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Cover Page Footnote

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ORIGINAL ARTICLE

Optimization of QuEChERS and high performance liquid chromatography-fluorescence detection conditions to assess the impact of preparation procedures on EU priority PAHs in coffee samples and their PAHs consumption risk

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Abstract

The good performance conditions for determination of EU priority PAHs in coffee samples were established to evaluate the effects of roasting degree on the PAHs in coffee beans and the brewing methods on the PAHs transfer from coffee beans to their brews. The consumption risk of the PAHs in coffee products was also assessed. The PAHs levels of the roasted coffee beans were in the order: 923.65 ng/g (dark roast) > 132.20 ng/g (medium roast) > 69.28 ng/g (light roast). Compared with general brewing with the drip bag (PAHs content, 0.30−0.62 ng/mL in coffee brews), the coffee machine brewing (set at 4 bar) induced higher PAHs release into coffee brews (PAHs content, 0.36−2.14 ng/g). The PAHs amounts of the commercial brewed and canned coffee products were 0.32−1.23 ng/g and 0.16−0.46 ng/g, respectively. The consumption risk of the PAHs in the coffee brews and products is a low level of concern.

Keywords: Analysis, Brewing, Risk assessment, Roasting, Polycyclic aromatic hydrocarbons (PAHs)

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are carcinogenic and mutagenic and can be produced during food processing [1]. European Food Safety Authority (EFSA) proposed the EU priority 15 + 1 PAHs based on 15 PAHs recommended by the Scientific Committee for Food (SCF) and the benzo[c]fluorene (BcL) recommended by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) [2]. EFSA [2] reported that the contents of benzo[a]pyrene (BaP), PAH 2 (BaP + chrysene (CHR)), PAH 4 (PAH 2 + benz[a]anthracene (BaA) + benzo[b]fluoranthene (BbF)) and PAH 8 (PAH 4 + benzo[k]fluoranthene (BkF) + benzo[g,h,i]perylene (BgP) + dibenz[a,h]antracene (DhA) + indeno [1−3]-cdpyrene (IcP)) of coffee powder were relatively high among all surveyed foods.

The purpose of roasting coffee beans is to change the physical and chemical properties of green beans to facilitate extraction and increase flavor; it is one of the important procedures for preparing coffee.

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Coffee roasting involves an intense thermal process that can be applied either directly (roasting with flame, gas oven, or coal) or indirectly (roasting with electric oven). As the degree of roasting increases, the color of the coffee beans becomes darker. The degree of roasting creates different flavors of coffee beans. However, the roasting process can lead to the formation of PAHs in coffee beans [3]. Coffee is very popular in Taiwan. Brewing with the drip bag and brewing by the coffee machine are the most commonly used ways for Taiwanese people to prepare coffee brews. However, there is no thorough report on the composition and content of the PAHs in coffee samples and the consumption risk of the PAHs in coffee products in Taiwan.

Food matrices are complex and the amounts of toxic compounds in food are low. Matrix interference makes detection of these compounds difficult. A good sample preparation procedure is essential for quantitative accuracy [4]. QuEChERS (quick, easy, cheap, effective, rugged and safe) technology can simplify and shorten the extraction process of organic compounds, improve extraction recovery, accuracy and precision, and reduce solvent use and waste generation [5,6]. This technology has been used to extract PAHs from food in recent years [7,8].

For the analysis of PAHs in food in previous studies, high performance liquid chromatography coupled with fluorescence detector (HPLC-FLD) was mostly used to analyze the EU priority PAHs [8−11] and gas chromatography coupled with mass spectrometry (GC−MS) was mostly used to analyze the US Environmental Protection Agency (EPA) PAHs [7,12−14].

In this study, the appropriate determination conditions of the EU priority PAHs in coffee samples were established with QuEChERS extraction and HPLC-FLD (temperature controllable) analysis. Moreover, the effect of roasting degree on the PAHs in coffee beans (light, medium and dark roast), the effect of different brewing methods (drip bag brewing (atmospheric pressure) and coffee machine brewing (relative high pressure, 4 bar)) on the PAHs transfer from coffee beans to their brews, and the levels of the PAHs in commercial coffee products were studied. The consumption risk of the PAHs from coffee drinking was also assessed.

2. Materials and methods

2.1. Chemicals

Solvents (HPLC grade) including acetonitrile (ACN), acetone, acetic acid (CH_3COOH) and tetrahydrofuran (THF) were purchased from Merck Co. (Darmstadt, Germany). The standards of the EU priority PAHs: BaA, BaP, BbF, BgP, BkF, CHR, DhA, IcP, benzo[j]fluoranthene (BjF), cyclopenta[cd]pyrene (CPP), dibenzo[a,e]pyrene (DeP), dibenzo[a,h]pyrene (DhP), dibenzo[a,i]pyrene (DiP), dibenzo[a,l]pyrene (DlP), 5-methylchrysene (5-MC) and benzo[c]fluorene (BcL) were purchased from Restek Co. (Bellefonte, PA, U.S.A.); their purity is greater than 99%. The QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) kits (Dikma ProElut QuEChERS) were obtained from Dikma Technologies Co. (Dikma Technologies Inc., Lake Forest, CA, USA). Distilled deionized water (dd H_2O) was prepared through Milli-DI® Water Purification System (Merck Co., Darmstadt, Germany).

2.2. Analysis of the EU priority PAHs

The Shimadzu HPLC system (Shimadzu Co., Kyoto, Japan) for analysis of the EU priority PAHs was equipped with an HPLC pump system (LC-20AT), a controller (SCL-20A), an auto-sampler (SIL-10A), an on-line degasser (DGU-12A) and a new type temperature-controllable fluorescent detector (FLD) (RF-20AXS). A photodiode array (PDA) detector (S-3210) (Schambeck SFD GmbH, Bad Honnef, Germany) was used to aid in the measurement of PAHs. A Pinnacle II PAH column (150 mm × 3.0 mm ID, 4 μm) and a Pinnacle II PAH Guard cartridge (Restek Co., Bellefonte, PA, USA) kept at 35 °C was used to separate the PAHs. The mobile phase was composed of dd H_2O (A) and ACN (4% THF) (B) (gradient condition, 70% B from 0.00 to 3.10 min, 70−85% B from 3.10 to 13.09 min, 85−90% from 13.10 to 15.10 min, 90−100% from 15.11 to 19.20 min; flow rate, 1.4 mL/min from 0.00 to 13.09 min and 2.0 mL/min from 13.10 to 19.20 min; injection volume, 10 μL). The FLD was set at 20 °C and the excitation (Ex)/emission (Em) wavelengths were set as follows: 304 nm/353 nm from 0 to 3.29 min (BcL), 273 nm/384 nm from 3.30 to 5.39 min (BaA, CHR and 5-MC), 312 nm/507 nm from 5.40 to 5.79 min (BjF), 302 nm/452 nm from 5.80 to 6.64 min (BbF), 305 nm/430 nm from 6.65 to 7.59 min (BkF), 290 nm/410 nm from 7.60 to 9.19 min (BaP), 300 nm/420 nm from 9.20 to 10.84 min (DlP, DhA, BgP), 300 nm/500 nm from 10.85 to 12.14 min (IcP), 297 nm/403 nm from 12.15 to 14.99 min (DeP), 292 nm/440 nm from 15.00 to 17.90 min (DiP), and 304 nm/457 nm from 17.91 to 19.20 min (DhP).

2.3. Roasting process of coffee beans, preparation of coffee brews and commercial coffee products

Gukeng (Yunlin, Taiwan) is a famous coffee cultivation area in Taiwan. The coffee beans (*Coffea*

Arabica) used in this investigation were gathered from coffee trees and dried in the sun. The peeled green coffee beans were stirred and roasted in a roasting pot to obtain three different roast levels of coffee beans (light roast, medium roast and dark roast). The different roast levels of coffee beans were referred to depending on the roasting temperature. During the roasting process, the beans change color from light green/yellow to brown and the development of the flavour. When the temperature reaches 200 °C, the first crack occurs, and the bean changes from a green/yellow color to a light brown color (light roast); when the temperature rises to 215 °C (the end of the first crack), the heat causes a chemical change inside the coffee beans, turning the color to dark brown (medium roast); when the temperature exceeds 230 °C, the second crack occurs intensively, and the beans are dark brown in color with an oily sheen (dark roast). The roasted coffee beans were ground with a grinder. The liquid coffee samples were prepared by brewing of each ground coffee beans (5 g) with 85 mL of 95 °C hot water using a drip bag (atmospheric pressure) and a coffee machine (set at 4 bar), respectively. These two coffee brewing methods are the most commonly used by Taiwanese people. In addition, ready-to-drink brewed and canned coffee products (pure coffee) commercially available in Taiwan were also collected. The coffee products are popular and well-known brands, including Starbucks, 85 °C, City Café, McCafé, Mr. Brown Coffee, UCC, Bernachon, etc. Three products were randomly selected for each brand. The PAHs in each product were determined in triplicate.

2.4. Extraction of the PAHs

The PAHs in each sample were extracted with the QuEChERS kit. For ground coffee beans, 1 g of the sample in a centrifuge tube was mixed with 5 mL of dd H_2O and a ceramic stone, then shaken for 1 min. After adding 5 mL of acetone, the mixture was shaken for 1 min. For liquid coffee, 10 g of the sample in a centrifuge tube was mixed with ceramic stone, then shaken for 1 min. After adding 5 mL of acetone (1% acetic acid), the mixture was shaken for 1 min. Immediately, each mixture (ground coffee bean or liquid coffee) was placed into a QuEChERS column (4 g of magnesium sulfate ($MgSO_4$) and 1 g of sodium acetate). After shaking for 1 min and centrifuging at 3000×*g* at 4 °C for 5 min, 3 mL of the supernatant was transferred into a QuEChERS clean-up column (900 mg of $MgSO_4$, 300 mg of endcapped octadecylsilane (C_{18}) silica gel particles and 300 mg of primary secondary amine (PSA)). After shaking for 1 min and centrifuging at 3000×*g* at 4 °C for 5 min, 1 mL of the supernatant was taken out for the PAHs analysis.

2.5. Method validation

The validation of the developed extraction conditions were performed with reference to the guidelines of International Union of Pure and Applied Chemistry (IUPAC) and Association of Official Analytical Communities (AOAC) for laboratory validation of methods of analysis; the parameters for limit of detection (LOD), limit of quantitation (LOQ), linearity, specificity, recovery and precision were measured [15,16]. The specificity of PAHs was estimated by comparing the blank sample to its corresponding sample spiked with the standards of PAHs. LOD and LOQ are 3 and 10 times the signal-to-noise ratio (S/N), respectively. Regression analysis was used to assess linearity. The establishment of each PAH calibration curve was carried out by plotting average peak area against injection level. The recoveries (%) of PAHs were evaluated through spiking the standard of PAHs (0.5, 1 and 2 ng/g (ground coffee bean) or (1, 5 and 10 ng/g for coffee brew)) into blank samples; intra- and inter assays were performed with five replicate tests and the values of coefficient of variation (%) (CV%) were counted.

The EU Regulation (Commission Regulation (EU) No 836/2011) [17] indicates that the performance criteria for the analytical methods of PAH 4 should be free from matrix (or spectral interferences) and verification of positive detection. The green coffee beans in the Taiwan market are mainly imported from countries such as Brazil, Indonesia, Colombia, Ethiopia, Guatemala and Nicaragua, mainly Arabica varieties [18]. Trace amounts of PAHs could be detected in general commercial green coffee beans in Taiwan (data not shown). PAHs were not detected in the sun-dried green coffee beans collected in Gukeng (Yunlin, Taiwan), and the bean background also did not interfere with the measurement of the PAHs (Fig. 1). The green coffee beans were used as a blank sample as well.

2.6. Health risk assessment

Food consumption data were collected form four sets of Nutrition and Health Survey in Taiwan (NAHSIT) (2005–2008, 2010, 2011, and 2012), covering all age groups (1 to over 65 (65^{+}) years old), with a valid number of 7580 (excluding specific ethnicities). The mean consumption levels of coffee for Whole group (WG) (n = 7580; average body

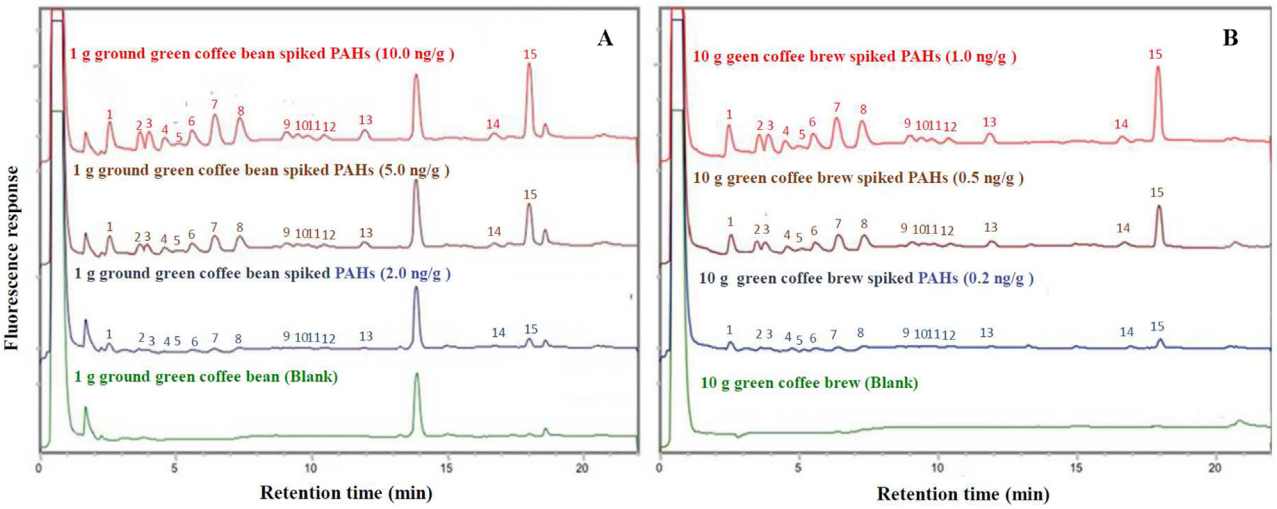

Fig. 1. Chromatograms of the extracts of coffee samples spiked with various levels of the EU priority PAHs. The analytical conditions were described in Section 2.2. (A) Coffee bean and (B) coffee brew. Peaks: 1: benzo[c]fluorene (BcL), 2: benzo[a]anthracene (BaA), 3: chrysene (CHR), 4: 5-methylchrysene (5-MC), 5: benzo[j]fluoranthene (BjF), 6: benzo[b]fluoranthene (BbF), 7: benzo[k]fluoranthene (BkF), 8: benzo[a]pyrene (BaP), 9: dibenzo[a,l]pyrene (DlP), 10: dibenzo[a,h]pyrene (DhA), 11: benzo[g,h,i]perylene (BgP), 12: indeno[c,d]pyrene (IcP), 13: dibenzo[a,e]pyrene (DeP), 14: Dibenzo[a,i]pyrene (DiP), 15: dibenzo[a,h]anthracene (DhP).

weight (BW), 51.59 kg) and Consumer only (CO) (n = 439; average BW, 60.42 kg) were 0.344 and 3.03 g/kg BW/day, respectively.

EFSA [2] reported that Exposure of margin (*MOE*) is suitable for use as a qualitative risk indicator to assess the trace level of genotoxic and carcinogenic substances in food. The equation for assessing the average daily dose of the hazardous substance (*j*) in food (*k*) ingested by an individual (*i*) was as follows:

$$MOE = \frac{BMDL_{j10}}{EDI_{i.j}} = \frac{BMDL_{j10}}{\sum_{k=1}^{n}\left[c_{k.j} \times \left(\frac{CR_k}{BW}\right)_i\right]}$$

Benchmark dose lower limit ($BMDL_{j10}$) (mg/kg BW/day): the lower bound of a 95% confidence interval on the benchmark dose (BMD) that corresponds to a 10% tumor incidence induced by the hazardous substance (*j*); $EDI_{i.j}$: the estimated average daily intake dose (mg/kg *BW*/day) of the hazardous substance (*j*) consumed by the individual (*i*). *n*: the amount of food (*k*) (g/day) consumed by the individual (*i*); $c_{k.j}$: the average concentration (mg/kg) of the hazardous substance (*j*) in the food (*k*); CR_k: the amount of food (*k*) (g/day) consumed by the individual (*i*); *BW*: the weight (kg) of the individual (*i*); *CR* and *BW* are from the same individual (*i*).

The $BMDL_{10}$ values for BaP, PAH 4 and PAH 8 were 0.07, 0.34 and 0.49 mg/kg BW/day, respectively [2].

2.7. Statistical analysis

The statistical analyses were performed by SAS statistical software, version 9.4 (SAS Institute Inc., Cary, NC, USA). The data were analyzed by analysis of variance (ANOVA) and the differences between the means of experimental results were assessed using Duncan's multiple range test. A p-value less than 0.05 ($p < 0.05$) is statistically significant.

3. Results and discussion

3.1. Establishment of appropriate conditions for analysis of EU priority PAHs in coffee samples

High performance liquid chromatography-fluorescence detection (HPLC-FLD) has high sensitivity, simple and easy operation, and reasonable operating cost. HPLC-FLD is suitable for routine analysis of the EU priority PAHs in samples. The HPLC condition presented good efficiency for the PAHs separation; the values of separation factor (α) and resolution (*Rs*) were all higher than 1 (Supplementary Table 1). The EU priority PAHs could be simultaneously determined in 18.1 min (Fig. 1 and Supplementary Table 1).

Food matrices are complex, and the use of QuEChERS technology in various types of food requires evaluation of its appropriate conditions [1]. In this work, QuEChERS technology was used to extract the PAHs in coffee samples including coffee bean and coffee brew. The extraction efficiencies for the spiked EU priority PAHs in blank ground coffee bean (1 ng/g) and blank coffee brew (1 ng/g) were assessed using six solvents: ACN, ACN acidified with 1% acetic acid (ACN (1% acetic acid)), acetone, acetone acidified with 1% acetic acid (acetone (1%

acetic acid)), ACN/acetone (1:1, v/v) and ACN/acetone (1:1, v/v) acidified with 1% acetic acid (ACN/Acetone (1:1, v/v) (1% acetic acid)). For ground coffee bean, 1 g of sample in a centrifuge tube was mixed with 10 mL of dd H_2O and a ceramic stone and shaken for 1 min; then, 10 mL of each solvent was added and shaken for an additional 1 min. For liquid coffee, 10 mL of the sample in a centrifuge tube was mixed with ceramic stone and shaken for 1 min; then, 10 mL of each solvent was added and shaken for an additional 1 min. Each mixture was further processed with QuEChERS kit for analysis (described in *Section 2.4*).

According to EU regulation (Commission Regulation (EU) No 836/2011) [17], the determination criteria for PAH 4 in foodstuffs are as follows: LOD ≤0.3 μg/kg; LOQ ≤0.9 μg/kg; acceptable recovery (%), 50–120%. The validation specification of food chemical analytical methods issued by Taiwan Food and Drug Administration (TFDA) [19] indicates that the acceptable performance criteria (similar to AOAC requirements [20]) are as follows: recovery (%), 60–125% (>1-<10 ppb) and 50–125% (≤1 ppb); coefficient of variation (CV) % of the repeatability (intra-assay), ≤30% (>1-<10 ppb) and ≤35% (≤1 ppb); CV % of the intermediate precision (inter-assay), ≤32% (>1-<10 ppb) and ≤36% (≤1 ppb); the correlation coefficient (r) of the linear regression equation, > 0.99.

Table 1 shows that the recoveries (%) of the EU priority PAHs were the best for coffee bean extracted with acetone (62%–96%) and coffee brew extracted with acetone (1% acetic acid) (94%–105%). The recoveries of the PAHs could be further improved when 5 mL of water was added to 1 g of coffee bean and then extracted with 5 mL of acetone (72%–112%), and 10 g of coffee brew was extracted with 5 mL of acetone (97%–106%) (Table 2). The optimized conditions, compliant with EU and TFDA analytical specifications, were used to extract the EU priority PAHs in coffee samples.

Since cyclopenta[c,d]pyrene (CPP) has no fluorescence absorption, PDA was used to assist FLD for the PAHs measurement. For CCP (retention time, 3.57 min), its maximum absorption wavelength was 222 nm. However, CCP detected at 222 nm had much lower sensitivity (LOD >21 ng/g and LOQ >66 ng/g for coffee been, and LOD >20 ng/g and LOQ >65 ng/g for coffee brew) than other PAHs detected with FLD (the LOD and LOQ values of the PAHs were 0.002–0.10 ng/g and 0.006–0.35 ng/g for the coffee bean, and 0.003–0.06 ng/g and 0.009–0.20 ng/g for the coffee brew, respectively) (Table 4). The LOD and LOQ values of PAH 4 were 0.01–0.09 ng/g and 0.04–0.30 ng/g for the coffee bean, and 0.004–0.04 ng/g and 0.01–0.12 ng/g for the coffee brew, respectively, which meet the EU regulation (Commission Regulation (EU) No 836/2011) [17] for the determination of PAH 4 in foodstuffs.

Due to the poor detection sensitivity of CPP and the large gap with other PAHs, CPP was not included in the determination method development. Additionally, CPP in coffee samples may be too low to be detected at 222 nm. EFSA [2] illustrated that BaP is the most important marker of carcinogenic PAHs in foodstuffs. The maximum level of BaP in food is also regulated by the TFDA [21]. As assessed by EFSA [2], PAH 8 did not provide a statistically significant added value compared to PAH 4; therefore, maximum levels of BaP and sum of PAH 4 in foodstuffs are regulated by the European Commission (Regulation (EU) No 835/2011) [22]. CPP is not included in PAH 4 and PAH 8. Although CPP cannot be analyzed by HPLC-FLD, it should have less effect on the determination of PAHs in the samples.

Table 3 shows that the average recoveries of PAH 4 were 99–104% for green coffee beans (spiked 2, 5 and 10 ng/g of the PAHs) and 98–100% for green coffee brews (spiked 0.2, 0.5 and 1.0 ng/g of the PAHs), respectively. The recoveries of PAH 4 were in compliance with EU regulation (Commission Regulation (EU) No 836/2011). Moreover, the average recoveries of the PAHs were 82–104% for the green coffee beans and 95–100% for the green coffee brews, respectively. The recoveries of the PAHs in the coffee samples could also meet TFDA specifications [19]. It indicates that the established conditions for extraction of the EU priority PAHs from the coffee samples have good accuracy.

For intra- and inter-assays, the CV (%) (known as relative standard deviation (RSD) (%)) values of the recoveries of the spiked PAHs in the respective coffee samples were 2–11% and 8–20% for coffee bean, and 2–9% and 7–20% for coffee brew, respectively (Table 3). All values of CV (%) were in line with TFDA specifications [19], indicating that the established conditions have good repeatability and intermediate precision. Table 3 shows that the linear regression equations of each PAH for coffee bean and coffee brew also exhibit good linearity (r > 0.99).

Skoog et al. [23] stated that increasing temperature leads to a decrease in fluorescence intensity. Because of the temperature control unit, the RF-20Axs FLD is not affected by temperature fluctuations, and can maintain the optimum detection sensitivity and ensure the optimum reproducibility. Our results revealed that the FLD indeed has good sensitivity for the PAHs detection. The LOD and LOQ values of PAH 4 in coffee samples detected by

Table 1. The recoveries (%) of EU priority PAHs in coffee bean and coffee brew extracted with various solvents.

Sample	PAH	Recovery (%) (CV%)					
		ACN	ACN (1% acetic acid)	Acetone	Acetone (1% acetic acid)	ACN/Acetone (1:1, v/v)	ACN/Acetone (1:1, v/v) (1% acetic acid)
Coffee bean	BcL	82 (1)	73 (10)	62 (25)	68 (13)	89 (28)	109 (5)
	BaA[a,b]	99 (4)	40 (24)	96 (6)	72 (24)	90 (23)	93 (8)
	CHR[a,b]	59 (10)	44 (11)	91 (16)	66 (13)	91 (39)	67 (3)
	5-MC	79 (13)	46 (6)	62 (8)	36 (8)	83 (45)	39 (2)
	BjF	78 (14)	72 (20)	72 (10)	70 (9)	86 (16)	64 (5)
	BbF[a,b]	80 (12)	71 (8)	93 (2)	89 (6)	95 (36)	86 (10)
	BkF [b]	75 (13)	67 (3)	92 (3)	84 (1)	90 (34)	79 (4)
	BaP[a,b]	73 (10)	68 (1)	95 (4)	93 (4)	77 (8)	83 (3)
	DlP	67 (12)	58 (3)	82 (3)	73 (3)	85 (37)	70 (4)
	DhA[b]	52 (11)	48 (8)	76 (4)	67 (5)	59 (6)	60 (11)
	BgP [b]	53 (17)	45 (13)	75 (6)	65 (4)	72 (37)	58 (14)
	IcP [b]	61 (7)	54 (2)	89 (1)	70 (7)	81 (36)	70 (1)
	DeP	54 (9)	51 (2)	85 (4)	72 (4)	73 (21)	65 (6)
	DiP	49 (9)	49 (6)	87 (4)	74 (4)	66 (7)	66 (2)
	DhP	55 (6)	47 (3)	99 (3)	76 (12)	70 (2)	70 (2)
Coffee brew	BcL	82 (0)	81 (2)	91 (1)	96 (3)	86 (1)	87 (2)
	BaA[a,b]	85 (2)	86 (2)	99 (1)	105 (4)	93 (2)	94 (4)
	CHR[a,b]	81 (1)	82 (3)	97 (1)	103 (3)	91 (2)	90 (4)
	5-MC	77 (4)	75 (4)	93 (2)	100 (3)	88 (2)	88 (2)
	BjF	70 (10)	66 (3)	88 (2)	96 (8)	80 (1)	86 (6)
	BbF[a,b]	74 (2)	75 (2)	97 (1)	102 (5)	87 (1)	88 (4)
	BkF [b]	72 (2)	73 (2)	97 (1)	102 (3)	87 (1)	88 (2)
	BaP[a,b]	69 (2)	71 (2)	99 (1)	103 (2)	87 (1)	86 (2)
	DlP	69 (4)	70 (3)	96 (1)	100 (3)	85 (1)	86 (3)
	DhA[b]	55 (4)	57 (4)	90 (1)	95 (4)	76 (1)	78 (3)
	BgP [b]	59 (4)	61 (4)	92 (1)	97 (3)	78 (2)	79 (3)
	IcP [b]	57 (8)	62 (5)	97 (4)	96 (6)	79 (2)	83 (4)
	DeP	52 (3)	54 (3)	92 (1)	94 (3)	76 (1)	76 (3)
	DiP	47 (5)	49 (4)	103 (2)	102 (2)	81 (1)	78 (3)
	DhP	47 (5)	50 (4)	103 (3)	99 (2)	80 (2)	77 (3)

The recoveries (%) of the PAHs were calculated through five replicate tests and expressed as mean (coefficient of variation %, CV%). .

[a] PAH 4 = BaA + CHR + BbF + BaP.

[b] PAH 8 = PAH 4 + BkF + DhA + BgP + IcP.

the FLD were well below the criteria of PAH 4 measurement of EU regulation (Commission Regulation (EU) No 836/2011) [17].

Regarding the determination of PAHs in coffee samples, different methods are used to extract PAHs (mostly traditional methods) and then analyze by FLD or GC−MS. Tfouni et al. [3] extracted PAH 4 in coffee brew samples through a mixture of cyclohexane and N, N-dimethylformamide/water (9/1, V/V) for liquid−liquid extraction and a silica gel solid phase extraction (SPE) cartridge for clean-up. The LOD values and recoveries for PAH 4 were 0.006−0.01 ng/g and 77−87% (RSD%, 9−20%), respectively, as determined by HPLC-FLD. Lee and Shin [24] utilized saponification (1 M potassium hydroxide (KOH) ethanol solution), liquid−liquid extraction (LLE) (ethanol/hexane (1/1, v/v) mixture, hexane, and distilled water) and purification (Florisil SPE cartridge) to extract 7 PAHs (BaA, CHR, BbF, BkF, BaP, DhA, BgP) in commercial roasted coffee beans. The PAHs were detected with HPLC-FLD. The LOD and LOQ values of the PAHs were 0.016−0.497 ng/g (PAH 4, 0.017−0.031 ng/g) and 0.054−1.656 ng/g (PAH 4, 0.055−0.104 ng/g), respectively. Jimenez et al. [25] extracted US EPA PAHs from roasted coffee using hexane and rotor mixer. After purification with a silica gel SPE cartridge, the PAHs were determined by HPLC-FLD. The LOD and LOQ values for the US EPA PAHs were 0.01−0.21 ng/g (PAH 4, 0.01−0.21 ng/g) and 0.03−0.71 ng/g (PAH 4, 0.04−0.71 ng/g), respectively. The recoveries for all PAHs were greater than 80%. Guatemala-Morales et al. [26] used ultrasound extraction (*n*-hexane and methylene chloride (9:1 v/v)), alkaline saponification (KOH ethanol solution) and purification (silica gel SPE cartridge) to extract US EPA PAHs from roasted coffee beans, and then analyzed with GC−MS. The LOD and LOQ values of the PAHs were 0.38−2.6 ng/g and 1.27−7.20 ng/g, respectively. The recoveries for the PAHs were

Table 2. The recoveries (%) of EU priority PAHs in coffee beans and coffee brews extracted with different levels of solvents.

Sample	PAH	Recovery (%) (CV%)			
		5 mL Water + 5 mL Acetone	10 mL Water + 5 mL Acetone	5 mL Water + 10 mL Acetone	10 mL Water + 10 mL Acetone
Coffee bean	BcL	87 (3)	74 (6)	75 (3)	62 (25)
	BaA[a,b]	112 (3)	102 (2)	110 (4)	96 (6)
	CHR[a,b]	102 (13)	77 (3)	81 (3)	91 (16)
	5-MC	72 (8)	41 (32)	69 (2)	62 (8)
	BjF	82 (25)	83 (10)	84 (4)	72 (10)
	BbF[a,b]	100 (4)	88 (3)	99 (2)	93 (2)
	BkF [b]	98 (1)	88 (2)	93 (3)	92 (3)
	BaP[a,b]	100 (4)	88 (2)	98 (1)	95 (4)
	DlP	89 (3)	79 (2)	89 (2)	82 (3)
	DhA[b]	78 (6)	70 (5)	80 (1)	76 (4)
	BgP [b]	84 (7)	69 (6)	90 (1)	75 (6)
	IcP [b]	92 (4)	78 (4)	89 (3)	89 (1)
	DeP	89 (3)	77 (2)	85 (2)	85 (4)
	DiP	76 (3)	70 (0)	82 (1)	87 (4)
	DhP	95 (1)	86 (2)	96 (5)	99 (3)
Sample	PAH	Recovery (%) (CV%) [a]			
		20 mL Acetone (1% Acetic acid)	10 mL Acetone (1% Acetic acid)	5 mL Acetone (1% Acetic acid)	
Coffee brew	BcL	95 (1)	96 (3)	102 (2)	
	BaA[a,b]	101 (2)	105 (4)	104 (2)	
	CHR[a,b]	104 (5)	103 (3)	103 (2)	
	5-MC	93 (5)	100 (3)	99 (2)	
	BjF	83 (8)	96 (8)	98 (2)	
	BbF[a,b]	96 (4)	102 (5)	100 (5)	
	BkF [b]	99 (0)	102 (3)	102 (1)	
	BaP[a,b]	100 (1)	103 (2)	104 (2)	
	DlP	97 (1)	100 (3)	101 (2)	
	DhA[b]	93 (2)	95 (4)	97 (2)	
	BgP [b]	95 (2)	97 (3)	99 (3)	
	IcP [b]	93 (2)	96 (6)	103 (11)	
	DeP	97 (1)	94 (3)	102 (2)	
	DiP	106 (9)	102 (2)	106 (2)	
	DhP	97 (4)	99 (2)	106 (2)	

The recoveries (%) of the PAHs were calculated through five replicate tests and expressed as mean (coefficient of variation %, CV%).
[a] PAH 4 = BaA + CHR + BbF + BaP.
[b] PAH 8 = PAH 4 + BkF + DhA + BgP + IcP.

39.8%–69.0%. Kamalabadi et al. [27] determined 7 PAHs (BaA, BbF, BcF, BaP, CHR, 5-MC, IcP) in roasted coffee samples using microwave-assisted extraction (MAE) (solution, water and 85% (v/v) KOH ethanol solution (1 M); microwave condition, 520 W for 8 min) and dispersive liquid–liquid micro-extraction (LLME) (solvent, acetone and tetrachloroethylene) coupled with GC–MS. LOD and LOQ values of the PAHs were 0.1–0.3 ng/g (PAH 4, 0.2–0.3 ng/g) and 0.3–0.9 ng/g (PAH 4, 0.6–0.9 ng/g), respectively. The recoveries for the PAHs were 88.1–101.3% (RSD%, 5.5–8.1%). Duedahl-Olesen et al. [28] adopted pressurized liquid extraction (PLE) with clean-up steps of gel permeation chromatography (SX-3) and SPE (silica) to extract PAHs from coffee brews. The results of GC–MS analysis showed that the LOD values and recoveries for PAH 4 were 0.1–0.3 μg/kg and 94–106%, respectively.

In this work, we optimized QuEChERS conditions for the convenient and rapid extraction of the EU priority PAHs from coffee beans and coffee brews, respectively, which not only exhibited good accuracy and precision, but also complied with EU and TFDA testing specifications. In addition, the temperature-controlled FLD also showed higher sensitivity for the determination of EU priority PAHs.

3.2. The content of EU priority PAHs in coffee samples and risk assessment

Houessou et al. [29] described that the presence of PAHs in coffee samples may be due to contamination of green beans or the formation of the compounds during roasting. In this study, we evaluated different roast level of coffee been on the formation of PAHs. Table 4 shows that the sequence for the

Table 3. Detection of limit (LOD), quantitation of limit (LOQ) and recoveries (%) for extraction of the spiked EU priority PAHs in each coffee matrix using the established QuEChRERS conditions.

Respective matrix	PAH	Intra-day			Inter-day			Average recovery (%)	LOD [a] (ng/g)	LOQ [b] (ng/g)	Linear regression equation [c]	Regression coefficient (r)
		Recovery (%) (CV%)			Recovery (%) (CV%)							
		2.0 ng/g	5.0 ng/g	10.0 ng/g	2.0 ng/g	5.0 ng/g	10.0 ng/g					
Coffee bean	BcL	88 (6)	93 (11)	96 (6)	86 (11)	94 (11)	98 (13)	93	0.04	0.11	$Y = 499{,}984\ X$ - 56,324	0.9992
	BaA [d,e]	110 (6)	107 (9)	104 (8)	103 (17)	99 (19)	102 (16)	104	0.09	0.29	$Y = 118{,}583\ X + 563$	0.9993
	CHR [d,e]	103 (9)	101 (10)	99 (8)	100 (16)	98 (20)	100 (16)	100	0.08	0.30	$Y = 128{,}442\ X + 3514$	0.9996
	5-MC	75 (10)	82 (8)	81 (9)	78 (16)	84 (19)	84 (15)	81	0.07	0.20	$Y = 22{,}686\ X + 855$	0.9994
	BjF	90 (11)	92 (7)	93 (7)	92 (12)	91 (14)	96 (10)	92	0.10	0.35	$Y = 11{,}986\ X + 436$	0.9997
	BbF [d,e]	101 (7)	98 (10)	102 (5)	96 (11)	99 (10)	99 (9)	99	0.01	0.04	$Y = 345{,}581\ X$ - 14,837	0.9995
	BkF [e]	100 (6)	98 (4)	96 (6)	97 (9)	100 (11)	102 (10)	99	0.002	0.006	$Y = 753{,}346\ X$ - 16,775	0.9993
	BaP [d,e]	98 (5)	97 (3)	100 (3)	98 (8)	99 (10)	102 (9)	99	0.01	0.04	$Y = 638{,}871\ X + 887$	0.9994
	DlP	93 (7)	92 (6)	94 (7)	92 (13)	96 (12)	97 (11)	94	0.01	0.04	$Y = 12{,}877\ X$+ 1024	0.9992
	DhA [e]	81 (7)	84 (7)	82 (8)	81 (16)	83 (14)	84 (14)	83	0.04	0.12	$Y = 46{,}833\ X + 15{,}632$	0.9991
	BgP [e]	88 (6)	92 (8)	91 (8)	90 (20)	94 (15)	94 (17)	92	0.05	0.16	$Y = 40{,}124\ X$ - 1101	0.9991
	IcP [e]	95 (7)	94 (6)	98 (5)	93 (11)	92 (10)	95 (13)	95	0.02	0.06	$Y = 53{,}424\ X + 8114$	0.9991
	DeP	94 (6)	92 (8)	95 (4)	95 (9)	94 (11)	96 (10)	94	0.05	0.16	$Y = 248{,}664\ X + 1599$	0.9996
	DiP	79 (5)	83 (5)	85 (4)	80 (12)	82 (12)	84 (13)	82	0.08	0.33	$Y = 145{,}569\ X$ - 14,321	0.9997
	DhP	98 (2)	100 (4)	99 (5)	99 (9)	99 (10)	101 (9)	99	0.004	0.02	$Y = 128{,}032\ X$ - 70,989	0.9997
Respective matrix	PAH	Intra-day			Inter-day			Average recovery (%)	LOD [a] (ng/g)	LOQ [b] (ng/g)	Linear regression equation [c]	Regression coefficient (r)
		Recovery (%) (CV%)			Recovery (%) (CV%)							
		0.2 ng/g	0.5 ng/g	1.0 ng/g	0.2 ng/g	0.5 ng/g	1.0 ng/g					
Coffee brew	BcL	97 (4)	99 (5)	102 (3)	94 (20)	96 (12)	99 (16)	98	0.015	0.05	$Y = 521{,}675\ X$ - 14,675	0.9996
	BaA [d,e]	97 (5)	98 (3)	102 (3)	96 (12)	95 (13)	102 (12)	98	0.006	0.02	$Y = 134{,}412\ X + 622$	0.9996
	CHR [d,e]	95 (5)	98 (6)	104 (4)	101 (14)	99 (15)	100 (19)	100	0.04	0.12	$Y = 156{,}441\ X + 1123$	0.9995
	5-MC	90 (3)	94 (4)	98 (2)	93 (13)	96 (17)	99 (14)	95	0.04	0.15	$Y = 244{,}519\ X$ - 613	0.9997
	BjF	97 (6)	93 (2)	99 (4)	92 (19)	96 (10)	96 (21)	96	0.06	0.20	$Y = 13{,}999\ X + 301$	0.9998
	BbF [d,e]	96 (4)	102 (4)	101 (3)	94 (11)	97 (12)	100 (11)	98	0.005	0.02	$Y = 333{,}112\ X + 1613$	0.9997
	BkF [e]	94 (3)	98 (5)	101 (4)	92 (15)	97 (15)	100 (14)	97	0.003	0.009	$Y = 767{,}554\ X$ - 9763	0.9998
	BaP [d,e]	97 (3)	100 (3)	102 (2)	98 (13)	100 (10)	98 (18)	99	0.004	0.01	$Y = 640{,}115\ X + 633$	0.9996
	DlP	98 (4)	97 (7)	101 (3)	95 (13)	99 (17)	98 (15)	98	0.03	0.10	$Y = 142{,}265\ X$ - 544	0.9995
	DhA [e]	93 (5)	92 (4)	98 (2)	95 (18)	94 (11)	99 (18)	95	0.06	0.20	$Y = 48{,}436\ X$ - 795	0.9993
	BgP [e]	96 (2)	95 (3)	99 (4)	95 (10)	98 (16)	98 (12)	97	0.06	0.20	$Y = 41{,}667\ X + 812$	0.9994
	IcP [e]	94 (3)	97 (6)	102 (5)	97 (14)	96 (13)	103 (11)	98	0.004	0.01	$Y = 58{,}904\ X$ - 1024	0.9992
	DeP	96 (7)	97 (9)	102 (4)	97 (10)	99 (7)	100 (15)	99	0.03	0.10	$Y = 234{,}866\ X + 15{,}331$	0.9998
	DiP	98 (5)	100 (4)	105 (3)	99 (13)	98 (14)	101 (16)	100	0.01	0.03	$Y = 158{,}949\ X + 4112$	0.9996
	DhP	100 (8)	98 (8)	104 (4)	99 (11)	97 (9)	103 (11)	100	0.005	0.02	$Y = 136{,}824\ X$ - 36,775	0.9994

The recoveries (%) for intra- and inter-assays were carried out through five repeated tests and expressed as mean (coefficient of variation %, CV%).

[a] LOD is based on S/N $\geq$ 3 of standard solution.

[b] LOQ is based on S/N $\geq$ 10 of standard solution.

[c] Y is the value of the peak area, X is the value of sample concentration (LOD-10 ng/g); injection volume, 10 μL.

[d] PAH 4 = BaA + CHR + BbF + BaP.

[e] PAH 8 = PAH 4 + BkF + DhA + BgP + IcP.

Table 4. The content of PAHs in the coffee beans of different roasting levels and the coffee brewed with the drip bag or the coffee machine.

Sample	Roast level	Content (ng/g)																
		BcL	BaA [a,b]	CHR [a,b]	5-MC	BjF	BbF [a,b]	BkF [b]	BaP [a,b]	DlP	DhA [b]	BgP [b]	IcP [b]	DeP	DiP	DhP	PAH 4	PAH 8	Σ
Coffee bean	light	3.92 ± 0.21A	6.34 ± 0.63 A	39.59 ± 2.69 C	N.D.[C]	N.D.	N.D.	N.D.	4.70 ± 0.25 C	N.D.	N.D.	4.40 ± 0.04 C	0.28 ± 0.02 A	8.22 ± 0.56 A	1.83 ± 0.17 A	N.D.	50.63 ± 3.57 C	55.31 ± 3.63 C	69.28 ± 4.57 C
	medium	3.57 ± 0.19 A	6.28 ± 0.17 A	54.70 ± 2.46 A	N.D.	N.D.	N.D.	N.D.	6.97 ± 0.18 B	N.D.	N.D.	52.52 ± 2.47 B	0.49 ± 0.01 A	7.07 ± 0.68 A	0.41 ± 0.04 B	0.19 ± 0.01 B	67.95 ± 2.81 A	120.96 ± 5.29 B	132.20 ± 6.21 B
	dark	1.09 ± 0.05 B	2.97 ± 0.23 B	49.25 ± 1.87 B	N.D.	N.D.	N.D.	N.D.	7.94 ± 0.29 A	0.76 ± 0.06 A	N.D.	853.14 ± 29.60 A	0.63 ± 0.05 A	7.30 ± 0.54 A	N.D.	0.57 ± 0.05 A	60.92 ± 2.45 B	914.69 ± 32.10 A	923.65 ± 32.74 A

Sample	Roast level	Content (ng/g)																	
		BcL	BaA [a,b]	CHR [a,b]	5-MC	BjF	BbF [a,b]	BkF [b]	BaP [a,b]	DlP	DhA [b]	BgP [b]	IcP [b]	DeP	DiP	DhP	PAH 4	PAH 8	Σ
Coffee brew (brewing with the drip bag, atmospheric pressure)	light	0.08 ± 0.00 B	0.09 ± 0.00 C	0.11 ± 0.01 C	N.D.	N.D.	N.D.	0.01 ± 0.00 A	0.01 ± 0.00 B	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.01 ± 0.00 A	0.21 ± 0.01 D	0.22 ± 0.02 E	0.30 ± 0.02 F
	medium	0.10 ± 0.01 A	0.17 ± 0.01 AB	0.12 ± 0.01 BC	N.D.	N.D.	N.D.	N.D.	0.01 ± 0.00 B	N.D.	N.D.	0.12 ± 0.01 C	N.D.	N.D.	N.D.	0.01 ± 0.00 A	0.30 ± 0.02 B	0.42 ± 0.03 D	0.53 ± 0.04 D
	dark	0.09 ± 0.00 AB	0.15 ± 0.01 B	0.06 ± 0.01 D	N.D.	N.D.	N.D.	0.01 ± 0.00 A	0.01 ± 0.00 B	N.D.	N.D.	0.29 ± 0.01 B	N.D.	N.D.	N.D.	0.01 ± 0.00 A	0.22 ± 0.02 CD	0.52 ± 0.03 C	0.62 ± 0.04 C
Coffee brew (brewing with the coffee machine, 4 bar)	light	0.10 ± 0.01 A	0.06 ± 0.00 D	0.16 ± 0.01 A	N.D.	N.D.	0.01 ± 0.00 A	0.01 ± 0.00 A	0.02 ± 0.00 A	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.25 ± 0.01 C	0.26 ± 0.02 E	0.36 ± 0.03 E
	medium	0.10 ± 0.00 A	0.16 ± 0.01 AB	0.17 ± 0.01 A	N.D.	N.D.	N.D.	0.01 ± 0.00 A	0.02 ± 0.00 A	N.D.	N.D.	0.33 ± 0.03 B	N.D.	0.03 ± 0.00 A	N.D.	0.01 ± 0.00 A	0.35 ± 0.02 A	0.69 ± 0.05 B	0.83 ± 0.05 B
	dark	0.10 ± 0.01 A	0.18 ± 0.01 A	0.13 ± 0.01 B	N.D.	N.D.	N.D.	N.D.	0.01 ± 0.00 B	N.D.	N.D.	1.72 ± 0.10 A	N.D.	N.D.	N.D.	0.01 ± 0.00 A	0.32 ± 0.02 AB	2.04 ± 0.12 A	2.14 ± 0.13 A

Values (mean ± SD, n = 3) in the same column followed by a different uppercase letter are significantly different ($P < 0.05$). Coffee brewed from the same batch of roasted coffee beans.

[a] PAH 4 = BaA + CHR + BbF + BaP.

[b] PAH 8 = PAH 4 + BkF + DhA + BgP + IcP.

[C] N.D. = not detected.

Table 5. The content of PAHs in commercial coffee products.

Type	Coffee product (Brand)	Content (ng/g)																	
		BcL	BaA [a,b]	CHR [a,b]	5-MC	BjF	BbF [a,b]	BkF [b]	BaP [a,b]	DlP	DhA [b]	BgP [b]	IcP [b]	DeP	DiP	DhP	PAH 4	PAH 8	Σ
Brewed coffee	I	0.05 ± 0.00 C	N.D.[c]	N.D.	N.D.	N.D.	N.D.	N.D.	0.01 ± 0.00 B	N.D.	N.D.	0.29 ± 0.02 C	N.D.	N.D.	N.D.	N.D.	0.01 ± 0.00 E	0.30 ± 0.02 D	0.35 ± 0.02 D
	II	0.05 ± 0.00 C	0.11 ± 0.01 A	0.05 ± 0.00 A	N.D.	N.D.	N.D.	N.D.	0.01 ± 0.00 B	N.D.	N.D.	0.95 ± 0.07 A	0.05 ± 0.00 A	N.D.	N.D.	N.D.	0.17 ± 0.01 A	1.17 ± 0.08 A	1.23 ± 0.09 A
	III	0.05 ± 0.00 C	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.02 ± 0.00 A	N.D.	N.D.	0.70 ± 0.03 B	N.D.	N.D.	N.D.	N.D.	0.02 ± 0.00 D	0.72 ± 0.03 B	0.77 ± 0.03 B
	IV	0.07 ± 0.00 A	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.01 ± 0.00 B	N.D.	N.D.	0.22 ± 0.02 D	0.02 ± 0.00 B	N.D.	N.D.	N.D.	0.01 ± 0.00 E	0.25 ± 0.02 E	0.32 ± 0.02 DE
Canned coffee	V	0.05 ± 0.00 C	0.09 ± 0.00 B	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.14 ± 0.01 E	N.D.	N.D.	N.D.	N.D.	0.09 ± 0.00 BC	0.23 ± 0.01 E	0.28 ± 0.02 E
	VI	0.07 ± 0.00 A	0.07 ± 0.00 C	0.04 ± 0.00 B	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.01 ± 0.00 C	N.D.	N.D.	N.D.	0.11 ± 0.01 B	0.12 ± 0.01 F	0.19 ± 0.01 F
	VII	0.06 ± 0.00 B	0.07 ± 0.00 C	N.D.	N.D.	N.D.	N.D.	0.01 ± 0.01 A	0.01 ± 0.00 B	N.D.	N.D.	0.31 ± 0.03 C	N.D.	N.D.	N.D.	N.D.	0.08 ± 0.00 C	0.40 ± 0.03 C	0.46 ± 0.03 C
	VIII	0.05 ± 0.00 C	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	0.01 ± 0.00 B	N.D.	N.D.	0.10 ± 0.01 F	N.D.	N.D.	N.D.	N.D.	0.01 ± 0.00 E	0.11 ± 0.01 F	0.16 ± 0.01 G

Values (mean ± SD, n = 3) in the same column followed by a different uppercase letter are significantly different ($P < 0.05$).

[a] PAH 4 = BaA + CHR + BbF + BaP.

[b] PAH 8 = PAH 4 + BkF + DhA + BgP + IcP.

[c] N.D. = not detected.

Table 6. Dietary risk assessments of BaP, PAH 4 and PAH 8 in the coffee samples for the general ethnic group (1–65^{+} years old, whole group (WG) and consumers only (CO)).

Sample	Roast level	MOE					
		WG			CO		
		BaP	PAH 4	PAH 8	BaP	PAH 4	PAH 8
Coffee brew (brewing with the drip bag, atmospheric pressure)	Light	20,349,000	4,707,000	6,475,000	2,310,000	534,000	735,000
	Medium	20,349,000	3,295,000	3,391,000	2,310,000	374,000	385,000
	dark	20,349,000	4,493,000	2,739,000	2,310,000	510,000	311,000
Coffee brew (brewing with the coffee machine, 4 bar)	Light	10,174,000	3,953,000	5,479,000	1,155,000	449,000	622,000
	Medium	10,174,000	2,824,000.	2,064,000	1,155,000	321,000	234,000
	dark	20,349,000	3,089,000	698,000	2,310,000	351,000	79,000
Commercial product	Brand	MOE					
		WG			CO		
		BaP	PAH 4	PAH 8	BaP	PAH 4	PAH 8
Brewed coffee	I	20,349,000	98,837,000	4,748,000	2,310,000	11,221,000	539,000
	II	20,349,000	5,814,000	1,217,000	2,310,000	660,000	138,000
	III	10,174,000	49,419,000	1,978,000	1,155,000	5,611,000	225,000
	IV	20,349,000	98,837,000	5,698,000	2,310,000	112,210,001	647,000
Canned coffee	V	-	10,982,000	6,193,000	-	1,247,000	703,000
	VI	-	8,985,000	11,870,000	-	1,020,000	1,348,000
	VII	20,349,000	12,355,000	3,561,000	2,310,000	1,403,000	404,000
	VIII	20,349,000	98,837,000	12,949,000	2,310,000	11,221,000	1470000W

MOE: margin of exposure; the MOE was calculated according to the equation described in *Section 2.6*; PAH 4 = BaA + CHR + BbF + BaP; PAH 8 = PAH 4 + BkF + DhA + BgP + IcP.

content of the EU priority PAHs in the roasted coffee beans was 923.65 ng/g (dark roast) > 132.20 ng/g (medium roast) > 69.28 ng/g (light roast). The content of PAH 8 also showed the same trend (dark roast, 914.69 ng/g; medium roast, 120.96 ng/g; light roast, 55.31 ng/g). The content of PAH 4 in the roasted coffee beans was ranked as 67.95 ng/g (medium roast) > 60.92 ng/g (dark roast) > 50.63 ng/g (light roast). The content of BaP in the coffee bean samples for dark roast, medium roast and light roast samples was 7.94 ng/g, 6.97 ng/g and 4.70 ng/g, respectively. While coffee beans had higher levels of PAHs, their brewed samples had much lower levels of PAHs. The PAH levels for the samples brewed using the drip bag (atmospheric pressure) were: 0.30 ng/g for light roast, 0.53 ng/g for medium roast, and 0.62 ng/g for dark roast. The PAH levels for the coffee machine (set at 4 bar) brewed samples were: 0.36 ng/g for light roast, 0.83 ng/g for medium roast, and 2.14 ng/g for dark roast. The contents of BaP, PAH 4 and PAH 8 in the samples brewed using the drip bag were 0.01 ng/g, 0.21 ng/g and 0.22 ng/g (light roast), 0.01 ng/g, 0.30 ng/g and 0.42 ng/g (medium roast), and 0.01 ng/g, 0.22 ng/g and 0.52 ng/g (dark roast), respectively. The contents of BaP, PAH 4 and PAH 8 in the samples brewed with the coffee machine were 0.02 ng/g, 0.25 ng/g and 0.26 ng/g (light roast), 0.02 ng/g, 0.35 ng/g and 0.69 ng/g (medium roast), and 0.01 ng/g, 0.32 ng/g and 2.04 ng/g (dark roast), respectively (Table 3). Higher roasting levels produced more PAHs in the coffee samples. Furthermore, brewing at high pressure resulted in more release of PAHs from the coffee beans into the coffee brews compared to brewing at atmospheric pressure (Table 4).

Table 5 shows that the content of the EU priority PAHs in the commercial coffee products was low (0.32–1.23 ng/g for the brewed coffee products and 0.19–0.46 ng/g for the canned coffee products). BaP was not detected in two brands of coffee products, and the content of BaP in the products that could be detected was 0.1–0.2 ng/g. The levels of PAH 4 and PAH 8 were 0.01–0.17 ng/g and 0.25–1.17 ng/g for the brewed coffee products, and 0.01–0.11 ng/g and 0.11–0.40 ng/g for the canned coffee products, respectively.

Coffee brew is the form of coffee consumption. Coffee brew samples were used to assess their consumption risk for the PAHs. Table 6 shows the results of dietary risk assessments for BaP, PAH 4 and PAH 8 in the coffee samples for the general ethnic group (1–65^{+} years old, whole group (WG) and consumers only (CO)). All samples had MOE values above the reference value of 10,000, indicating the consumption risk is a low level of concern. EFSA [2] pointed out that when the MOE value of hazardous substances in food is less than 10,000, it indicates that the risk is a high level of concern and relevant risk management strategies must be formulated. When the MOE value is greater than 10,000, it means that the risk is a low level of concern, but the quality control still needs to be prudent.

Houessou et al. [29] roasted coffee beans in a pilot spouted bed roaster (the inlet air temperature varying from 180 to 260 °C) using both dark (20 min) and light (5 min) roasting conditions. They determined the US EPA PAHs in the coffee samples and found that the transformation of low molecular PAHs to high molecular PAHs occurred with increasing roasting degree. The coffee brew samples were prepared using an electric coffee maker (50 g of the ground roasted coffee was treated by passing 300 mL of water). The levels of PAHs in coffee beans and their brews increased with increasing roasting temperature and time. Compared with the light-roasted coffee beans, the transfer rate of PAHs from dark-roasted coffee beans was slightly lower. The transfer of PAHs into the brews was less than 35% due to the low solubility of PAHs in water, resulting in low values of the toxicity equivalent (TEQ) for the coffee brews. Tfouni et al. [3] roasted coffee beans of two cultivars (*C. arabica* and *C. canephora*) using the light, medium and dark roasting conditions. The coffee brews were prepared (50 g of ground coffee beans/500 mL of boiled water) with the procedures for filtered coffee (prepared by dripping boiling water onto the ground coffee beans held in a paper filter) and boiled coffee (prepared by boiling the mixture of the ground coffee beans and water then filtering through a paper filter), respectively. The authors found that the levels of PAH 4 were 0.015–0.105 mg/L for *C. arabica* and 0.011–0.111 mg/L for *C. canephora*, respectively. For both cultivars, the filtered coffee brews had higher PAH 4 levels than the boiled coffee brews. There were no statistically significant differences in PAH 4 levels detected in the brews of different roasting degrees, except between the filtered brews of dark roast (their PAH 4 levels were relatively low). Jimenez et al. [25] determined the US EPA PAHs in roasted coffee samples and observed that the concentration of total PAHs in coffee samples was related to the degree of roasting, with the lowest concentration for light roast sample and the highest concentration for dark roast sample. Different brands of coffee samples had different levels of PAHs.

Previous reports of PAHs in coffee samples were mostly the US EPA PAHs [25,29,30]. The structures of the US EPA PAHs consist of 2–6 fused aromatic rings, while structures of the EU priority PAHs consist of 4–6 fused aromatic rings (all high molecular PAHs). This study focused on the determination of the EU priority PAHs in coffee beans and coffee brews. Our results showed that there was a positive correlation between the total amounts of EU priority PAHs and the degree of roasting. The transfer rate of the PAHs from the roasted coffee beans to their brews were 1.14–10.67%. Brewing with the coffee machine released more PAHs than brewing with the drip bag. Although the dark roast coffee beans released more PAHs into their coffee brews, the light roast samples had higher PAHs transfer (7.36–8.83%) than the dark roast samples (1.14–3.94%) (Table 4). Different brands of coffee products had different content of the PAHs. The amount of the PAHs in the commercial brewed coffee products was slightly higher than that in the commercial canned coffee products (Table 5). The consumption risk of the PAHs in the coffee brew samples is a low level of concern (Table 6) due to low content of PAHs (Tables 5 and 6).

4. Conclusions

The HPLC system equipped with the temperature-controlled FLD had higher efficiency and sensitivity for measurement of the EU priority PAHs in coffee samples. For the QuEChERS procedure, acetone (5 mL) and acetone (1% acetic acid) (5 mL) were more appropriate for the extraction of the PAHs from coffee bean (1 g of sample + 5 mL of water) and coffee brew (10 g of sample), respectively. The determination results for LOD, LOQ, recovery (%), linearity, repeatability, and intermediate precision of the PAHs in coffee samples using our developed conditions were also in line with the EU and TFDA regulations. The total amount of the EU priority PAHs in coffee samples increased with the degree of roasting. For the PAH 4 content in the coffee samples, the order from high to low was medium roast, dark roast and light roast. Due to the relatively high pressure, the coffee machine brewing resulted in the release of more PAHs from the coffee beans into the coffee brews compared to the drip bag brewing; nevertheless, the amounts of the PAHs were still relatively low. Although different brands of commercial canned and brewed coffee products had different levels of the PAHs, the levels of the PAHs were also not high. Even though the consumption risk of the PAHs in coffee brews and coffee products is a low level of concern, brewing and roasting methods should be considered to reduce the intake of PAHs.

Conflict of interest

The authors have declared that there is no conflict of interest.

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Appendix.

Supplementary Table 1. Retention time (RT), separation factor (α) and resolution (Rs) for the PAHs determined with the developed HPLC-FLD conditions

Peak No.	PAH	*RT*	α [a]	*Rs* [b]
1	BcL	2.61	–	–
2	BaA	3.81	1.632 (1/2)	4.948 (1/2)
3	CHR	4.07	1.084 (2/3)	1.104 (2/3)
4	5-MC	4.76	1.205 (3/4)	2.453 (3/4)
5	BjF	5.18	1.104 (4/5)	1.247 (4/5)
6	BbF	5.82	1.143 (5/6)	1.753 (5/6)
7	BkF	6.69	1.170 (6/7)	2.795 (6/7)
8	BaP	7.48	1.132 (7/8)	2.943 (7/8)
9	DlP	9.19	1.253 (8/9)	4.870 (8/9)
10	DhA	9.36	1.020 (9/10)	1.001 (9/10)
11	BgP	9.91	1.064 (10/11)	1.003 (10/11)
12	IcP	10.35	1.048 (11/12)	1.504 (11/12)
13	DeP	11.93	1.164 (12/13)	3.365 (12/13)
14	DiP	16.74	1.429 (13/14)	10.781 (13/14)
15	DhP	18.05	1.082 (14/15)	2.817 (14/15)

The analytical conditions of PAHs were described in *Section 2.2.*

[a] $\alpha = t_{R2}\text{-}t_0/t_{R1}\text{-}t_0$, where t_{Rn} = retention time of an analyte, t_0 = retention time of an unretained peak.

[b] $Rs = 2\,(t_{R2}\text{-}t_{R1})/(w_1+w_2)$, where w_n = band widt h of an analyte at the baseline.

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