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Evaluation of Analysis of Polycyclic Aromatic Hydrocarbons by the QuEChERS Method and Gas Chromatography–Mass Spectrometry and Their Formation in Poultry Meat As Affected by Marinating and Frying

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ABSTRACT: The objectives of this research were to develop a method for the determination of 16 polycyclic aromatic hydrocarbons (PAHs) in poultry meat by combining the quick, easy, cheap, effective, rugged, and safe (QuEChERS) method with gas chromatography–mass spectrometry (GC–MS) and study their formation during marinating and frying. The recoveries of 16 PAHs ranged from 94.5 to 104% in blank samples and from 71.2 to 104% in poultry meat samples. The quantitation limits of 16 PAHs were from 0.02 to 1 ng/mL, with the intraday variability being from 2.4 to 6.6% [percent relative standard deviation (RSD%)] and interday variability being from 3.3 to 7.1% (RSD%). Most PAHs followed a time-dependent increase over a 24 h marinating period, with naphthalene being generated in the largest amount. Among the various poultry meat, chicken gizzard produced the highest level of total PAHs after 24 h of marinating. A similar tendency was observed for most PAHs during frying of poultry meat, but a high amount of total PAHs was shown in duck drumstick after 15 min of frying.

KEYWORDS: *polycyclic aromatic hydrocarbons, poultry meat, GC–MS, QuEChERS, marinating, frying*

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are mainly formed by incomplete combustion of hydrocarbon compounds during pyrolysis or pyrosynthesis reaction.[1] Structurally, PAHs contain two or more benzene rings connected with each other to form a stable compound. Because of their nondegradable nature, PAHs have been classified as vital environmental pollutants because they may interfere with the normal function of DNA.[2] Many PAHs have been characterized in nature, in which benzo[*a*]-anthracene, benzo[*a*]pyrene, benzo[*b*]fluoranthene, benzo[*k*]-fluoranthene, chrysene, dibenzo[*a*,*h*]anthracene, and indeno-[1,2,3-*c*,*d*]pyrene were probable carcinogens to animals or humans, according to a report by the International Agency for Research on Cancer (IARC).[3] Among them, both benzo[*a*]-anthracene and benzo[*a*]pyrene were shown to be the most carcinogenic.[3] In addition, the 16 PAHs, including acenaphthene, acenaphthylene, anthracene, benzo[*a*]anthracene, benzo-[*a*]pyrene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*g*,*h*,*i*]perylene, chrysene, dibenzo[*a*,*h*]anthracene, fluoranthene, fluorene, indeno[1,2,3-*c*,*d*]pyrene, naphthalene, phenanthrene, and pyrene, were listed as priority organic pollutants by the United States Environmental Protection Agency (U.S. EPA).[4]

Because of the presence of trace amounts in the complex matrix of foods, the analysis of PAHs, especially in meat products, has been difficult. Traditionally, PAHs were often extracted with nonpolar or low-polar solvents, such as hexane or methylene chloride, or with the Soxhlet method, followed by saponification or liquid–liquid partition to remove water-soluble impurities and purification using a solid-phase cartridge with silica gel or C18 as the packing material. However, these methods are time-consuming, and the presence of many impurities can interfere with subsequent chromatographic analysis, resulting in low recovery.[5] Some other improved techniques, such as solid-phase microextraction,[6] hollow-fiber liquid-phase microextraction,[7] and supercritical fluid extraction,[8] were developed in recent years to reduce solvent consumption and shorten analysis time. Nevertheless, these methods still encounter difficulty in determining PAHs in meat products. More recently, Purcaro et al.[9] developed a microwave-assisted extraction method to analyze PAHs in smoked meat products and reported a substantial reduction in the extraction time, but the elevated extraction temperature (115 °C) may induce volatilization of several PAHs, such as naphthalene, acenaphthene, and fluorene. In view of the drawbacks associated with analysis of PAHs in foods, the development of a reliable method to shorten the analysis time with maximum accuracy is urgent. The quick, easy, cheap, effective, rugged, and safe (QuEChERS) method is a promising technique widely used for extraction and purification of pesticides in food commodities, including fruit, vegetable, olive oil, milk, baby food, barley, and egg;[10] however, the feasibility of using the QuEChERS method in combination with gas chromatography–mass spectrometry (GC–MS) for PAH analysis remains unexplored.

Because PAHs can be formed during incomplete combustion of oil, wood, and coal, it is quite possible that meat products can be readily contaminated with PAHs during frying, smoking, and grilling.[11,12] To address this important issue, the Codex Alimentarius Commission[13,14] issued "Code of Practice for the Reduction of Contamination of Food with PAHs from Smoking and Direct Drying Processes", stating that the difference in PAH contents among various food products can be

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dependent upon the heating time, temperature, distance between the food and heating medium, as well as oil dripping onto meat. Marinating is a traditional Chinese cooking method by soaking meat in a mixture of juice containing soy sauce, sugar, and/or some other flavoring ingredients and heating at about 100 °C for an extended period of time to impart characteristic color, flavor, and texture to meat products. However, the effects of marinating and frying on PAH formation in poultry meat remain unknown. The objectives of this study were to develop a QuEChERS and GC−MS method for the determination of PAHs in chicken and duck meat and study their formation as affected by marinating and frying.

EXPERIMENTAL SECTION

Materials and Chemicals. Poultry meat, including chicken heart, chicken drumstick, chicken gizzard, chicken breast, and duck drumstick, with a total amount of 1.6, 14.4, 6.4, 8.0, and 14.4 kg, respectively, were purchased from a local supermarket in Taipei, Taiwan. Palm oil (150 L) used for frying was obtained from the Chiang-Kuan Co. (Kaohsiung, Taiwan). Soy sauce (7 L) was procured from the Gin-Lan Food Co. (Taoyuan, Taiwan). Crystal sugar (700 g) was from the Taiwan Sugar Co. (Tainan, Taiwan). A total of 16 PAH standards, including acenaphthene, acenaphthylene, anthracene, benzo[*a*]anthracene, benzo[*a*]pyrene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*g*,*h*,*i*]perylene, chrysene, dibenzo[*a*,*h*]anthracene, fluoranthene, fluorene, indeno[1,2,3-*c*,*d*]pyrene, naphthalene, phenanthrene, and pyrene, were from Supelco (Bellefonte, PA). QuEChERS kits were from Agilent Technologies (Palo Alto, CA). Solvents, such as acetonitrile and acetone, were from Merck (Darmstadt, Germany). Deionized water was made using a Barnstead Easypure II water purification system from the Thermo Scientific Co. (Waltham, MA).

Instrumentation. The GC−MS system was composed of a gas chromatograph (model 6890) equipped with a mass spectrometer (model 5973) from Agilent Technologies. The temperature-controlled oil bath (B503) was from the I-Seng Technology Co. (Taipei, Taiwan). The shaker (VM-2000) was from the Shiang-Tai Co. (Taipei, Taiwan). An Agilent HP-5MS column (30 m × 0.25 mm inner diameter and 0.25 μm film thickness) used to separate 16 PAHs was from Agilent Technologies.

Marinating of Poultry Meat. A method similar to that described by Lee et al.[15] was used to marinate poultry meat. A total amount of 0.2, 0.8, 1.0, 1.8, and 1.8 kg each of chicken heart, chicken gizzard, chicken breast, chicken drumstick, and duck drumstick was poured into five separate cookers with each containing 0.6, 2.4, 3.0, 5.4, and 5.4 L of juice preheated to 100 °C. The juice was composed of 10% soy sauce, 1% crystal sugar, and 89% water, which is the standard formula used for marinating poultry meat in most restaurants in Taiwan. Then, the marinating treatment proceeded for 12 and 24 h, during which the juice was replenished with water to maintain it at a constant level, as indicated every 1 h. After marinating for 12 and 24 h, the marinated chicken heart, chicken drumstick, chicken breast, chicken gizzard, and duck drumstick were deboned separately, cut into pieces, vacuum-packed, and stored at −20 °C for subsequent GC−MS analysis. Duplicate experiments were performed for each marinating treatment.

Frying of Poultry Meat. A total amount of 15 L of palm oil was poured into a fryer and preheated to 180 °C, after which 0.2, 0.8, 1.0, 1.8, and 1.8 kg each of chicken heart, chicken gizzard, chicken breast, chicken drumstick, and duck drumstick were added to a fryer separately for frying, with the frying times being 4 and 10 min, 2 and 10 min, 5 and 10 min, 10 and 20 min, and 15 and 30 min, respectively. After frying, the various chicken and duck meat samples were deboned separately, cut into slices, vacuum-packed, and stored at −20 °C for subsequent GC−MS analysis. Duplicate experiments were carried out for each frying treatment.

Extraction and Purification of PAHs. Initially, 1 kg each of various raw and heated chicken and duck meat samples were ground into pieces in a blender prior to extraction. Then, 5 g of meat sample was collected and mixed with 10 mL of deionized water in a centrifuged tube and shaken vigorously for 1 min, after which 10 mL of acetonitrile was added and shaken again for 1 min. Next, the QuEChERS method containing 6 g of magnesium sulfate and 1.5 g of sodium acetate was added, followed by shaking for 1 min and centrifuging at 4000 rpm for 5 min. Then, 6 mL of supernatant was collected and poured into a centrifuged tube (QuEChERS) containing 400 mg of PSA, 1200 mg of $MgSO_4$, and 400 mg of C18EC for purification. After centrifugation at 4000 rpm for 5 min, the supernatant was collected and 1 μL was injected for PAH analysis by GC−MS.

GC−MS Analysis. An Agilent HP-5MS column (30 m × 0.25 mm inner diameter and 0.25 μm film thickness) was connected to a 5 m guard column to extend column life. A total of 16 PAHs were separated within 40 min with helium as the carrier gas at a flow rate of 1.0 mL/min, injector temperature of 290 °C, and spiltless mode, with the following temperature programming condition: 70 °C in the beginning, raised to 195 °C at 15 °C/min while maintained for 2.5 min, raised to 240 °C at 15 °C/min while maintained for 17 min, raised to 270 °C at 5 °C/min, and raised to 310 °C at 15 °C/min while maintained for 10 min. The various PAHs in poultry meat samples were identified by comparing retention times and mass spectra of unknown peaks to those of reference standards. In addition, the unknown PAHs were further identified by co-chromatography and selected ion monitoring (SIM) mode to minimize interference by sample impurities and enhance sensitivity.

For quantitation of PAHs, 14 concentrations of 0.1, 0.2, 0.3, 0.5, 1, 2, 5, 10, 25, 30, 50, 70, 100, and 120 ng/mL were each prepared for naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, and benzo[*b*]fluoranthene separately, whereas 13 concentrations of 0.2, 0.3, 0.5, 1.0, 2.0, 5.0, 10, 25, 30, 50, 70, 100, and 120 ng/mL were each prepared for benzo[*a*]anthracene and chrysene separately. Likewise, 10 concentrations of 1.0, 2.0, 5.0, 10, 25, 30, 50, 70, 100, and 120 ng/mL were each prepared for benzo[*k*]fluoranthene and benzo[*a*]pyrene separately, while 11 concentrations of 0.5, 1.0, 2.0, 5.0, 10, 25, 30, 50, 70, 100, and 120 ng/mL were each prepared for indeno[1,2,3-*c*,*d*]pyrene and dibenzo[*a*,*h*]anthracene separately. In addition, 12 concentrations of 0.3, 0.5, 1.0, 2.0, 5.0, 10, 25, 30, 50, 70, 100, and 120 ng/mL were each prepared for benzo[*g*,*h*,*i*]perylene. After GC−MS analysis, the standard curve of each PAH was obtained by plotting the concentration against area. The regression equations and correlation coefficient were determined automatically with an Excel software system. The concentrations of various PAHs were calculated as follows:

$$\text{concentration of PAH (ng/g)} = \frac{\left(\frac{A-b}{a}\right)V \times \text{dilution factor/recovery}}{W}$$

where A is the peak area of PAH, b is the intercept of the regression equation, a is the slope of the regression equation, V is the volume of the extract, and W is the weight of the sample (g).

Detection Limit and Quantitation Limit. Five concentrations of 0.04, 0.1, 0.18, 0.2, and 0.4 ng/mL were prepared separately for naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, and pyrene, while four concentrations of 0.1, 0.2, 0.3, and 0.4 ng/mL were prepared separately for benzo[*a*]anthracene and chrysene. In addition, three concentrations of 1.0, 2.0, and 3.0 ng/mL were prepared separately for benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, indeno[1,2,3-*c*,*d*]pyrene, dibenzo[*a*,*h*]anthracene, and benzo[*g*,*h*,*i*]perylene. Each concentration was injected onto GC−MS 3 times, and the limit of detection (LOD) was calculated on the basis of PAH standards in solvents and S/N ≥ 3, whereas the limit of quantitation (LOQ) was calculated on the basis of PAH standards dissolved in sample extract and S/N ≥ 3.

Precision Study. The intraday variability was determined by preparing 25 ng/mL each of 16 PAH standards and injecting into GC−MS 3 times each in the morning, afternoon, and evening for a total of nine replicates on the same day. Likewise, the interday variability was measured by preparing the same concentration and injecting into GC−MS on the first, second, and third day with three replicates each day for a total of nine replicates.

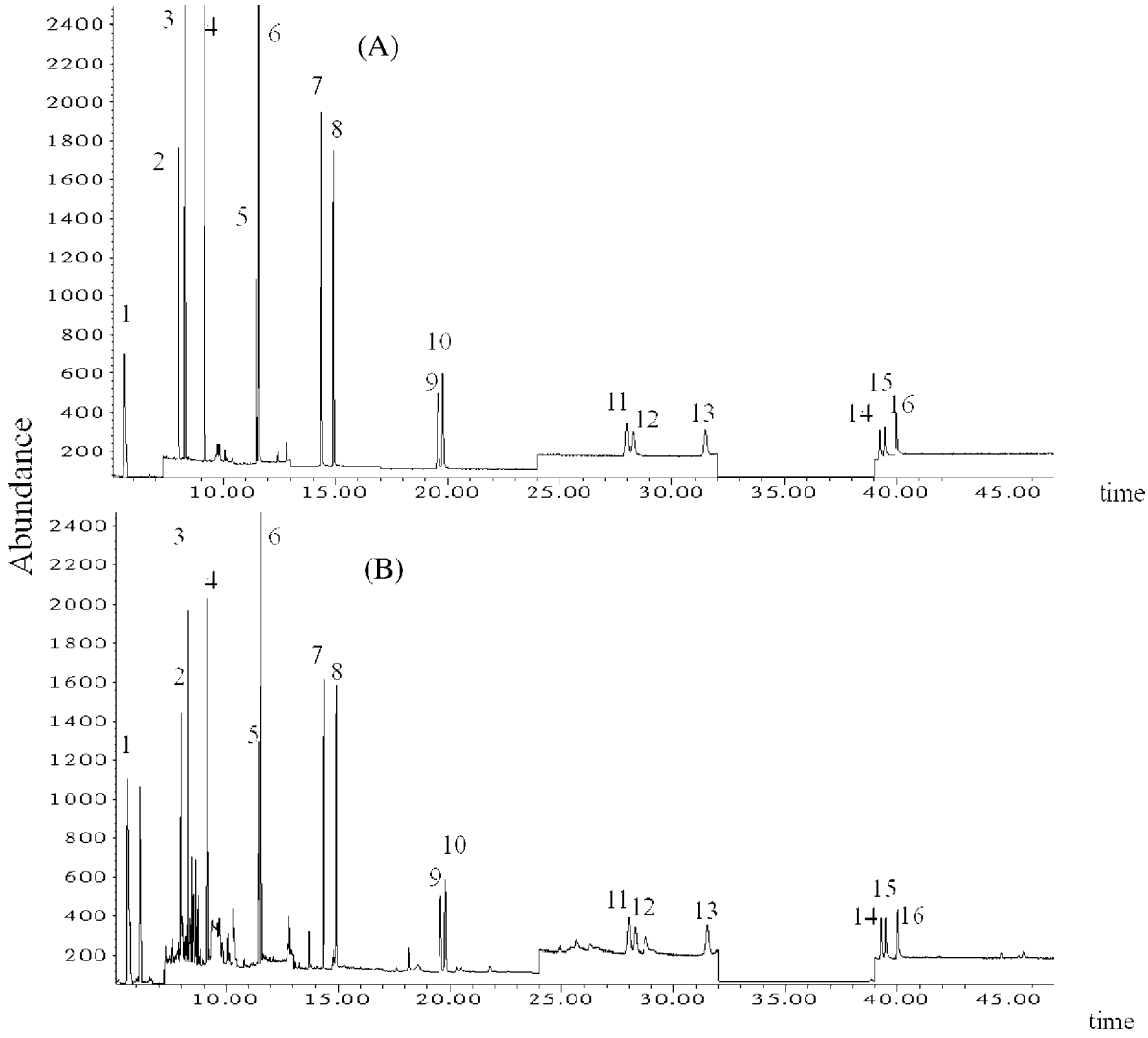

Figure 1. GC−MS chromatogram of (A) 16 PAH standards and (B) marinated chicken gizzard spiked with 16 PAH standards detected with SIM mode. Peaks: 1, naphthalene; 2, acenaphthylene; 3, acenaphthene; 4, fluorene; 5, phenanthrene; 6, anthracene; 7, fluoranthene; 8, pyrene; 9, benzo[*a*]anthracene; 10, chrysene; 11, benzo[*b*]fluoranthene; 12, benzo[*k*]fluoranthene; 13, benzo[*a*]pyrene; 14, indeno[1,2,3-*c*,*d*]pyrene; 15, dibenzo[*a*,*h*]anthracene; and 16, benzo[*g*,*h*,*i*]perylene.

Recovery. The recoveries were determined for both blank and poultry meat samples, with the former being carried out by preparing a mixture of 16 PAH standards with 250 and 1000 ng/g each and mixing with deionized water in a centrifuged tube, following the same extraction and purification procedure with the QuEChERS method as described in the preceding section. For poultry meat samples, the procedures were the same, except a mixture of 16 PAH standards was mixed with 5 g of meat sample and deionized water in a centrifuged tube. After GC−MS analysis, the recovery of each PAH was determined on the basis of the ratio of the amount of the standard after GC−MS relative to the amount of the standard spiked before GC−MS.

Statistical Analysis. Duplicate analyses were performed for each meat sample, and the data were subjected to analysis of variance (ANOVA) and Duncan's multiple range test for statistical significance ($p < 0.05$) using a SAS software system.[16]

RESULTS AND DISCUSSION

Evaluation of the GC−MS Method. Figure 1 shows the GC−MS chromatogram of (A) 16 PAH standards and (B) marinated chicken gizzard spiked with 25 ng/mL each of 16 PAH standards detected with SIM mode. An adequate resolution of 16 PAHs was attained within 40 min with a substantial amount of sample impurities being minimized by employing a SIM detection mode. The separation time was similar to that reported by Bordajandi et al.,[17] who used a DB-5MS column (30 m × 0.25 mm inner diameter and 0.25 μm film thickness) to separate 15 PAHs within 42 min, but the resolution was inferior to ours because several PAH peaks overlapped. In our study, the SIM mode was used for PAH detection according to elution time and *m*/*z*: 5−7.3 min, *m*/*z* 127 and 128.2 for naphthalene; 7.3−8.7 min, *m*/*z* 152, 153, and 154 for acenaphthylene and acenaphthene; 8.7−10.5 min, *m*/*z* 163, 165, and 166 for fluorene; 10.5−13 min, *m*/*z* 176, 178, and 179 for phenanthrene and anthracene; 13−17 min, *m*/*z* 200, 202, and 203 for fluoranthene and pyrene; 17−24 min, *m*/*z* 226, 228, and 229 for benzo[*a*]-anthracene and chrysene; 24−39 min, *m*/*z* 250, 252, and 253 for benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, and benzo[*a*]-pyrene; and 39−45 min, *m*/*z* 276, 277, 278, and 279 for indeno[1,2,3-*c*,*d*]pyrene, dibenzo[*a*,*h*]anthracene, and benzo-[*g*,*h*,*i*]perylene. More recently, Gratz et al.[18] developed a high-performance liquid chromatograph (HPLC) method to separate 15 PAHs within 17.5 min using a short Zorbax Eclipse column (50 × 4.6 mm inner diameter, with a particle size

Table 1. Quality Control Data of 16 PAHs by GC−MS Analysis

	PAHs	retention time (min)	LOD[a] (ng/mL)	LOQ[b] (ng/mL)	LOQ[c] (ng/g)	intraday variability mean ± SD (ng/mL)	intraday variability RSD (%)	interday variability mean ± SD (ng/mL)	interday variability RSD (%)
1	naphthalene	5.61	0.1	0.02	0.04	81.8 ± 2.0	2.4	80.6 ± 2.6	3.3
2	acenaphthylene	8.00	0.1	0.09	0.18	26.3 ± 1.1	4.1	25.7 ± 1.3	5.0
3	acenaphthene	8.29	0.1	0.02	0.04	20.8 ± 0.8	3.9	20.3 ± 1.0	4.7
4	fluorene	9.16	0.1	0.09	0.18	24.5 ± 1.0	4.1	23.9 ± 1.1	4.8
5	phenanthrene	11.45	0.1	0.15	0.3	29.1 ± 1.2	4.0	28.5 ± 1.3	4.5
6	anthracene	11.57	0.1	0.2	0.4	21.5 ± 0.8	3.9	21.0 ± 0.9	4.3
7	fluoranthene	14.35	0.1	0.1	0.2	18.3 ± 0.6	3.5	18.0 ± 0.8	4.5
8	pyrene	14.91	0.1	0.02	0.04	17.4 ± 0.6	3.2	17.1 ± 0.8	4.5
9	benzo[*a*]anthracene	19.55	0.3	0.2	0.4	18.2 ± 0.8	4.6	17.7 ± 1.0	5.4
10	chrysene	19.76	0.3	0.2	0.4	16.6 ± 0.7	4.0	16.2 ± 0.7	4.5
11	benzo[*b*]fluoranthene	27.9	2	1	2	26.2 ± 1.1	4.3	26.0 ± 1.2	4.5
12	benzo[*k*]fluoranthene	28.25	2	1	2	11.1 ± 0.7	6.3	10.8 ± 0.8	7.0
13	benzo[*a*]pyrene	31.46	2	1	2	20.7 ± 1.2	5.8	19.8 ± 1.4	6.9
14	indeno[1,2,3-*c*,*d*] pyrene	39.22	2	0.5	1	20.2 ± 1.0	4.9	19.4 ± 1.3	6.6
15	dibenzo[*a*,*h*] anthracene	39.42	2	0.5	1	19.3 ± 1.3	6.6	18.6 ± 1.3	7.1
16	benzo[*g*,*h*,*i*]perylene	39.99	2	0.5	1	18.8 ± 1.0	5.2	18.1 ± 1.2	6.5

[a]The LOD was based on S/N ≥ 3 of standard solution. [b]The LOQ was based on S/N ≥ 3 of sample extract. [c]The LOQ was the limit of quantitation of PAHs in the sample based on wet weight.

Table 2. PAH Recoveries (%) Obtained Using the QuEChERS Method for Blank and Marinated Chicken Gizzard Samples with the Addition of Two Levels of PAH Standards[a]

	PAHs	blank sample 50 ng/g	blank sample 200 ng/g	blank sample mean	chicken gizzard sample 50 ng/g	chicken gizzard sample 200 ng/g	chicken gizzard sample mean
1	naphthalene	99.6 ± 4.2	97.5 ± 0.7	98.6 ± 4.6	85.6 ± 1.2	89.6 ± 1.6	87.6 ± 1.4
2	acenaphthylene	98.0 ± 2.4	103 ± 0.4	100 ± 2.6	99.1 ± 1.2	92.5 ± 3.1	95.8 ± 2.2
3	acenaphthene	97.7 ± 3.6	99.0 ± 0.6	98.4 ± 3.9	93.9 ± 0.3	89.8 ± 3.0	91.9 ± 1.7
4	fluorene	103 ± 0.3	97.1 ± 0.5	99.8 ± 0.6	105 ± 0.5	96.0 ± 0.4	100 ± 0.5
5	phenanthrene	99.6 ± 3.3	101 ± 0.6	100 ± 3.6	107 ± 1.5	101 ± 0.6	104 ± 1.1
6	anthracene	99.0 ± 5.8	98.6 ± 2.8	98.8 ± 7.2	97.6 ± 0.2	93.9 ± 2.0	95.8 ± 1.1
7	fluoranthene	103 ± 1.9	101 ± 1.1	102 ± 2.5	89.1 ± 0.6	84.4 ± 2.4	86.8 ± 1.5
8	pyrene	102 ± 0.4	101 ± 2.6	102 ± 1.7	89.3 ± 0.4	81.0 ± 3.1	85.2 ± 1.8
9	benzo[*a*]anthracene	102 ± 6.2	105 ± 4.4	104 ± 8.4	88.1 ± 1.5	90.8 ± 2.8	89.5 ± 2.2
10	chrysene	102 ± 3.9	103 ± 5.8	103 ± 6.8	87.8 ± 0.9	89.7 ± 4.4	88.8 ± 2.7
11	benzo[*b*]fluoranthene	99.4 ± 9.0	102 ± 0.4	101 ± 9.2	91.9 ± 0.6	98.8 ± 0.7	95.4 ± 0.7
12	benzo[*k*]fluoranthene	98.3 ± 0.0	98.5 ± 2.5	98.4 ± 1.3	101 ± 7.9	93.7 ± 0.8	97.2 ± 4.4
13	benzo[*a*]pyrene	93.8 ± 5.7	95.1 ± 5.1	94.5 ± 8.3	83.7 ± 5.3	87.4 ± 3.4	85.6 ± 4.4
14	indeno[1,2,3-*c*,*d*]pyrene	98.6 ± 4.0	95.3 ± 0.4	97.0 ± 4.2	72.5 ± 6.1	69.8 ± 4.1	71.2 ± 5.1
15	dibenzo[*a*,*h*]anthracene	93.3 ± 1.9	102 ± 5.9	97.7 ± 4.9	83.0 ± 4.2	90.7 ± 7.9	86.9 ± 6.1
16	benzo[*g*,*h*,*i*]perylene	101 ± 5.6	97.3 ± 5.2	99.1 ± 8.2	71.7 ± 4.2	72.1 ± 5.8	71.9 ± 5.0

[a]Average of duplicate analyses ± standard deviation.

of 1.8 μm) with fluorescence detection. Although the separation time was reduced greatly, a lot of impurities were present on the HPLC chromatogram, which may decrease the quantitation accuracy and shorten column life.

Quality Control Data. Table 1 shows the quality control data of 16 PAH standards, with LOD ranging from 0.1 to 2 ng/mL based on standard solution and LOQ ranging from 0.02 to 1 ng/mL based on sample extract. Because of the matrix effect involved in meat sample extraction, we observed a larger peak response for PAH standards dissolved in sample extract than in solvent. Thus, the LOQ (ng/mL) was determined by dissolving PAH standards in sample extract, and this is why the LOQ of most PAHs was lower than the LOD. However, when the sample weight (wet) was taken into account, the LOQ was estimated from 0.04 to 2 ng/g. In comparison to some other published reports using HPLC and QuEChERS for PAH extraction and purification in meat, the LOQ ranged from 0.84 to 16, from 0.24 to 4.7, from 0.39 to 22, and from 0.71 to 44 ng/g for oyster, finfish, shrimp, and crab, respectively,[18] as well as from 0.12 to 1.9 ng/g for horse mackerel.[19] However, the LOQ of acenaphthylene was not detected in both studies.[18,19] Additionally, in our experiment, the LOQ was lower than that reported by Gratz et al.[18] and Ramalhosa et al.,[19] probably caused by the difference in extraction, purification, and meat sample variety, as well as the detection mode. The intraday variability for 16 PAHs ranged from 2.4 to 6.6% [percent relative standard deviation (RSD%)], while the interday variability ranged from 3.3 to 7.1% (RSD%), demonstrating that a high reproducibility was achieved by our method.

Table 2 shows the recoveries of 16 PAHs in blank and poultry meat samples, with the former ranging from 94.5 to

104% and the latter ranging from 71.2 to 104%. In the poultry meat sample, with the exception of indeno[1,2,3-*c*,*d*]pyrene and benzo[*g*,*h*,*i*]perylene, the recoveries of the other 14 PAHs were higher than 85.2%. A slightly lower recovery of indeno[1,2,3-*c*,*d*]pyrene and benzo[*g*,*h*,*i*]perylene is probably due to the high molecular weight, which may encounter difficulty in vaporization during GC separation at high temperatures. In several previous studies, Ramalhosa et al.[19] used the QuEChERS method to extract and purify 15 PAHs in fish for HPLC analysis and found that the recoveries ranged from 84.8 to 110.5%, whereas the recoveries of 15 PAHs in oyster were from 76 to 101% for a low concentration (50.0 ng/g each) of PAHs and from 85 to 90% for a high concentration (10.0 μg/g each) of PAHs by adopting a QuEChERS and HPLC technique with fluorescence detection.[18] Instead of the QuEChERS method, Castro et al.[20] employed a microwave-assisted extraction technique to determine 16 PAHs in outdoor particulates by HPLC, and the recoveries were from 62.3 to 112%. Apparently, the difference in recovery can be greatly affected by sample variety and methods of extraction and purification, as well as detection.

Extraction of PAHs. As mentioned above, the QuEChERS method is frequently applied to pesticide determination in vegetable and fruit samples, owing to short extraction and purification time, as well as low solvent consumption. Until recently, the QuEChERS method was applied to extract and purify PAHs in fish and oyster for subsequent HPLC analysis with fluorescence detection.[18,19] However, because of the difference in the matrix effect among various meat commodities, the extraction and purification conditions have to be evaluated carefully when adopting the QuEChERS method. In our experiment, both variety and volume of solvents were compared for extraction efficiency. Initially 5, 10, and 15 mL of acetonitrile were compared for extraction efficiency of PAHs for 5 g of meat sample, with both volumes of 10 and 15 mL resulting in a higher yield of total PAHs (Table 3). Next, both solvents of acetonitrile and acetone with the same volume were compared, and 10 mL was shown to generate a higher recovery than 15 mL (Table 4). Thus, 10 mL of acetonitrile and acetone were further compared for subsequent extraction and purification using the QuEChERS method. On the basis of the GC−MS chromatogram, a lesser amount of impurities was present for acetonitrile than for acetone after purification (Figure 2). Although there was only a slight difference in recovery between purified and nonpurified meat sample with acetonitrile as the extraction solvent (Table 5), the purification step by the QuEChERS method was deemed to be necessary to extend column life, following acetonitrile extraction of PAHs from chicken gizzard. In a similar study, Ramalhosa et al.[19] used 10 mL of acetonitrile to extract PAHs from 5 g of fish meat but reported no difference in extraction efficiency with and without purification by the QuEChERS method. Likewise, Gratz et al.[18] suggested that the purification step by the QuEChERS method may not be necessary following extraction of PAHs from seafoods with 15 mL of acetonitrile. Because both studies used HPLC with fluorescence detection for PAH determination,[18,19] the column lifetime may be reduced substantially amid the presence of a large amount of impurities on the chromatogram. Thus, in our study, the most appropriate extraction and purification condition of PAHs from chicken gizzard as described in the Experimental Section was adopted for a subsequent study of PAH formation in various poultry meat by GC−MS as affected by marinating and frying.

Table 3. Effects of Different Volumes of Acetonitrile on the Extraction Efficiency of PAHs (ng/g) in Marinated Chicken Gizzard[a]

	PAHs	acetonitrile 15 mL	acetonitrile 10 mL	acetonitrile 5 mL
1	naphthalene	48.6 ± 11.0	45.3 ± 4.8	29.4 ± 0.7
2	acenaphthylene	nd[b]	nd	nd
3	acenaphthene	nd	nd	nd
4	fluorene	17.2 ± 0.6	14.1 ± 0.1	11.5 ± 0.3
5	phenanthrene	22.4 ± 3.4	18.7 ± 1.9	9.0 ± 0.2
6	anthracene	nd	nd	nd
7	fluoranthene	0.4 ± 0.0	0.6 ± 0.2	0.2 ± 0.0
8	pyrene	1.1 ± 0.3	1.4 ± 0.2	0.6 ± 0.0
9	benzo[*a*]anthracene	nd	nd	nd
10	chrysene	nd	nd	nd
11	benzo[*b*]fluoranthene	34.6 ± 1.4	39.2 ± 2.1	29.1 ± 2.3
12	benzo[*k*]fluoranthene	nd	nd	nd
13	benzo[*a*]pyrene	4.6 ± 0.3	3.0 ± 0.6	2.0 ± 0.0
14	indeno[1,2,3-*c*,*d*]pyrene	nd	nd	nd
15	dibenzo[*a*,*h*]anthracene	nd	nd	nd
16	benzo[*g*,*h*,*i*]perylene	nd	nd	nd
	total	129.1 ± 14.2	122.4 ± 4.6	81.9 ± 3.1

[a]Average of duplicate analyses ± standard deviation, expressed as ng/g. [b]nd = not detected, below the LOQ.

Table 4. Recoveries of PAHs in Marinated Chicken Gizzard with Acetonitrile or Acetone as the Extraction Solvent and Volume at 10 and 15 mL[a]

	PAHs	acetonitrile 15 mL	acetonitrile 10 mL	acetone 15 mL	acetone 10 mL
1	naphthalene	111.7 ± 1.5	108.1 ± 1.7	94.3 ± 3.4	102.5 ± 4.9
2	acenaphthylene	94.8 ± 2.0	93.6 ± 1.5	105.7 ± 0.7	95.6 ± 0.9
3	acenaphthene	91.9 ± 0.0	89.3 ± 1.2	99.4 ± 2.1	93.8 ± 3.8
4	fluorene	82.1 ± 5.2	90.5 ± 0.6	117.0 ± 3.0	99.7 ± 4.6
5	phenanthrene	93.8 ± 1.8	89.4 ± 3.6	95.0 ± 9.3	105.2 ± 4.8
6	anthracene	80.4 ± 3.5	96.4 ± 4.7	105.8 ± 9.8	94.1 ± 4.3
7	fluoranthene	91.6 ± 2.9	90.8 ± 1.2	97.1 ± 1.5	97.4 ± 6.7
8	pyrene	100.4 ± 8.6	97.8 ± 1.1	96.2 ± 0.6	106.1 ± 3.7
9	benzo[*a*]anthracene	99.2 ± 1.8	97.2 ± 5.3	88.9 ± 4.2	102.9 ± 1.8
10	chrysene	91.3 ± 2.9	94.4 ± 2.3	94.7 ± 2.4	97.8 ± 1.9
11	benzo[*b*]fluoranthene	80.9 ± 9.0	94.2 ± 3.0	103.2 ± 1.5	105.8 ± 0.4
12	benzo[*k*]fluoranthene	96.1 ± 8.8	101.1 ± 1.7	88.4 ± 0.9	92.1 ± 3.0
13	benzo[*a*]pyrene	72.8 ± 2.4	85.6 ± 1.6	97.7 ± 3.1	101.7 ± 0.8
14	indeno[1,2,3-*c*,*d*]pyrene	69.2 ± 7.8	91.3 ± 1.7	96.4 ± 3.0	109.0 ± 0.8
15	dibenzo[*a*,*h*]anthracene	62.6 ± 1.1	81.5 ± 2.9	91.7 ± 0.2	92.0 ± 0.8
16	benzo[*g*,*h*,*i*]perylene	60.8 ± 5.8	66.4 ± 4.5	86.5 ± 5.7	74.2 ± 0.3

[a]Average of duplicate analyses ± standard deviation.

Effects of the Heating Time on PAH Contents in Marinated and Deep-Fried Poultry Meat. Table 6 shows the effect of the marinating time on PAH formation in various poultry meat, with the total PAHs being generated from 14.4 to 124.5 ng/g. Only a minor amount of PAHs were detected in raw poultry meat (data not shown). Comparatively, a higher content of total PAHs was produced for all of the poultry meat commodities after 24 h of marinating than after 12 h of marinating. Also, the lowest total PAHs were shown in duck drumstick, which equaled 14.4 and 17.8 ng/g after 12 and 24 h

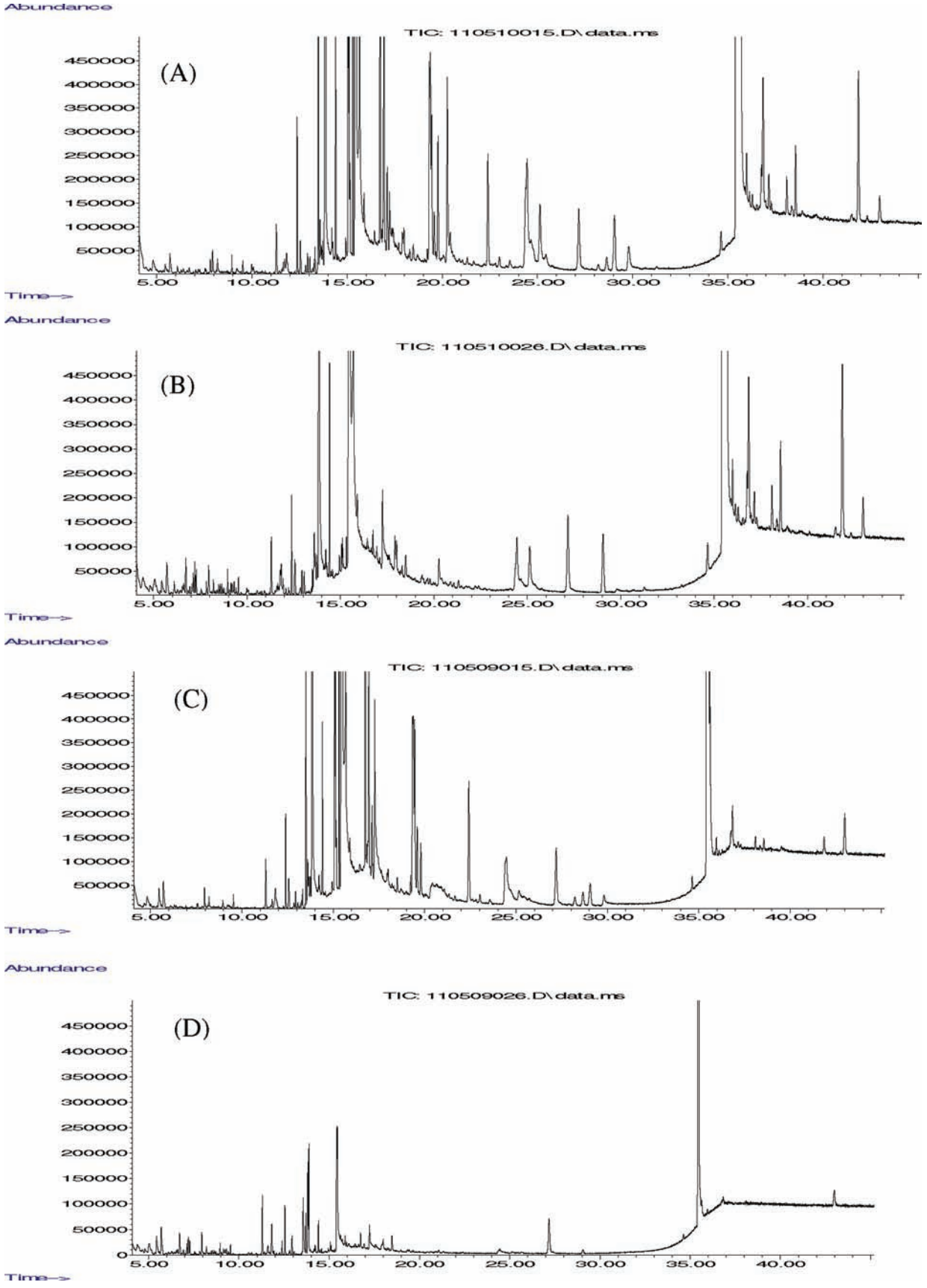

Figure 2. GC−MS chromatogram of PAHs with acetone as the extraction solvent (A) without and (B) with the QuEChERS method for purification or acetonitrile as the extraction solvent (C) without and (D) with the QuEChERS method for purification.

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Table 5. Recoveries of PAHs in Marinated Chicken Gizzard with Acetonitrile or Acetone as the Extraction Solvent with and without the QuEChERS Method for Purification[a]

	PAHs	acetonitrile		acetone	
		without QuEChERS	with QuEChERS	without QuEChERS	with QuEChERS
1	naphthalene	113.2 ± 10.5	122.1 ± 0.8	107.7 ± 12.1	49.5 ± 0.3
2	acenaphthylene	100.7 ± 1.4	99.1 ± 1.2	88.9 ± 0.3	92.6 ± 5.6
3	acenaphthene	99.8 ± 1.0	97.2 ± 0.1	91.8 ± 1.3	98.9 ± 4.6
4	fluorene	104.5 ± 0.9	109.7 ± 0.7	107.6 ± 3.2	107.7 ± 4.3
5	phenanthrene	113.6 ± 4.0	126.1 ± 7.8	122.0 ± 2.1	123.2 ± 0.8
6	anthracene	97.7 ± 0.7	95.9 ± 0.8	90.5 ± 1.8	100.2 ± 5.1
7	fluoranthene	95.8 ± 1.0	92.3 ± 0.7	78.4 ± 0.4	99.4 ± 3.8
8	pyrene	91.7 ± 1.9	89.9 ± 0.6	76.8 ± 2.7	86.3 ± 6.2
9	benzo[*a*]anthracene	87.3 ± 0.7	86.5 ± 1.5	90.0 ± 2.0	98.3 ± 3.9
10	chrysene	85.3 ± 1.1	85.0 ± 0.5	88.0 ± 1.0	98.6 ± 3.8
11	benzo[*b*]fluoranthene	90.4 ± 1.7	94.8 ± 3.5	76.9 ± 1.2	79.2 ± 3.2
12	benzo[*k*]fluoranthene	79.8 ± 0.1	74.2 ± 0.9	88.1 ± 1.7	93.0 ± 1.7
13	benzo[*a*]pyrene	84.2 ± 0.9	77.4 ± 3.9	87.9 ± 0.8	88.4 ± 2.0
14	indeno[1,2,3-*c*,*d*]pyrene	75.1 ± 0.3	70.6 ± 4.8	82.4 ± 0.6	88.1 ± 1.5
15	dibenzo[*a*,*h*]anthracene	76.4 ± 0.4	83.8 ± 1.1	89.5 ± 4.4	93.8 ± 3.5
16	benzo[*g*,*h*,*i*]perylene	75.2 ± 3.2	71.7 ± 4.2	82.3 ± 0.3	84.4 ± 1.2

[a]Average of duplicate analyses ± standard deviation.

of marinating, respectively. In contrast, a high amount of total PAHs was present in chicken guts, especially chicken gizzard (124.5 ng/g) and chicken heart (77.5 ng/g), after prolonged marinating for 24 h (Table 6), which may be due to the fat-soluble nature of PAHs. A similar outcome was observed by Chen et al.,[21] reporting a larger level of total PAHs to be accumulated in chicken liver than in the other organs of the chicken. Likewise, both Donn and Fee[22] and Lawrence and Weber[23] pointed out that PAHs were more susceptible to accumulation in digestive organs of chicken, which was in agreement with our finding. Among the various PAHs in chicken and duck meat, naphthalene was generated at a greater amount than the other PAHs, whereas seven PAHs, namely, benzo[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, indeno[1,2,3-*c*,*d*]pyrene, and benzo[*g*,*h*,*i*]perylene remained undetected over a 24 h marinating period. It has been well-documented that naphthalene is more prone to formation than the other PAHs as a result of the presence of two benzene rings, because the lipid oxidation degradation products, such as cyclohexene, may be oxidized to form benzene during heating, which, in turn, reacts with the C4 compound for naphthalene generation.[1] This finding is similar to that reported by Chen et al.,[21] demonstrating naphthalene to be the major PAH detected in several commercial meat products, including stewed chicken liver, stewed chicken wing, grilled duck, grilled chicken, and stewed pork stomach.

A similar tendency was observed for PAH formation in poultry meat as affected by frying, with the total PAHs being higher in chicken heart than in chicken breast and chicken gizzard after 10 min of frying (Table 7). In comparison to chicken drumstick fried for 12 min, a larger amount of total PAHs was found in duck drumstick fried for 15 min, which may be caused by the extensive frying time of the latter. However, this phenomenon was not observed during 20 and 30 min of frying of chicken drumstick and duck drumstick, respectively. Interestingly, there was no significant difference ($p > 0.05$) in naphthalene and total PAH levels in duck drumstick between 15 and 30 min of frying, as well as chicken gizzard between 2 and 10 min of frying. The difference in meat commodity and frying time length may account for this effect. Two frying times were selected, with the first denoting the time length required for meat to be edible and the second denoting the time length required for meat to be overcooked but still acceptable. Similar to marinated meat products, naphthalene was produced at a larger amount than the other PAHs during frying, while eight PAHs, including benzo[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, indeno[1,2,3-*c*,*d*]pyrene, dibenzo[*a*,*h*]anthracene, and benzo[*g*,*h*,*i*]perylene, remained undetected. Surprisingly, benzo[*a*]pyrene, the major carcinogenic PAH used as an indicator in food products, was not detected in marinated and fried poultry meat. Because the benzo[*a*]pyrene formation can be dependent upon the cooking condition and meat maturity,[24,25] the absence of benzo[*a*]pyrene in marinated and fried poultry meat may be associated with less smoke formation during heating. According to literature reports, most studies are focused on benzo[*a*]pyrene determination in smoked and charcoal-grilled meat products because the production of smoke during heating may play a critical role for benzo[*a*]pyrene generation.[25,26] As pointed out by Kazerouni et al.,[25] grilling could induce a much larger amount of benzo[*a*]pyrene in meat products than oven-broiling and pan-frying, probably caused by oil dripping onto charcoal for smoke production, resulting in the adhesion of PAHs in the smoke onto the meat surface. In addition, benzo[*a*]pyrene containing five benzene rings may not be formed when the temperature of wood pyrolysis in a smoke generator was below 425 °C and when the temperature of oxidation of volatile products of pyrolysis was below 375 °C.[27] In a study dealing with the effects of frying, grilling, roasting, and boiling on PAH formation in meat products, Perello et al.[28] reported that phenanthrene, naphthalene, fluoranthene, and pyrene were the major PAHs formed, with roasting generating a high amount of total PAHs in hake (19.26 μg/kg) and chicken meat (27.93 μg/kg) and frying producing a large level of total PAHs (16.91−35.42 μg/kg) in sardine, tuna, veal steak, pork loin meat, and lamb. In comparison, in our experiment, marinating could induce a higher amount of total PAHs in chicken heart, chicken drumstick, and chicken gizzard than

Table 6. Concentrations of PAHs (ng/g)[a] in Poultry Meat Products during Marinating

		chicken heart		chicken drumstick		chicken gizzard		chicken breast		duck drumstick	
	PAHs	12 h	24 h	12 h	24 h	12 h	24 h	12 h	24 h	12 h	24 h
1	naphthalene	36.7 ± 1.8 Aa	47.5 ± 4.9 Aa	39.9 ± 3.7 Aa	42.8 ± 2.5 Aa	48.5 ± 2.7 Aa	90.5 ± 13.8 Aa	30.5 ± 1.8 Ba	39.4 ± 0.3 Aa	4.9 ± 0.3 Ab	6.1 ± 0.5 Aa
2	acenaphthylene	3.9 ± 0.0 Bc	4.4 ± 0.0 Ac	3.1 ± 0.3 Abc	3.1 ± 0.1 Ac	3.6 ± 0.0 Bc	5.6 ± 0.5 Ab	2.3 ± 0.0 Acd	2.4 ± 0.3 Ac	1.3 ± 0.1 Bcd	1.6 ± 0.0 Abc
3	acenaphthene	1.2 ± 0.1 Ad	1.5 ± 0.1 Ac	1.0 ± 0.1 Ac	1.1 ± 0.0 Ac	0.7 ± 0.0 Bc	1.1 ± 0.1 Ab	0.7 ± 0.0 Acd	0.8 ± 0.1 Ad	0.5 ± 0.0 Ade	0.5 ± 0.0 Abc
4	fluorene	4.1 ± 0.0 Ac	6.6 ± 1.3 Ac	2.8 ± 0.3 Abc	3.2 ± 0.0 Ac	2.5 ± 0.2 Ac	4.3 ± 0.4 Ab	2.7 ± 0.1 Ac	3.0 ± 0.0 Ac	1.7 ± 0.0 Ac	1.9 ± 0.6 Ab
5	phenanthrene	9.4 ± 0.0 Ab	14.2 ± 0.4 Ab	7.3 ± 0.7 Ab	7.7 ± 0.4 Ab	8.8 ± 1.1 Bb	15.4 ± 1.2 Ab	6.6 ± 0.3 Bb	9.7 ± 0.5 Ab	5.6 ± 0.7 Aa	6.2 ± 0.3 Aa
6	anthracene	0.6 ± 0.2 Ad	0.8 ± 0.0 Ac	0.4 ± 0.1 Ac	0.4 ± 0.0 Ac	0.5 ± 0.0 Bc	0.8 ± 0.0 Ab	0.5 ± 0.1 Acd	0.5 ± 0.0 Ad	0.4 ± 0.0 Ae	0.4 ± 0.0 Ac
7	fluoranthene	0.3 ± 0.0 Bd	0.6 ± 0.0 Ac	0.2 ± 0.0 Ac	0.2 ± 0.0 Ac	0.5 ± 0.0 Bc	0.7 ± 0.0 Ab	nd[b]	0.6 ± 0.3 d	nd	0.3 ± 0.1 c
8	pyrene	0.9 ± 0.1 Ad	1.9 ± 0.3 Ac	0.1 ± 0.1 Ac	0.2 ± 0.0 Ac	0.2 ± 0.0 Bc	6.0 ± 0.1 Ab	0.1 ± 0.0 Ad	0.3 ± 0.2 Ad	nd	0.8 ± 0.0 bc
9	benzo[*a*]anthracene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
10	chrysene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
11	benzo[*b*]fluoranthene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
12	benzo[*k*]fluoranthene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
13	benzo[*a*]pyrene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
14	indeno[1,2,3-*c*,*d*] pyrene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
15	dibenzo[*a*,*h*] anthracene	2.0 ± 0.1 cd	nd	nd	nd	nd	nd	nd	nd	nd	nd
16	benzo[*g*,*h*,*i*]perylene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	total	59.1 ± 1.8 A	77.5 ± 6.8 A	54.8 ± 5.4 A	58.7 ± 3.1 A	65.2 ± 4.0 A	124.5 ± 16.1 A	43.4 ± 2.4 B	56.7 ± 1.7 A	14.4 ± 1.2 A	17.8 ± 1.7 A

[a]Mean of duplicate analyses ± standard deviation. Symbols bearing different captial letters (A and B) within each poultry meat commodity in the same row are significantly different ($p < 0.05$). Symbols bearing different lowercase letters (a−e) in the same column are significantly different ($p < 0.05$). [b]nd = not detected, below the LOQ.

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Table 7. Concentrations of PAHs (ng/g)[a] in Poultry Meat Products during Frying

		chicken heart		chicken drumstick		chicken gizzard		chicken breast		duck drumstick	
	PAHs	4 min	10 min	12 min	20 min	2 min	10 min	5 min	10 min	15 min	30 min
1	naphthalene	29.0 ± 0.0 Ba	56.4 ± 0.3 Aa	33.5 ± 3.7 Aa	48.8 ± 2.5 Aa	43.7 ± 2.3 Aa	30.9 ± 2.3 Aa	35.8 ± 0.0 Ba	42.4 ± 0.3 Aa	70.9 ± 10.4 Aa	48.0 ± 1.9 Aa
2	acenaphthylene	6.3 ± 0.4 Ab	7.8 ± 0.1 Ab	7.4 ± 0.3 Ab	7.1 ± 0.1 Ab	5.4 ± 0.6 Ab	7.0 ± 0.6 Bb	7.0 ± 0.1 Ab	7.8 ± 0.3 Ab	6.8 ± 0.3 Ab	6.7 ± 0.0 Ab
3	acenaphthene	0.4 ± 0.0 Bc	0.5 ± 0.0 Ae	0.4 ± 0.1 Ac	0.5 ± 0.0 Ac	0.4 ± 0.0 Ad	0.4 ± 0.1 Ac	0.4 ± 0.0 Ade	0.4 ± 0.1 Ad	0.6 ± 0.0 Ab	0.3 ± 0.0 Bc
4	fluorene	0.6 ± 0.0 Bc	1.4 ± 0.1 Ad	0.4 ± 0.3 Ac	0.6 ± 0.0 Ac	0.6 ± 0.2 Ac	0.6 ± 0.2 Ac	0.6 ± 0.1 Ad	0.7 ± 0.0 Ad	nd[b]	nd
5	phenanthrene	0.7 ± 0.2 Bc	2.2 ± 0.1 Ac	1.0 ± 0.7 Ac	1.9 ± 0.4 Ac	0.9 ± 0.1 Ac	1.9 ± 0.1 Bc	1.6 ± 0.0 Ac	2.2 ± 0.5 Ac	1.4 ± 0.0 Ab	0.6 ± 0.0 Bc
6	anthracene	0.3 ± 0.0 Ac	0.6 ± 0.1 Ae	nd	nd	nd	nd	nd	0.4 ± 0.0 d	nd	0.4 ± 0.0 c
7	fluoranthene	nd	0.2 ± 0.0 e	nd	0.3 ± 0.0 c	0.2 ± 0.0 d	nd	0.2 ± 0.0 Ae	0.2 ± 0.0 Ad	nd	nd
8	pyrene	nd	nd	0.1 ± 0.1 Ac	0.3 ± 0.0 Ac	nd	nd	0.2 ± 0.1 Ae	0.3 ± 0.0 Ad	nd	0.1 ± 0.0 c
9	benzo[*a*]anthracene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
10	chrysene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
11	benzo[*b*]fluoranthene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
12	benzo[*k*]fluoranthene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
13	benzo[*a*]pyrene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
14	indeno[1,2,3-*c*,*d*]pyrene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
15	dibenzo[*a*,*h*]anthracene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
16	benzo[*g*,*h*,*i*]perylene	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
	total	37.1 ± 0.6 B	69.1 ± 0.8 A	42.8 ± 0.5 B	59.5 ± 0.5 A	51.2 ± 3.1 A	40.9 ± 2.7 A	45.8 ± 0.5 B	54.5 ± 0.8 A	79.7 ± 12.8 A	56.1 ± 2.3 A

[a]Mean of duplicate analyses ± standard deviation. Symbols bearing different captial letters (A and B) within each poultry meat commodity in the same row are significantly different ($p < 0.05$). Symbols bearing different lowercase letters (a–e) in the same column are significantly different ($p < 0.05$). [b]nd = not detected, below the LOQ.

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frying, which may be due to the extensive heating time of the former.

In conclusion, an improved analytical method for PAH determination in poultry meat was developed by mixing 5 g of meat sample with 10 mL of deionized water in a centrifuged tube, followed by adding 10 mL of acetonitrile for extraction for 1 min, adding the QuEChERS method for further extraction for 1 min, purifying in a centrifuged tube containing the QuEChERS method, and injecting into GC−MS for separation and quantitation of 16 PAHs. Naphthalene was generated at a much larger amount than the other PAHs during marinating and frying. The longer the marinating or frying time, the greater the formation of PAHs. With the exception of chicken breast and duck drumstick, marinating could produce a higher level of total PAHs than those in chicken heart, chicken drumstick, and chicken gizzard during frying.

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REFERENCES

(1) Chen, B. H.; Chen, Y. C. Formation of polycyclic aromatic hydrocarbons in the smoke from heated model lipids and food lipids. *J. Agric. Food Chem.* **2001**, *49*, 5238−5243.

(2) Alexandrov, K.; Rojas, M.; Satarug, S. The critical DNA damage by benzo(*a*)pyrene in lung tissues of smokers and approaches to preventing its formation. *Toxicol. Lett.* **2010**, *198*, 63−68.

(3) International Agency for Research on Cancer (IARC). Formaldehyde, 2-butoxyethanol and 1-*tert*-butoxypropan-2-ol. *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*; IARC: Lyon, France, 2006; Vol. *88*.

(4) United States Environmental Protection Agency (U.S. EPA). *Appendix A to 40 CFR Part 423*; U.S. EPA: Washington, D.C., 2010; http://www.epa.gov/waterscience/methods/pollutants.htm.

(5) Pan, H.; Cao, Y. Optimization of pretreatment procedures for analysis of polycyclic aromatic hydrocarbons in charcoal-grilled pork. *Anal. Lett.* **2010**, *43*, 97−109.

(6) Bianchi, F.; Careri, M.; Mangia, A.; Mattarozzi, M.; Musci, M. Experimental design for the optimization of the extraction conditions of polycyclic aromatic hydrocarbons in milk with a novel diethoxydiphenylsilane solid-phase microextraction fiber. *J. Chromatogr., A* **2008**, *1996*−*1997*, 41−45.

(7) Ratola, N.; Alves, A.; Kalogerakis, N.; Psillakis, E. Hollow-fibre liquid-phase microextraction: A simple and fast cleanup step used for PAHs determination in pine needles. *Anal. Chim. Acta* **2008**, *618*, 70−78.

(8) Lage Yusty, M. A.; Cortizo Davina, J. L. Supercritical fluid extraction and high-performance liquid chromatography−fluorescence detection method for polycyclic aromatic hydrocarbons investigation in vegetable oil. *Food Control* **2005**, *16*, 59−64.

(9) Purcaro, G.; Moret, S.; Conte, L. S. Optimisation of microwave assisted extraction (MAE) for polycyclic aromatic hydrocarbon (PAH) determination in smoked meat. *Meat Sci.* **2009**, *81*, 275−280.

(10) Sobhanzadeh, E.; Bakar, N. K. A.; Abas, M. R.; Nemati, K. Sample preparation methods for pesticides analysis in food matrices and environmental samples by chromatography-based techniques: A review. *J. Fundam. Sci.* **2009**, *5*, 106−128.

(11) Wenzl, T.; Simon, R.; Kleiner, J.; Anklam, E. Analytical methods for polycyclic aromatic hydrocarbons (PAHs) in food and the environment needed for new food legislation in the European Union. *Trends Anal. Chem.* **2006**, *25*, 716−725.

(12) Stumpe-Viksna, I.; Bartkevics, V.; Kukare, A.; Morozovs, A. Polycyclic aromatic hydrocarbons in meat smoked with different types of wood. *Food Chem.* **2008**, *110*, 794−797.

(13) Codex Alimentarius Commission. *Joint FAO/WHO Food Standards Programme Codex Committee on Food Additives and Contaminants. Discussion Paper on Polycyclic Aromatic Hydrocarbons (PAH) Contamination*; Codex Alimentarius Commission: Rome, Italy, 2005; CX/FAC 05/37/34.

(14) Codex Alimentarius Commission. *Code of Practice for the Reduction of Contamination of Food with Polycyclic Aromatic Hydrocarbons (PAH) from Smoking and Direct Drying Processes*; Codex Alimentarius Commission: Rome, Italy, 2009; CAC/RCP 68/2009.

(15) Lee, H. W.; Chien, J. T.; Chen, B. H. Formation of cholesterol oxidation products in marinated foods during heating. *J. Agric. Food Chem.* **2006**, *54*, 4873−4879.

(16) SAS. *SAS Procedures and SAS/Graph User's Guide, Version 6*; SAS Institute, Inc., Cary, NC, 2010.

(17) Bordajandi, L. R.; Dabrio, M.; Ulberth, F.; Emons, H. Optimisation of the GC−MS conditions for the determination of the 15 EU foodstuff priority polycyclic aromatic hydrocarbons. *J. Sep. Sci.* **2008**, *31*, 1769−1778.

(18) Gratz, S.; Mohrhaus, A.; Gamble, B.; Gracie, J.; Jackson, D.; Roetting, J.; Ciolino, L.; McCauley, H.; Schneider, G.; Crockett, D.; Krol, W.; Terri, A.; White, J.; Flottmeyer, M.; Johnson, Y.; Heitkemper, D.; Fricke, F. *Screen for the Presence of Polycyclic Aromatic Hydrocarbons in Select Seafoods Using LC−Fluorescence*; Forensic Chemistry Center (FCC), Food and Drug Administration (FDA): Cincinnati, OH, 2010; FDA/ORA/DFS 4475.

(19) Ramalhosa, M. J.; Paiga, P.; Moraia, S.; Delerus-Matos, C.; Oliveire, M. B. P. P. Analysis of polycyclic aromatic hydrocarbons in fish: Evaluation of a quick, easy, cheap, effective, rugged, and safe extraction method. *J. Sep. Sci.* **2009**, *32*, 3529−3538.

(20) Castro, D.; Slezakova, K.; Oliva-Teles, M. T.; Delerue-Matos, C.; Alvim-Ferraz, M. C.; Morais, S.; Pereira, J. Analysis of polycyclic aromatic hydrocarbons in atmospheric particulate samples by microwave-assisted extraction and liquid chromatography. *J. Sep. Sci.* **2009**, *32*, 501−510.

(21) Chen, B. H.; Wang, C. Y.; Chiu, C. P. Evaluation of analysis of polycyclic aromatic hydrocarbons in meat products by liquid chromatography. *J. Agric. Food Chem.* **1996**, *44*, 2244−2251.

(22) Donn, B. P.; Fee, J. Polycyclic aromatic hydrocarbon carcinogens in commercial seafoods. *Can. J. Fish. Aquat. Sci.* **1979**, *36*, 1469−1476.

(23) Lawrence, J. F.; Weber, D. F. Determination of polycyclic aromatic hydrocarbons in Canadian samples of processed vegetable and dairy products by liquid chromatography with fluorescence detection. *J. Agric. Food Chem.* **1984**, *32*, 794−797.

(24) Larsson, B. K.; Sahlberg, G. P.; Eriksson, A. T.; Busk, L. A. Polycyclic aromatic hydrocarbons in grilled food. *J. Agric. Food Chem.* **1983**, *31*, 867−873.

(25) Kazerouni, N.; Sinha, R.; Hsu, C. H.; Greenberg, A.; Rothman, N. Analysis of 200 food items for benzo[*a*]pyrene and estimation of its intake in an epidemiologic study. *Food Chem. Toxicol.* **2001**, *39*, 423−436.

(26) Phillips, D. H. Polycyclic aromatic hydrocarbons in the diet. *Mutat. Res.* **1999**, *443*, 139−147.

(27) Stolyhwo, A.; Sikorski, Z. E. Polycyclic aromatic hydrocarbons in smoked fish—A critical review. *Food Chem.* **2005**, *91*, 303−311.

(28) Perello, G.; Marti-Cid, R.; Castell, V.; Llobet, J. M.; Domingo, J. L. Concentrations of polybrominated diphenyl ethers, hexachlorobenzene and polycyclic aromatic hydrocarbons in various foodstuffs before and after cooking. *Food Chem. Toxicol.* **2009**, *47*, 709−715.