DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS IN BARBECUED PORK USING QUECHERS EXTRACTION AND GAS CHROMATOGRAPHY – MASS SPECTROMETRY

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Abstract

Polycyclic aromatic hydrocarbons (PAHs) are chemical compounds that contain aromatic rings and do not contain heteroaryl or substituted groups. PAHs are found in petroleum, coal, and plastics; Moreover, PAHs are also found in foods, especially in cooked or grilled meats. These compounds are pollutants and identified as carcinogenic, mutagenic and teratogenic in humans. The goal of this paper was to develop a process to analyze the content of 16 common PAHs in grilled pork by mass spectrometry (GC-MS) using standard internal substances. Some specifications such as sample preparation - analysis conditions, analysis limits (MDL, MQL) and recovery rates were discussed. Results indicated that the combination of QuECheRS (Quick, Easy, Cheap, Effective, Rugged and Safe) extraction and GC-MS in SIM mode analysis allowed for quantification of 16 PAHs in very small concentrations (10 - 100ppb) with recoveries of 83.8 to 113.4% on spiked sample and the limit of detection ranged from 0.3 to 0.8µg/kg.

***Keywords**: grilled pork, GC-MS, PAHs, QuECheRS*

INTRODUCTION

PAHs are found in our living environment and the major route of exposure to humans is through food. PAHs are found in food chains from production environment (air, soil and water) or from food processing (Zelinkova & Wenzl, 2015; Ledesma et al., 2014; Orecchio et al., 2009; Luzardo et al., 2013). Food processing techniques contribute to the increase in PAH levels including drying, roasting and baking ...(Ledesma et al., 2014; Martin Rose et al., 2015). Sixteen common PAH compounds are frequently found in environmental monitoring samples and can pose a risk to human health, as shown in Figure 1.

Currently, many organizations such as the United States Environmental Protection Agency (U.S.EPA), the European Food Safety Authority (EFSA) and the International Agency for Research on Cancer (IACR)... have classified PAHs on the list of priority pollutants due to their carcinogenic and mutagenic properties human (EC., 2006; EFSA., 2008; FDA., 2006). The study by Daniel et al.

(2011) showed a risk of increased renal cell carcinoma by consuming by beef, boiled meat through histologic studies. Other studies have shown that Benzo[a]pyrene compounds (BaP) have adverse and toxic effects on the cells, tissues, development and immune system of animals (Essumang et al., 2013; Manda et al., 2012).

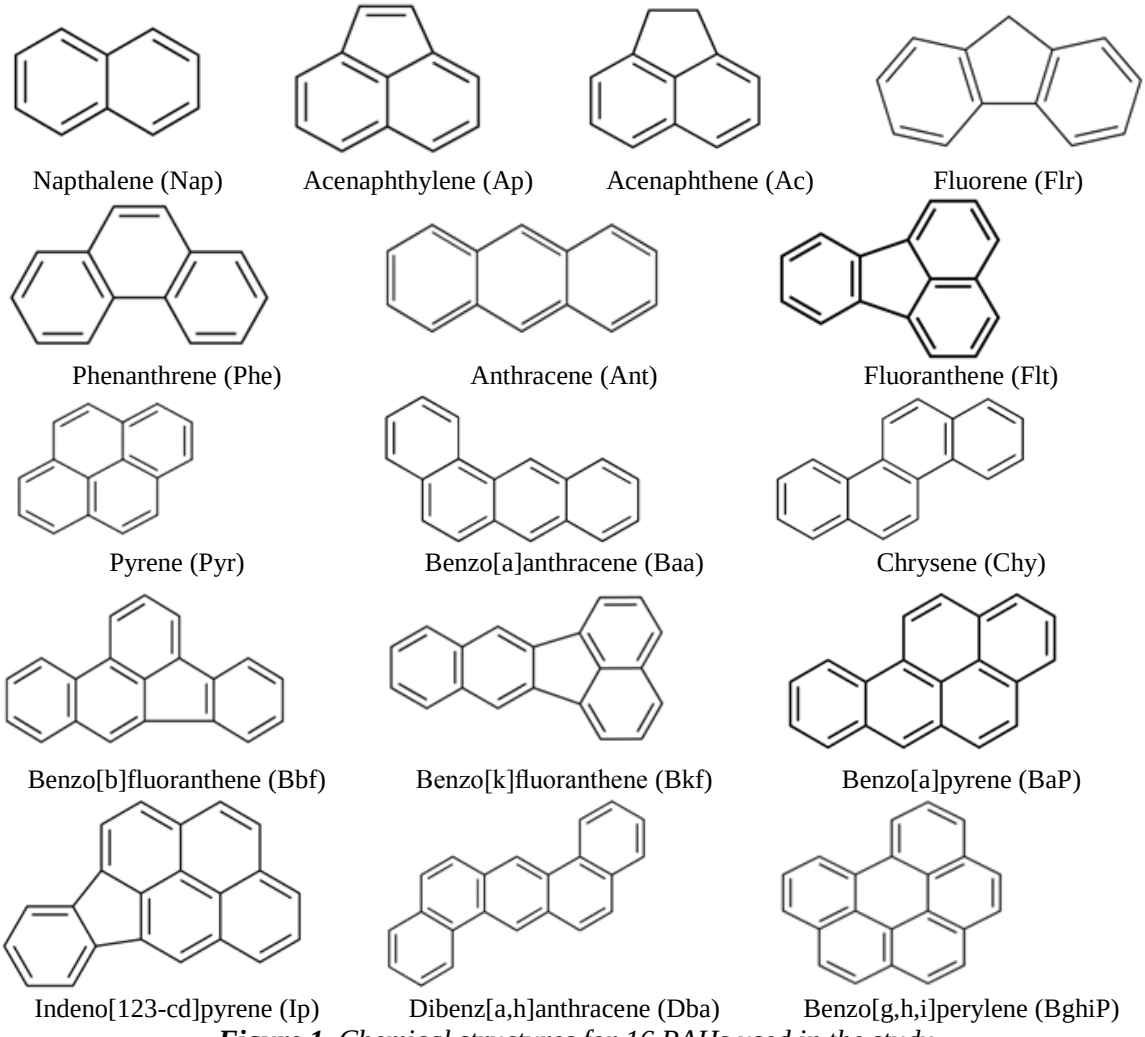


Figure 1. *Chemical structures for 16 PAHs used in the study*

Alomirah et al. (2011) and Alomirah et al. (2010) found that PAHs were highly pathogenic in roasted vegetables, chicken and smoked foods. Results showed that cancer risk related to the consumption of food for child, adolescent and adult with $2.63.10^{-7}$ and $9.3.10^{-7}$ the amount of BaP respectively. Studies have shown that levels of exposure for human to BaP are 2-500ng/day. Globally, the average consumption of PAHs ranges from 0.02 to 3.6 μg/person/day, while in countries such as India, Nigeria and China, it is 11, 6.0 and 3.56 μg/person/day due to the consumption of fried food by cooking oil (Diggs et al., 2011).

Currently, the number of studies on PAHs in food was limited in Vietnam. Le Hong Dung et al., (2012) and Tran Cao Son et al., (2016) showed that most samples of food processed by roasting, frying and baking in Vietnam were contaminated with PAHs, including BaP. Food samples were found with the level of BaP >2ng/g (European Union Regulations No 2011) including: fish fillets,

salads, fried tofu, grilled meat, fried noodles, grilled chicken and pancake rolls (Le Hong Dung et al., 2012).

The aim of this study was to understand and develop a method for the analysis of 16 PAHs on the meat samples, thereby, in the future, evaluating PAHs content on different foods in Ho Chi Minh City and giving a warning for human health.

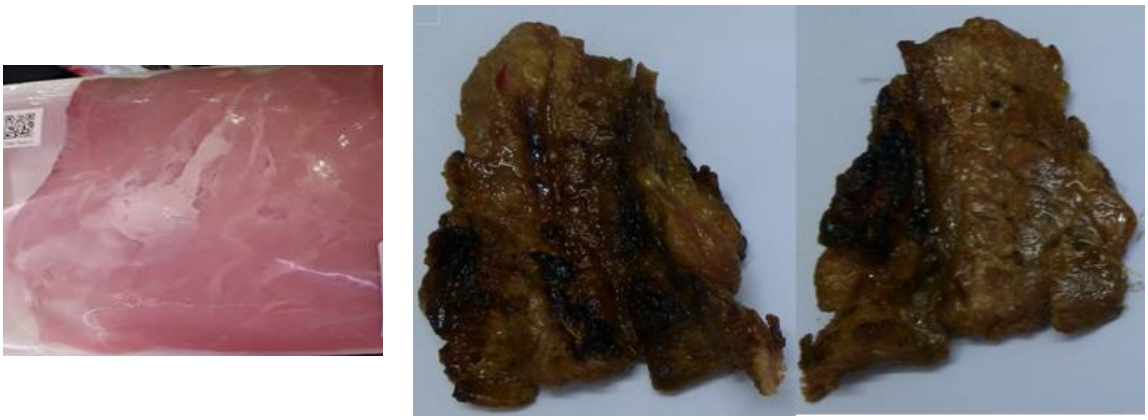
MATERIAL AND METHODS

Reagents and materials

Reagents: A mixture of 16 PAHs (2000 $mg.L^{-1}$ in hexane and a purity of> 99%) was supplied by Merck (Germany) and stored at 4^oC.. Solvents used for sample preparation and analysis process such as n-hexane, acetonitrile (ACN) were at HPLC grade (Merck). The chemicals: magnesium sulfate ($MgSO_4$) (Scharlab - USA), sodium chloride (Scharlab - USA), primary secondary amine (PSA) and octaecylsilane (C18) (Agilent Technologies – USA) were used as absorbed compounds. Phenanthrene-D10, perylene-D12, chrysene-D12 and acenaphthene-D10 were used as internal standards for instrumental quantitation.

Materials: PAH analysis was carried out using a gas chromatography equipped with MS 5980C inert XL/CI MSD (Agilent Technologies). In addition, some equipment such as: vortex machine, centrifuge Hermle Z336 (Germany) and 4-digit Ohaus balance (USA) were used for sample preparation. All sample preparation equipment (e.g. centrifuge tubes) were washed with soap and water and rinsed with acetone and n-hexane prior to use and between samples.

Samples: Blank sample was lean pork meat and grilled pork was purchased from a local grocery store in Tan Phu, Ho Chi Minh City (Figure 2). These samples were used to examine the sample processing procedures, calculate the PAHs recovery rate and test the method on the actual samples. The meat samples were cut into small pieces and roasted to a mash, packed in foil bags and stored in a freezer at -18°C to avoid the decomposition (Fig. 3).



(a) *(b)* *(c)*

***Figure 2**. Black sample (a); grilled pork sample (two sides: b and c)*

***Figure 3**. Blank meat sample (left); grilled meat sample (right) after grinded*

Analysis method

Sample preparation: Sample preparation in this study was followed a QuEChERS (QUick Easy Cheap Effective Rugged Safe) method (Rejczak & Tuzimski, 2015). Figure 4 shows sample extraction procedures: firstly, the samples are homogenized then extracted and fractionated using a mixture of water-ACN and salts ($MgSO_4$, NaCl) in centrifuge tubes. Polar compounds were soluble in water which was absorbed by the salt mixture.

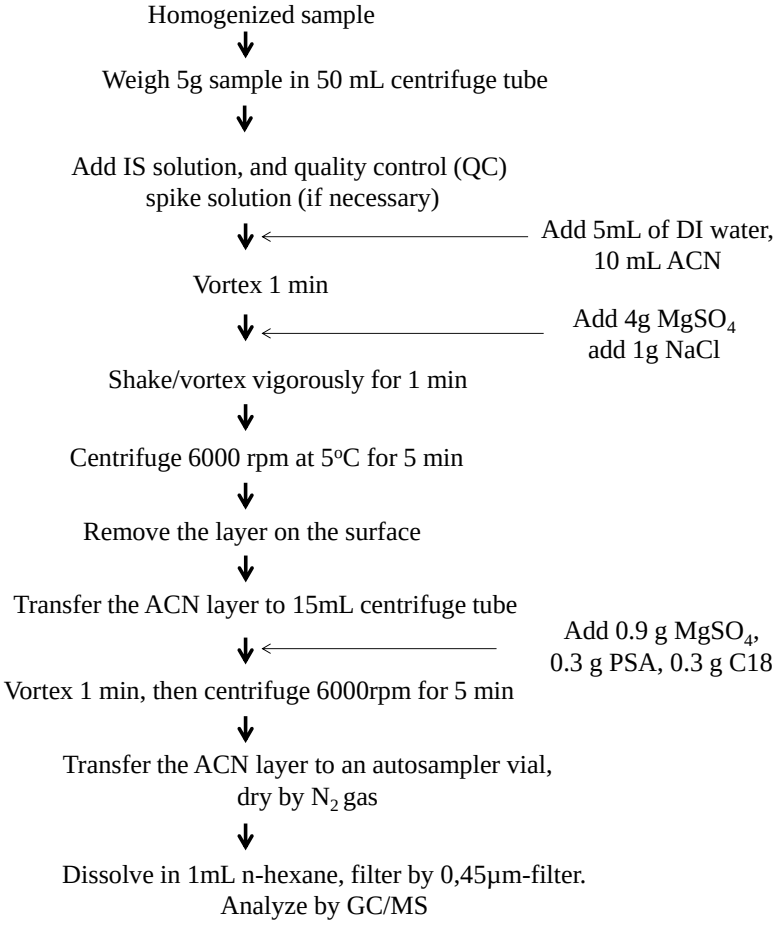


***Figure 4.** Sample preparation procedure by QuEChERS method Calibration*

PAHs – insoluble/poorly soluble in water – were extracted in ACN solvent and other compounds were removed by using absorbent (PSA, C18 and $MgSO_4$). This extraction was shaken/vortexed to increase the contact between the solvent/adsorbent and then separated by a difference in density when centrifuged. The centrifuge tubes were covered with aluminum foil to avoid contact with light, reducing the influences to the substances.

The final evaluate was purged with N_2 gas to dryness, then dissolved in 1mL n-hexane and filtered through a 0.45µm filter prior to GC/MS analysis.

Blank sample: Blank meat samples were treated by QuEChERS method (Figure 4) and no PAHs were added.

Standard samples: Blank meat samples were also treated with QuEChERS, but at the beginning, mixture of internal standards (phenanthrene-D10, perylene-D12, chrysene-D12 and acenaphthene-D10) at concentration of 1ppb and mixture of PAHs at concentrations of 10, 25, 50, 75 and 100ppb respectively, were added.

Analysis method: Separation of the compounds was achieved on an Agilent J&W DB-1MS column (30m x 0.25mm, 0.25µm) with helium (purity> 99.99%) as the carrier gas at a flow rate of 1.2µl/min, mass spectrometer transfer line, source and quadrupole temperatures were 280^{o}C, 230^{o}C and 150^{o}C respectively. The initial oven temperature was kept at 55^{o}C, 0.5 min hold, ramp to 235^{o}C at 30^{o}C/ min, then ramp to 300^{o}C at 5^{o}C/min, 0.5 min hold for a total run time of 20min. A 2µL sample was injected to an inlet by auto sampler system with a pulsed splitless mode.

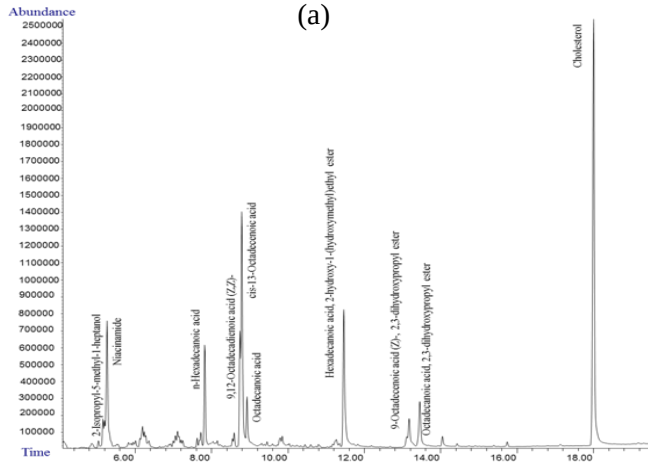
The MS spectrum analyzed the PAHs in two forms: TIC (total ion current) and SIM (selective ion monitoring) in positive ion mode (Table 1).

RESULTS AND DISCUSSION

Quality assurance/control

Blank samples: Analytical results of blank meat samples in TIC and SIM modes were presented in Figure 5. The blank chromatogram showed that there were some compounds such as: ester, cholesterol ... but no PAHs was detected. Therefore, the blank meat sample could be used for the calibration curve and the recovery rate.

(a)



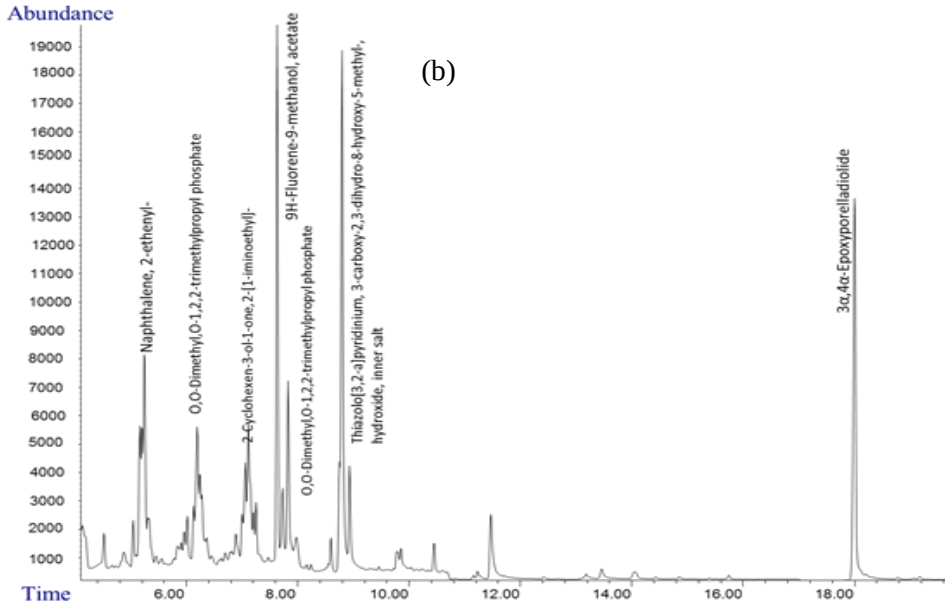


Figure 5. *Chromatogram of the blank meat sample: TIC mode (a) and SIM mode (b)*

Calibration

Figure 6 is an example chromatogram generated by spiking the sample blanks. The linear calibration curves were obtained by plotting the ratio $S_{(i)}/S_{IS}$ for each analytic versus its concentration ratio $C_{(i)}/C_{IS}$, where $S_{(i)}$, $C_{(i)}$ is the peak area and concentration of each PAH and IS is the corresponding internal standard. All curves had coefficients of determination r^2 >0.99 with the calibration range of 10-100ppb (Table 1).

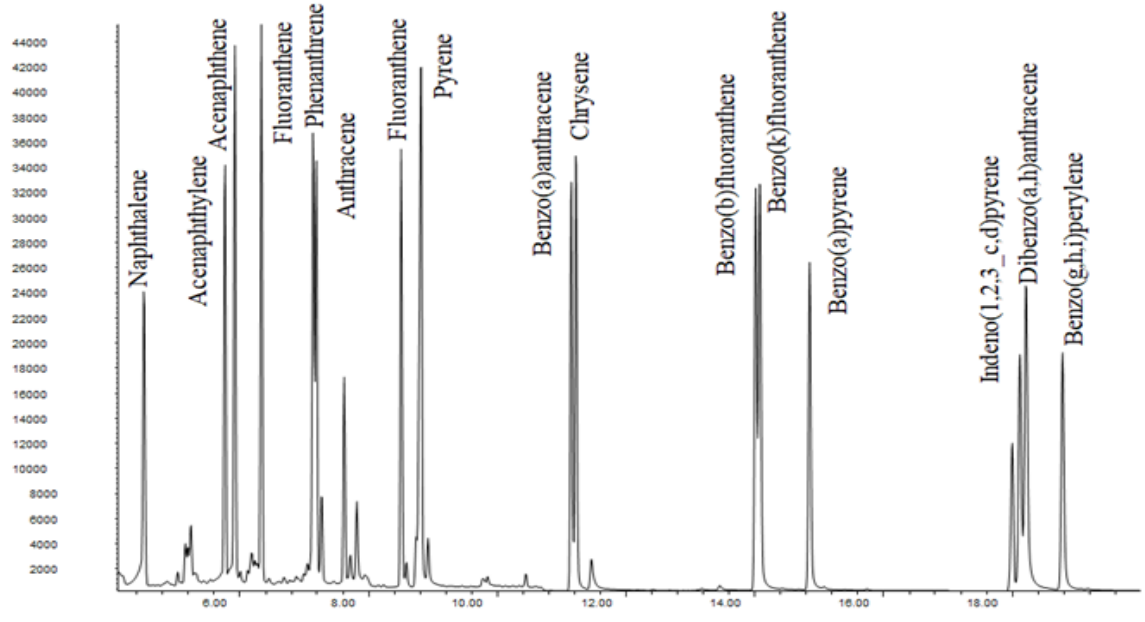


Figure 6. *Distribution of PAHs (concentration of 100ppb) in the spiked sample blank for SIM mode*

Table 1. *Retention time, monitored SIM ion and regression equation for 16 PAHs by GC-MS*

N°	PAH	t_R (min)	SIM ion (m/z)	Regression equation	r^2	Internal standard (IS)
1	Naphthalene	4.51	128	y = 0.0162x + 0.6698	0.9969	Phenanthrene-D10
2	Acenaphthylene	5.77	152	y = 0.1316x - 0.4261	0.9969	Phenanthrene-D10
3	Acenaphthene	5.93	153	y = 0.2074x - 0.4979	0.9985	Phenanthrene-D10
4	Fluorene	6.34	166	y = 0.5705x + 3.2148	0.9987	Phenanthrene-D10
5	Phenanthrene	7.15	178	y = 0.2717x + 6.3254	0.9988	Phenanthrene-D10
6	Anthracene	7.19	178	y = 0.2734x + 0.4092	0.9997	Phenanthrene-D10
7	Fluoranthene	8.51	202	y = 0.3139x + 0.5823	0.9993	Phenanthrene-D10
8	Pyrene	8.82	202	y = 0.2654x - 0.4807	0.9997	Phenanthrene-D10
9	Benz[a]anthracene	11.15	228	y = 0.3636x - 0.9548	0.9985	Chysene-D12
10	Chrysene	11.23	228	y = 0.4572x - 0.8096	0.9993	Chysene-D12
11	Benzo[b]fluoranthene	14.02	252	y = 0.5538x - 0.6097	0.9990	Perylene-D12
12	Benzo[k]fluoranthene	14.08	252	y = 0.6886x - 0.5331	0.9979	Perylene-D12
13	Benzo[a]pyrene	14.85	252	y = 0.4294x - 0.3898	0.9998	Perylene-D12
14	Indeno[1,2,3_cd]pyrene	18.12	276	y = 0.1171x - 0.3482	0.9970	Perylene-D12
15	Dibenz[a,h]anthracene	18.21	278	y = 0.3600x - 0.3985	0.9970	Perylene-D12
16	Benzo[g,h,i]perylene	18.78	276	y = 0.2278x - 0.1975	0.9980	Perylene-D12

Recovery, reproducibility and the analysis limits

The recovery and reproducibility (RSD) were evaluated on spiked samples at concentration of 10ppb and in replicates of five. The results analysis in Table 2 showed that the recoveries of 16 PAHs varied from 83.3% to 115.5% and the reproducibility (RSD) was 2.7% to 14.9%. These relatively high values suggested that recoveries of PAHs were highly complex when the sample matrix was in food form. This result was similar with some studies over the world on PAH analysis in foods (Martin Rose et al., 2015; Alomirah et al., 2011; Ge Li et al., 2016).

Table 2. *Recoveries, RSDs, limits of detection and quantification (MDL – MQL)*

N°	PAH	Recovery (%)	RSD (%)	Analysis limits	
				MDL (µg/kg)	MQL (µg/kg)
1	Naphthalene	87.0	6.5	0.4	1.2
2	Acenaphthylene	87.3	6.5	0.4	1.2
3	Acenaphthene	83.3	14.0	0.3	1.0
4	Fluorene	97.9	7.9	0.3	1.0
5	Phenanthrene	104.7	9.1	0.6	1.8
6	Anthracene	91.3	11.6	0.4	1.3
7	Fluoranthene	88.5	14.9	0.8	2.3
8	Pyrene	112.4	10.4	0.3	0.9
9	Benzo(a)anthracene	107.8	5.7	0.4	1.1
10	Chrysene	109.1	12.9	0.5	1.4
11	Benzo(b)fluoranthene	113.4	2.7	0.3	1.0

12	Benzo(k)fluoranthene	97.2	8.7	0.6	1.7
13	Benzo(a)Pyrene	98.5	6.0	0.4	1.1
14	Indeno(1,2,3_c,d)pyrene	115.5	7.9	0.5	1.5
15	Dibenzo(a,h)anthracene	111.8	12.5	0.4	1.2
16	Benzo(g,h,i)perylene	111.1	11.0	0.4	1.2

The analytical limits of the method were obtained by reducing the PAHs concentration in spike samples. The detection limit (MDL) and the quantitative limit (MQL) of the method were estimated from the signal-to-noise ratio (S/N) of 3 and 10 respectively. In this study, the limits of detection ranged from 0.3µg/kg to 0.8 µg/kg, which were satisfied with determination the low levels of PAHs in food.

Method application on actual meat samples

PAHs are usually formed during food processing (drying, smoking) and food cooking at high temperature (grilling, frying, roasting, baking) (Ledesma et al., 2014; Martin Rose et al., 2015). Therefore, in this study some samples of grilled pork were used to test the analytical method by determining PAHs content (Table 3).

Table 3. *PAH concentration (ppb) in some grilled pork samples*

Concentration (ppb) PAH	**Samle 1**	**Sample 2**	**Sample 3**	**Sample 4**
Naphthalene	0.70	18.56	0.26	13.27
Acenaphthylene	0.78	0.09	13.74	14.23
Acenaphthene	0.21	0.08	0.46	0.71
Fluorene	0.43	3.10	1.63	2.23
Phenanthrene	0.00	5.33	10.71	12.51
Anthracene	0.00	0.85	2.48	2.70
Fluoranthene	0.00	0.76	5.85	6.22
Pyrene	0.04	0.69	7.88	8.24
Benz[a]anthracene	0.00	0.03	0.65	0.66
Chrysene	0.41	0.00	1.26	1.31
Benzo[b]fluoranthene	0.00	0.00	1.78	1.85
Benzo[k]fluoranthene	0.00	0.00	1.41	1.46
Benzo[a]pyrene	0.00	0.00	1.03	0.97
Dibenz[a,h]anthracene	0.22	0.00	0.00	0.00
Indeno[1,2,3_c,d]pyrene	0.00	0.00	1.43	1.49
Benzo[g,h,i]perylene	0.00	0.00	1.18	1.09
Total 16 PAHs (ppb)	***2.79***	***29.49***	***51.76***	***68.93***

Results showed that there was the formation of PAH compounds in pork after grilling. The total 16 PAHs concentrations increased from 2.79ppb to 68.93ppb when an increase in grilling temperature and time was applied from sample 1 to sample 4. According to European Regulations, the permitted levels of PAHs in meat products are: total PAHs <35µg/kg and benzo[a]pyrene <2µg/kg (EC., 2006; EFSA., 2008). Thus, the third and fourth samples were likely to affect the health of the consumer, especially in the case of frequently consumption of grilled meat. This results were similar with some studies in the world when they found the high content of PAHs produced by baking meat (Ledesma et al., 2014; Martin Rose et al., 2015; Essumang et al., 2013; Alomirah et al., 2011).

CONCLUSION

The research had successfully developed the PAHs analysis process in meat products by combining QuEChERS extraction and GC-MS analysis. The procedure used internal standard reagents to quantify PAHs with a linear concentration from 10 to 100ppb and coefficients of determination $r^2 > 0.99$. In addition, the limit of detection for PAHs concentrations (0.3-0.8ppb) allowed the quantification of the small level of PAHs present in food. Therefore, the method should be applied and developed for related studies such as level of PAHs pollution in soil, water samples, different matrix foods...

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