eluent, decreasing the dosage of silica gel and utilizing moderate polarity

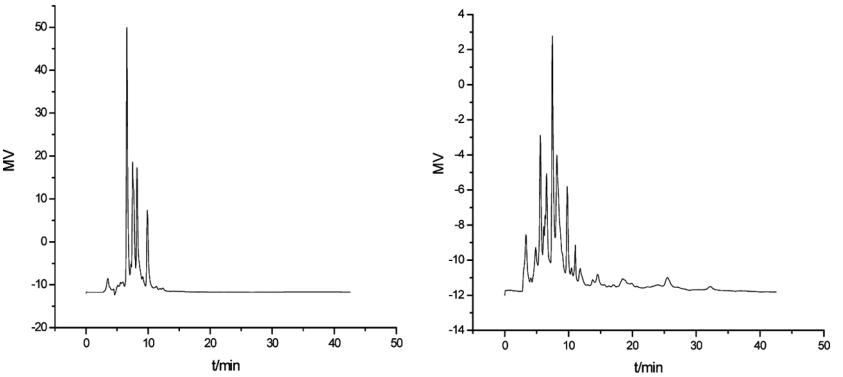

Figure 2. HPLC chromatogram of samples grilled for 4 minutes pretreated using the optimal conditions achieved in this work with silica gel as the column packing (a) and neutral alumina as the column packing (b). Chromatographic experimental conditions were the same as Fig. 1.

solvent such as benzene to replace cyclohexane had been tried. However, nothing can be done. As a result, neutral alumina was also deemed unsuitable for cleanup of the sample in this present work.

Some works have reported to utilize silica gel column following the neutral alumina column to pretreat samples of meat or fish. Obviously, it was unable to enhance the recoveries of PAHs since one column had too strong an adsorption to PAHs, let alone two columns in tandem.

Finally, a commercial available C-18 cartridge was investigated for the purpose. A C-18 bonded phase stationary was nonpolar, for which PAHs in the sample had an affinity. The main impurities, such as proteins and tallates, were polar, of which little were retained in the C-18 cartridge. As a result, once the sample was loaded, the C-18 cartridge was rinsed with water to get rid of the polar impurities, and then eluted with a weak polarity solvent to collect the target PAHs. Thus, nonpolar PAHs could be isolated from polar impurities in the sample rich with proteins and fats. According to the procedure described in the Solid Phase Extraction, the typical chromatograms were obtained as shown in Fig. 3, which revealed that the cleanup step had greatly reduced interferents, and a relatively smooth baseline and cleanly separated PAH peaks were obtained. It was testified that C-18 as the packing material was feasible to depurate samples of meat. Furthermore, a C-18 solid phase extraction cartridge used in the experiment could be purchased in the market and regenerated easily. To some extent, it was simple, convenient, rapid, and reusable.

In order to obtain high recovery, the influence of the volume of elution solvent on the recoveries of the target analytes was investigated. Pretreated by rinsing with

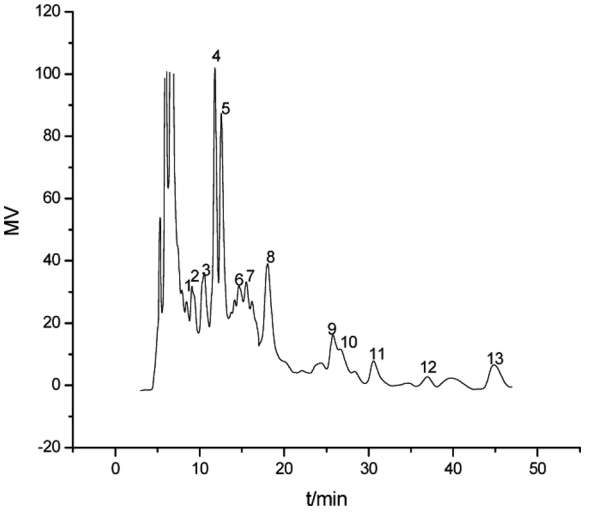

Figure 3. HPLC chromatogram of samples grilled for 4 minutes pretreated using the optimal conditions achieved in this work with C-18 as the column packing. Chromatographic experimental conditions were the same as Fig. 1. Peaks identification: 1, naphthalene; 2, acenaphthylene; 3, fluorene; 4, phenanthrene; 5, anthracene; 6, fluoranthene; 7, pyrene; 8, benz[a]anthracene; 9, benzo[b]fluoranthene; 10, benzo[k]fluoranthene; 11, benzo[a]pyrene; 12, dibenz[a,h]anthracene; 13, benzo[g,h,i]perylene.

Table 1. The effect of eluent volume on the recoveries of solid phase extraction

	The recovery with different volume of dichloromethane (%)		
Compound	2 ml	4 ml	6 ml
Naphthalene	18.5	38.9	89.3
Acenaphthylene	17.7	41.5	101.6
Fluorene	14.1	34.3	102.3
Phenanthrene	16.9	39.0	100.7
Anthracene	19.1	45.9	98.9
Fluoranthene	7.3	27.6	100.8
Pyrene	ND	17.4	98.3
Benz[a]anthracene	10.7	30.6	85.6
Benzo[b]fluoranthene	11.1	29.0	85.0
Benzo[k]fluoranthene	22.3	49.2	89.3
Benzo[a]pyrene	9.7	31.1	92.1
Dibenz[a,h]anthracene	11.3	38.2	99.2
Benzo[ghi]perylene	6.0	21.0	91.0

5 ml of dichloromethane, 5 ml of methanol, and 5 ml of water, a 1 ml of hundredfold dilution solution of PAHs multicomponent standard solution was loaded and flowed through the C-18 cartridge at a flow rate of 1 ml/min. Then, the cartridge was washed with 5 ml of water at a flow rate of 5 ml/min and then vaporized to dryness according to the procedure described in the Solid Phase Extraction section. Finally, PAHs retained in the C-18 cartridge were eluted using different volumes of dichloromethane at a flow rate of 1 ml/min. As shown in Table 1, the recoveries of PAHs were increased, correspondingly, with the increase of elution solvent. When the solvent volume was up to 6 ml, almost all of the targets were eluted completely. The recoveries had no obvious improvement by further increasing the eluent volume.

Analytical Performance

Under the optimum experimental conditions, the mixed standard solutions of multicomponents were determined with HPLC-UV, and a typical chromatogram was shown in Fig. 4. Thirteen kinds of PAHs could be separated within 45 min.

The method was validated based on the criteria of repeatability, linearity, and LOD. The results obtained were shown in Table 2. The linear ranges of PAHs had two magnitudes, and detection limits, based on a ratio of signal to noise of 3, were between 0.1 and 8.9 μg/l. Especially heavy PAHs and mutagenic and carcinogenic compounds had detection limits at a level of 0.1 μg/l. When the samples were preconcentrated by 120-fold, the detection limits in this scheme could meet the requirement of the European Union (EU) of 5 μg/kg PAHs. The precision of the method, expressed as relative standard deviation (RSD), was evaluated by consecutive injection of the PAHs multicomponent standard solution in replicates of three. The RSDs of peak areas were ranged between 1.2% and 4.6%, respectively.

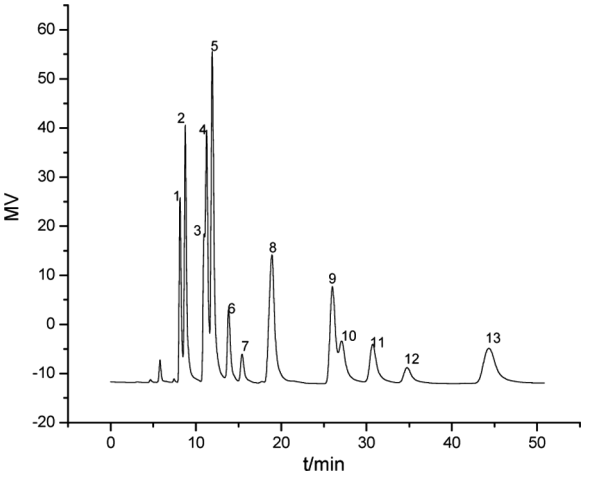

Figure 4. Chromatogram of standard mixed solution. Standard mixed solution: 1 ml of PAHs mixture standards solution described in Preparation of PAHs Multicomponent Standard Solution was taken and diluted with dichloromethane to 100 mL in volumetric flask, an aliquot (20 μl) of it was injected into HPLC system. Chromatographic experimental conditions were the same as Fig. 1.

Analysis of Charcoal-Grilled Pork Sample

The samples were pretreated according to the procedure described in Sample Pretreatment and determined with HPLC-UV according to the procedure described in HPLC-UV Determination. The determination results were listed in Table 3. When the pork was not processed by grilling, the content of heavy PAHs in pork, which have mutagenic and carcinogenic properties, was low, mostly undetectable. But, with the extension of roasting time, the content of all PAHs in the samples increased.

Table 2. Regression equations of PAHs and detection limits

Compound	Regression equations*	Correlation coefficient	Detection limit (μg/l)	Linear range (μg/l)
Naphthalene	$y = 5.4 \times 10^4 x - 3.8 \times 10^4$	0.999	6.8	20–1000
Acenaphthylene	$y = 4.3 \times 10^4 x - 2.6 \times 10^3$	0.998	8.9	20–1000
Fluorene	$y = 2.1 \times 10^5 x + 3.2 \times 10^3$	0.997	0.2	4–200
Phenanthrene	$y = 1.0 \times 10^6 x - 6 \times 10^4$	0.996	0.3	2–100
Anthracene	$y = 2.0 \times 10^6 x - 1.3 \times 10^4$	0.998	0.6	2–100
Fluoranthene	$y = 1.8 \times 10^5 x - 9.6 \times 10^3$	0.998	0.8	4–200
Pyrene	$y = 1.4 \times 10^5 x - 1.0 \times 10^4$	0.998	1.4	2–100
Benz[a]anthracene	$y = 1.0 \times 10^6 x - 1.6 \times 10^4$	0.997	0.2	2–100
Benzo[b]fluoranthene	$y = 3.2 \times 10^5 x - 2.1 \times 10^4$	0.997	1.3	4–200
Benzo[k]fluoranthene	$y = 5.1 \times 10^5 x - 9.1 \times 10^4$	0.998	0.7	2–100
Benzo[a]pyrene	$y = 3.6 \times 10^5 x - 6.3 \times 10^3$	0.998	0.1	2–100
Dibenz[a,h]anthracene	$y = 7.0 \times 10^4 x - 9.0 \times 10^3$	0.998	0.6	4–200
Benzo[ghi]perylene	$y = 2.7 \times 10^5 x - 2.0 \times 10^4$	0.997	0.3	4–200

*The unit of lateral axis X was μg/l.

Table 3. The determination results of charcoal-grilled pork sample

Compound	Toxicity	The content of PAHs with the grilling time (μg/kg)			
		0 min	2 min	3 min	4 min
Naphthalene	−	55.1	71.2	84.2	106.7
Acenaphthylene	−	35.9	51.1	67.4	241.3
Fluorene	−	8.5	50.3	60.4	67.6
Phenanthrene	−	ND	ND	16.6	26.5
Anthracene	−	1.9	4.8	ND	13.0
Fluoranthene	+	1.6	26.	37.9	55.8
Pyrene	−	2.4	50.3	55.2	71.3
Benz[a]anthracene	+	1.4	5.7	23.1	26.9
Benzo[b]fluoranthene	++	ND	ND	15.6	23.0
Benzo[k]fluoranthene	++	ND	ND	ND	11.1
Benzo[a]pyrene	++++	ND	2.4	4.5	10.2
Dibenz[a,h]anthracene	++	ND	19.9	23.6	25.3
Benzo[ghi]perylene	++	ND	13.0	21.5	29.1

−: Not carcinogenic.
+: weak carcinogen.
++: carcinogenic.
++++: highly carcinogenic carcinogenic.
ND = not found.

When the roasting time reached 4 minutes under these experimental conditions, the content of carcinogen BaP had exceeded the limitation recommended by the EU.

In order to evaluate the accuracy of the method, the recoveries were determined by the standard addition approach. Amounts of 10 μl of PAHs standard mixture solution were added into 60 g samples for analysis, other procedures were as the same as the procedures described in Sample Pretreatment. As shown in Table 4, the recoveries from 68.5% to 102.8% were acceptable for analysis of actual samples.

Table 4. The results of recoveries

Compound	Original (μg/kg)	Added (μg/kg)	Found (μg/kg)	Recovery (%)
Naphthalene	55.1	166.7	227.1	102.8
Acenaphthylene	35.9	166.7	159.5	74.1
Fluorene	8.5	33.3	35.2	79.2
Phenanthrene	ND	16.7	11.7	68.5
Anthracene	1.9	16.7	15.3	78.8
Fluoranthene	1.6	33.3	25.0	69.4
Pyrene	2.4	16.7	15.8	84.2
Benz[a]anthracene	1.4	16.7	13.1	74.9
Benzo[b]fluoranthene	ND	33.3	25.1	72.8
Benzo[k]fluoranthene	ND	16.7	11.7	72.4
Benzo[a]pyrene	ND	16.7	11.7	72.2
Dibenz[a,h]anthracene	ND	33.3	25.1	75.8
Benzo[ghi]perylene	ND	33.3	26.7	81.4

ND = not found.

CONCLUSIONS

In this paper, 13 PAHs in charcoal-grilled pork were analyzed by HPLC-UV. With cleanup and preconcentration procedures including soaponification, liquid-liquid extraction, and solid phase extraction using commercial available C-18 cartridge, the chromatograms revealed a relatively smooth baseline and cleanly separated PAH peaks. The PAHs could be determined at trace level with recoveries between 68.5% and 102.8%. The investigation into the influence of grilling time on PAHs content in charcoal-grilled pork testified that when the roasting time reached 4 minutes under the experimental conditions, the content of benzo(a)pyrene had exceeded the limit of the EU.

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