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Determination of polycyclic aromatic hydrocarbons in smoked and non-smoked black teas and tea infusions

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ABSTRACT

This study describes the occurrence of polycyclic aromatic hydrocarbons (PAHs) in smoked tea and tea infusions, *via* the monitoring of benzo(a)anthracene, chrysene, benzo(b)fluoranthene and benzo(a)pyrene (PAH4) that have been chosen as indicators for the occurrence of PAHs in food by the European Food Safety Agency. The concentrations ranged from 1.2 μg/kg for benzo(b)fluoranthene to 125.0 μg/kg for benzo(a)anthracene in smoked tea leaves, and from 0.6 μg/L for benzo(a)anthracene to 1.2 μg/L for benzo(b)fluoranthene in smoked tea infusions. Benzo(a)pyrene was never detected in infusions. The concentrations in non-smoked tea leaves ranged from 0.6 μg/kg for benzo(a)anthracene to 10.8 μg/kg for benzo(b)fluoranthene. It was shown that the concentrations of benzo(a)anthracene and chrysene were higher in smoked tea than in non-smoked tea while no difference was observed for benzo(b)fluoranthene and benzo(a)pyrene. The concentrations of PAHs in tea infusions are low compared to other foodstuffs, but the migration rates from leaves into water are high (82–123%).

1. Introduction

Tea (*Camellia sinensis*) is the most widely consumed beverage in the world, next to water. Due to the presence of flavonoids tea has many health benefits, such as antioxidative effects, and antimutagenic and anticarcinogenic activities (e.g., Da Silva Pinto, 2013; Jain, Manghani, Kohli, Nigam, & Rani, 2013), though recent studies also showed that tea leaves may contain contaminants that can be released into infusions and might be harmful to human health. In particular, a wide variety of polycyclic aromatic hydrocarbons (PAHs) was detected on tea leaves (Fiedler, Cheung, & Wong, 2002; Schlemitz & Pfannhauser, 1997). Most PAHs are toxic, and some of them have been proven carcinogenic and genotoxic (e.g., benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene; Sevastyanova et al., 2007; Spink et al., 2008).

However, PAHs are a very large class containing hundreds of different compounds, and it is impossible to monitor all of them simultaneously. In order to overcome this problem, the CONTAM (Contaminants in the Food Chain) Panel of the European Food Safety Authority (EFSA) reviewed the data on PAHs in food over several years and concluded that four PAHs (chrysene [Chr], benzo(a)anthracene [BaA], benzo(b)fluoranthene [BbF] and benzo(a)pyrene [BaP]), commonly called PAH4, can serve as an indicator for the occurrence of PAHs in food (EFSA, 2008). Following the scientific opinion of the EFSA, the European Commission fixed limits for benzo(a)pyrene and the sum of PAH4 in different foodstuffs in the amendment 835/2011 of the regulation 1881/2006. Tea is not yet covered by this regulation; however, as PAHs are very abundant in the air across the world and in tea-growing regions like India (Masih, Singhvi, Taneja, Kumar, & Masih, 2012), Sri Lanka (Wickramasinghe, Karunaratne, & Sivakanesan, 2011), China (Bi, Sheng, Peng, Cheng & Fu, 2005; Hu, Liu, Zhang, & Zhang, 2012) and Tibet (Liu et al., 2013), PAH contamination of tea leaves is an issue. Concentrations of PAH4 in black teas measured in previous studies varied from 4.9 to 103.6 μg/kg (Schlemitz and Pfannhauser, 1997), from 9.0 to 44.6 μg/kg (Ziegenhals, Jira, & Speer, 2008), from 6.4 to 700 μg/kg (Dabrova et al., 2012), and from 21.6 to 65.8 μg/kg (Ishizaki, Saito, Hanioka, Narimatsu, & Kataoka, 2010). Even higher concentrations were measured on Mate teas, with concentrations of PAH4 ranging from 184.6 to 1615 μg/kg (Ziegenhals et al., 2008). These concentrations largely exceed the highest authorised limits of PAHs defined in the regulation 835/2011/EC (6 μg/kg for BaP and 35 μg/kg for the sum of PAH4 in bivalve molluscs). Mate (*Ilex paraguariensis)* is a tea-like plant native to subtropical South America and is dried over a wood fire, which might explain the high PAH concentrations measured on these plants.

Tea leaves are normally not consumed themselves but as an infusion, and recent studies showed that the PAH concentrations in infusions are far lower than those measured on the tea leaves themselves. Concentrations of PAH4 in infusions of black teas ranged from 5.2 to 17.6 μg/L (Ishizaki et al., 2010) and from 6.1 to 135.9 μg/L for Mate tea infusions (Zuin, Montero, Bauer, & Popp, 2005). Even though these concentrations are far lower than in

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tea leaves, they remain high compared to the limits set for other foodstuffs in the regulation 835/2011/EC. However, as tea is consumed in far higher amounts than other foodstuffs, it might be more appropriate to refer to the PAH limits set for drinking water, which are much lower: the Commission Regulation 98/83/EC limits the amount of BaP in drinking water to 0.01 μg/L, and the sum of 4 PAHs (though not the PAH4 recommended by EFSA, but the sum of benzo(b)fluoranthene, BkF, benzo(g,h,i)perylene and indeno(1,2,3-c,d)pyrene) to 0.1 μg/L. Taking these limits as a reference for PAH content in tea infusions, PAHs in tea infusions are a real issue, and a risk to human health may exist, particularly for persons with a high consumption of Mate tea.

Much information about tea and Mate tea is available in the scientific literature, which helps when assessing the potential risk to human health caused by tea consumption. However, to our knowledge, no data are yet available for smoked black tea, a kind of tea originating from Asia and becoming more and more popular in western countries. The particularity of these black teas is that, after fermentation, the leaves are first dried on a hot iron plate before being smoked over a pine, spruce or bamboo fire, giving a heavy smoky taste and smell to the tea leaves and its infusions. The most famous of these smoked teas is Lapsang Souchong, a black tea native to south-eastern China and the Formosan Straight in Taiwan, though other versions like smoked Earl Grey can also be found on the market. Smoking the tea might increase the concentrations of PAHs on the tea leaves, similar to Mate tea, and knowledge about the PAH concentrations on smoked tea leaves as well as in the infusions of these tea leaves is important, in order to be able to evaluate their potential risk to human health.

The aim of this study was to monitor BaA, Chr, BbF and BaP on smoked black tea leaves and in infusions of smoked black teas, and to compare the results to those obtained for non-smoked black teas and to the data available in the scientific literature for Mate tea. The method used in this study was based on the procedure described in ISO 15753 (2006), though with a slight modification in the extraction of the PAHs from the matrix, where a modified QuEChERS extraction was used. The analysis of the final extracts was made by GC–MS/MS.

2. Materials and methods

2.1. Chemicals

A mix of BaA, Chr, BbF and BaP in methylene chloride at certified concentration of 2000 μg/mL, and benzo(a)pyrene-D_{12} (BaP-D_{12}) that served as internal standard, were purchased from Sigma–Aldrich (Bornem, Belgium). Acetonitrile, acetone, methanol, methylene chloride and *n*-hexane, all HPLC-grade, were purchased from Biosolve (Paris, France). Ultra-pure water was obtained using a Millipore laboratory water purification system (Overijse, Belgium). Anhydrous $MgSO_4$ was purchased from Merck (Overijse, Belgium), NaCl was purchased from Chem-Lab (Zedelgem, Belgium), and sodium citrate sesquihydrate ($C_6H_6Na_2O_7{\cdot}1.5H_2O$) and sodium citrate dihydrate ($C_6H_5Na_3O_7{\cdot}2H_2O$) were purchased from Sigma–Aldrich (Bornem, Belgium). Sep-Pak C_{18} (12 cm^3, 2 g) and Florisil (6 cm^3, 500 mg) extraction cartridges were purchased from Waters (Zellig, Belgium). Helium and nitrogen at a purity of 99.9999% were purchased from Air Liquide (Luxembourg).

A stock solution containing each PAH at 10 mg/L was prepared in hexane, and stored in the dark at 4 °C for a maximum of 4 weeks. Working solutions at 1 mg/L, 100 and 10 μg/L were prepared weekly in hexane. A stock solution of BaP-D_{12} at 1 g/L was prepared in hexane, and stored in the dark at 4 °C for a maximum of 4 weeks. A working solution at 50 μg/L was prepared weekly in hexane.

2.2. Samples

Ten smoked black teas and 5 non-smoked black teas were purchased on the local market. Infusions were prepared by incubating 2 g of tea leaves in 200 mL tap water at 95 °C for 5 min. The absence of PAH4 in the water used for preparing the infusions was checked. No PAH4 (limits of detection <0.3 μg/L) was identified in the tap water. The hardness of the water was also measured, and it was found to contain 108 mg of calcium and 65 mg of magnesium per litre.

2.3. Sample preparation

The method used in this study was based on the procedure described in ISO 15753 (2006), though with a slight modification in the extraction of the PAHs from the matrix, where a modified QuEChERS extraction was used. Briefly, entire tea leaves were ground in a laboratory blender from Retsch (Aartselaar, Belgium), and 5 g of tea powder or 10 mL of tea infusion were introduced into 50-mL centrifuge tubes with screw caps. For the tea powder, 10 mL of ultrapure water were added. Following this, 10 mL of a mixture of acetonitrile and acetone (60:40) were added and the tubes were shaken for 1 min. The salts (4 g $MgSO_4$, 1 g NaCl, 1 g $C_6H_5Na_3O_7{\cdot}2H_2O$ and 0.5 g $C_6H_6Na_2O_7{\cdot}1.5H_2O$) were added in order to separate the organic phase and the water phase. The tubes were shaken for 1 min and centrifuged at 4000 rpm for 5 min; 5 mL of the supernatant organic phase were loaded onto a C_{18} cartridge previously conditioned with 24 mL methanol and 24 mL acetonitrile. The eluant was collected, and the cartridge was washed with 5 mL acetonitrile:acetone 60:40 (V/V). Both eluants were combined and evaporated to dryness in a rotary evaporator (40 °C; 250 mbar). The residue was dissolved in 2 times 1 mL of a mixture of hexane and methylene chloride (75:25) and loaded on a Florisil cartridge previously conditioned with 5 mL methylene chloride and 5 mL hexane. The eluant was collected, and the cartridge was washed with 5 mL hexane:methylene chloride 75:25. Both eluants were combined and evaporated to dryness at 40 °C under a gentle stream of nitrogen. The residue was dissolved in 50 μL BaP-D_{12} (50 μg/L in hexane) and 50 μL hexane, and injected into GC–MS/MS. The internal standard was added at this stage and not at the beginning of the procedure as it broke down during the evaporation steps. One possible reason is that the molecule reacted with the ambient air and exchanged a deuterium atom with a hydrogen atom and was therefore no longer detected in the mass spectrometer.

The proposed extraction and purification protocol is very long and labour-intensive, though it is necessary because of the complexity of the tea matrix and the very low (sub-ppb) expected PAH concentrations. Therefore the final extract must be very clean, in order to reduce interferences in the GC–MS/MS to a minimum. The problem starts with the extraction step where (1) the dried sample must be wetted with water in order to limit solvent uptake by the tea leaves and (2) the organic solvent must be chosen in order to reach a maximal extraction of PAHs with a minimal extraction of matrix components. The QuEChERS approach was chosen for this because of the good recoveries of pesticides from tea leaves (recoveries between 70% and 120% (Chen, Cao, & Liu, 2011) and 70–100% for most compounds (Lozano et al., 2012)). Following this step, the extract was still very coloured, probably due to heavy contaminations with photosynthetic pigments like chlorophyll. These compounds have a polar porphyrin ring and a long hydrocarbon chain and their polarity depends on the length of the hydrocarbon chain. Therefore, two different SPE purification steps are required: an initial step on a C_{18}-support, in order to extract non-polar compounds, and a second step on a polar support (Florisil) in order to trap polar impurities. This

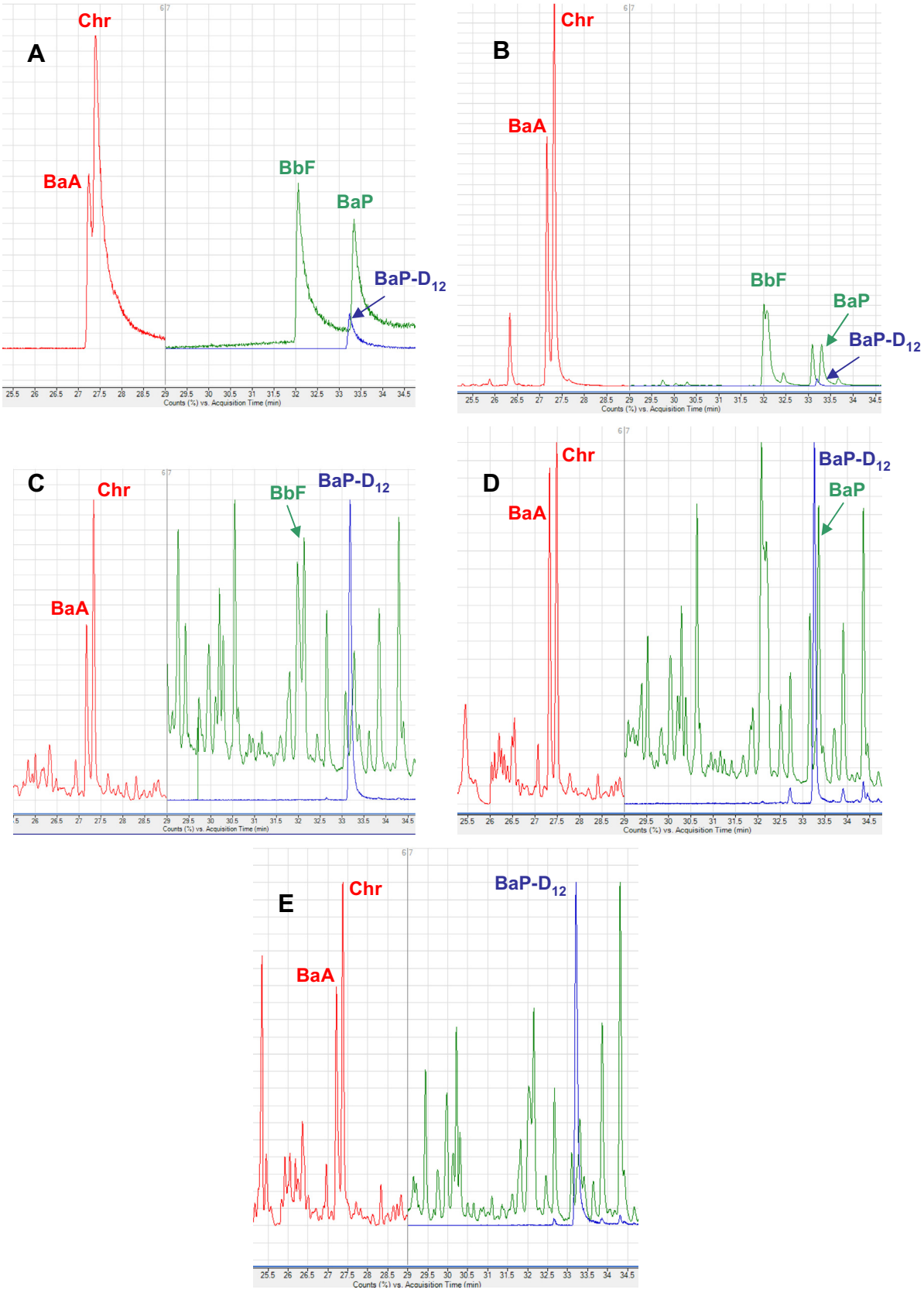

Fig. 1. Chromatograms of PAH4 standards at 25 μg/L: (A), a non-smoked tea spiked with PAH4 at 1 μg/kg (B), a non-spiked non-smoked tea [sample L] (C), a non-spiked smoked tea [sample A] (D) and a non-spiked infusion of a smoked tea [infusion of sample A] (E).

double SPE-purification step is also the method of choice presented in ISO standard 15735.

2.4. GC–MS/MS analysis

PAHs were analysed using an Agilent GC (7890 A) coupled to an Agilent tandem mass spectrometer (7000 A). The GC was equipped with an HP-5MS column (30 m × 0.25 mm × 0.25 μm) from Agilent (Santa Clara, CA), and the carrier gas was helium at a flow rate of 1.8 mL/min. The injector was kept at 260 °C, the transfer line at 250 °C and the ion source at 230 °C. The oven temperature was programmed as follows: 5 min at 70 °C, to 200 °C at 10 °C/min, hold 0.5 min, to 244 °C at 5 °C/min, hold 0.5 min, to 266 °C at 5 °C/min, hold 0.5 min, and to 300 °C at 5 °C/min, hold 5.5 min. Total run time was 48 min. Examples of chromatograms of all PAH4 standards at 25 μg/L (A), a non-smoked tea spiked with PAH4 at 1 μg/kg (B), a non-spiked non-smoked tea (C), a non-spiked smoked tea (D) and a non-spiked infusion of a smoked tea (E) are given in Fig. 1.

MS/MS analysis was carried out in electron impact mode. Tandem mass spectrometry (MS/MS) was operated in MRM (multiple reaction monitoring) mode. The monitored transitions of the PAH4 and BaP-D_{12} are given in Table 1.

2.5. Validation

2.5.1. Linearity, specificity and matrix effect

Blank samples (samples with amounts of PAH4 below the limit of detection) of non-smoked tea and non-smoked tea infusion were spiked with different amounts of PAHs (0, 0.1, 0.5, 1, 5 and 10 μg/kg and μg/L) and analysed as described above. Quantification of the peaks was done by integrating the area of the peaks and dividing it by the area of the peak of BaP-D_{12}. In order to assess the specificity of the method, the absence of parasite peaks on the chromatograms was verified (in quintuple: five aliquots of blank tea individually extracted and injected). Linearity was checked by calculating the correlation coefficient (r^2), and the matrix effect was investigated by comparing the slopes of the matrix-matched calibration curves with the slope of non-matrix-matched calibration curves.

2.5.2. Repeatability, reproducibility, accuracy and recovery

Blank samples of non-smoked tea were spiked with 1 μg/kg (μg/L) of each PAH, extracted and analysed, as described above. The quantification of the compounds was done on the basis of peak areas normalised with the areas of the respective internal standards, and comparison with a matrix-matched calibration curve. Tests were carried out in quintuplicate (five aliquots of the same sample individually extracted and injected). Repeatability (RSD_r) and reproducibility (RSD_R) were tested by calculating the HORRAT(r) and HORRAT(R) values, respectively. Accuracy was determined by measuring the degree of closeness of the measured concentration to the spiked concentration, and recovery was calculated by dividing the measured amount of PAH by the amount of spiking.

2.5.3. LOD and LOQ

The limit of detection (LOD) was defined as being the lowest concentration where both the quantifying and the qualifying transition presented a signal-to-noise ratio of 3. The limit of quantification (LOQ) was defined as being the lowest concentration where both the quantifying and the qualifying transition presented a signal-to-noise ratio of 10.

3. Results and discussion

3.1. Validation

All validation data are given in Table 2a (tea leaves) and Table 2b (infusions).

3.1.1. Linearity, specificity and matrix-effect

Calibration was done from 0.1 to 10 μg/kg for tea leaves, and from 0.1 to 10 μg/L for infusions. Correlation coefficients (r^2) are higher than 0.98 (leaves) and 0.99 (infusions), showing the linearity of the method over the entire calibration ranges. The chromatograms of the blank tea samples did not show any peak at the respective retention times, indicating that the method is very specific and the risk of wrong positives is very low. The slopes of the calibration curves in leaf samples diverge by 32% (BaA), 19% (Chr), 39% (BbF) and 33% (BaP), showing that there is a matrix effect for all compounds. Therefore the quantification of the PAHs in tea leave samples was made with matrix-matched calibration curves. For infusions, the slopes diverge less with differences of 18% (BaA), 19% (Chr), 15% (BbF) and 20% (BaP), probably because of the less complex matrix. However, it was decided to also use matrix-matched calibrations for tea infusions.

3.1.2. Repeatability, reproducibility, accuracy and recovery

The RSD_r and RSD_R produced HORRAT-values ranging from 0.4 to 1.0 (leaves) and 0.2 to 0.6 (infusions) and thus meet the criteria specified for tea leaves in Regulation 836/2011 of the European Commission ($HORRAT_r$ and $HORRAT_R$ less than 2). The accuracy of the method is acceptable with values ranging from 78% to 122% (leaves) and 89% to 105% (infusions).

The recovery values ranged from 53% to 82% (leaves) and from 67% to 88% (infusions), and thus meet the criteria specified in the Regulation 836/2011 for tea leaves (recovery between 50% and 120%). In leaves, the recovery values of BbF and BaP were lower than those of BaA and Chr and therefore the different steps of the sample preparation were evaluated separately. It was found that the recoveries of the QuEChERS extraction ranged from 74% to 83% for tea leaves and from 81% to 89% for infusions, the recoveries of the first SPE (on C_{18}) from 76% to 80%, the recoveries of the concentration in the rotary evaporator from 92% to 104%, the recoveries of the second SPE (on Florisil) from 75% to 86% and the recoveries of the final concentration under N_2 ranged from 80% to 109%. The recoveries of BbF and BaP in the QuEChERS extraction and the two SPEs were always lower than those of BaA and Chr, which is probably due to a lower solubility of these

Table 1
Quantifying and qualifying MRM transitions.

Compound	Quantifying transition			Qualifying transition		
	Parent ion [*m/z*]	Colision energy [*V*]	Daughter ion [*m/z*]	Parent ion [*m/z*]	Colision energy [*V*]	Daughter ion [*m/z*]
Benzo(a)anthracene	228	4	228	228	33	202
Chrysene	228	4	228	228	33	202
Benzo(b)fluoranthene	252	4	252	126	13	113
Benzo(a)pyrene	252	4	252	126	13	113
Benzo(a)pyrene-D_{12}	264	46	260			

Table 2a
Validation data for tea leaves.

	Benzo(a)anthracene	Chrysene	Benzo(b)fluoranthene	Benzo(a)pyrene
Matrix effect (slope without matrix)	5.5	8.6	1.6	1.3
Matrix effect (slope with matrix)	3.7	7.0	2.7	2.0
Linearity (r^2)	0.9878	0.9811	0.9975	0.9953
HORRAT (R)	0.5	0.5	0.4	0.4
HORRAT (r)	0.9	0.9	0.9	1.0
Accuracy (%)	114	122	109	78
Recovery	71	82	54	53
LQ (μg/kg)	0.3	0.4	0.5	0.6
LD (μg/kg)	0.2	0.2	0.3	0.3

Table 2b
Validation data for tea infusions.

	Benzo(a)anthracene	Chrysene	Benzo(b)fluoranthene	Benzo(a)pyrene
Matrix effect (slope without matrix)	5.0	8.5	2.7	2.3
Matrix effect (slope with matrix)	4.1	6.9	2.3	1.8
Linearity (r^2)	0.9947	0.9924	0.9991	0.9986
HORRAT (R)	0.4	0.6	0.4	0.4
HORRAT (r)	0.3	0.3	0.3	0.2
Accuracy (%)	98	105	92	89
Recovery	84	88	67	72
LQ (μg/L)	0.3	0.2	0.3	0.4
LD (μg/L)	0.1	0.1	0.1	0.1

compounds in the mix of acetonitrile:acetone 60:40 and a higher affinity for the SPE clean-up sorbents.

In a study with a similar sample preparation (QuEChERS extraction and SPE clean-up) the reported recovery values were slightly higher (80–103%, Dabrova et al., 2012) while in another study, on the analysis of PAHs in salmon, the recovery values were comparable to those obtained in the present study (56–96%; Forsberg, Wilson, & Anderson, 2011). Another study reported recovery values ranging from 82% to 109% (Danyi et al., 2009), though the extraction protocol consisted of only a solid–liquid extraction and concentration of the extract, without extensive purification (only a slight clean-up with diatomaceous earth and aluminium oxide in the extraction solvent), which results, according to the experience of the authors, in a fast fouling of the ion source in the mass spectrometer. Other studies also reported higher recovery values (88–162% in Schlemitz and Pfannhauser, 1997; 75–117% in Ziegenhals et al., 2008), though they used a different extraction method (supercritical fluid extraction) and only reported the relative recoveries.

3.1.3. LOD and LOQ

The LODs in leaves ranged from 0.2 to 0.3 μg/kg and the LOQs from 0.3 to 0.6 μg/kg and thus fulfil the performance criteria defined in the Regulation 836/2011/EC (limit of detection $\leqslant$0.3 μg/kg; limit of quantification $\leqslant$0.9 μg/kg). The LODs in infusions were 0.1 μg/L for all PAHs and the LOQs ranged from 0.2 to 0.4 μg/L.

Considering the presented validation data and the fact that the criteria specified in the Regulation 836/2011/EC are all fulfilled, it was concluded that the method was suitable for the proposed analyses.

3.2. PAH4 analysis in tea leaves

Ten samples of smoked tea (A–J) and 5 samples of non-smoked tea (K–O) were analysed as described above. When results were outside the calibration range, the sample was diluted tenfold or hundredfold in hexane and re-injected. The results are presented in Table 3.

BaA and Chr were detected in all samples of smoked an non-smoked teas, and their concentrations ranged from 11.3 to 125.0 μg/kg for BaA and from 12.3 to 93.0 μg/kg for Chr in smoked tea, and from 0.6 to 9.5 μg/kg for BaA and from 0.9 to 9.1 μg/kg for Chr in non-smoked tea. BbF was detected in 6 out of 10 samples for smoked tea and in 4 out of 5 samples for the non-smoked tea, and BaP was detected in 7 out of 10 samples for smoked tea and in 3 out of 5 samples for the non-smoked tea. The concentrations in smoked tea ranged from 1.2 to 34.4 μg/kg for BbF and from 2.8 to 21.9 μg/kg for BaP, and in non-smoked tea the concentrations ranged from 1.24 to 10.8 μg/kg for BbF and from 0.6 to 5.6 μg/kg for BaP.

The measured concentrations for non-smoked black tea are in line with previous findings of other research groups. Schlemitz and Pfannhauser (1997) measured concentrations ranging from 0.4 μg/kg (for BaP) to 45.4 μg/kg (for Chr); Ziegenhals et al. (2008) measured concentrations ranging from 0.8 μg/kg (for BaP) to 18.1 μg/kg (for Chr); Ishizaki et al. (2010) measured concentrations ranging from 4.3 μg/kg (for BaA) to 73.2 μg/kg (for BaP); Li et al. (2011) measured a mean BaP concentration of 9.4 μg/kg in black tea. Dabrova et al. (2012) measured higher maximum concentrations, with values ranging from 0.2 (for BaP) to 229.0 μg/kg (for Chr), though median values [from 1.4 μg/kg (BaA) to 10.4 μg/kg (Chr)] and mean values [from 20.7 μg/kg (BbF) to 41.9 μg/kg (Chr)] were similar to the concentrations measured in the present study. Lin, Tu, and Zhu (2005) also measured slightly higher concentrations, with values ranging from 37.6 μg/kg (for BbF) to 241.0 μg/kg (for Chr), although their median and mean values were not presented.

The concentrations measured in smoked tea cannot be compared to previous results as no such results have been published so far. However, when the concentrations measured in the present study are compared to the concentrations measured for the non-smoked tea, it seems that the concentrations of PAH4 on the smoked tea are considerably higher. Therefore, an α-risk statistical hypothesis test was done, with α equal to 95%, in order to conclude whether the chance that the observed differences are not due to random sampling is higher than 95% (when the p-value is below 0.05).

Table 3
PAH concentrations (in μg/kg) in smoked (A–J) and non-smoked (K–O) tea samples, and the sum of PAH4 (in μg/kg).

Sample	Benzo(a)anthracene	Chrysene	Benzo(b)fluoranthene	Benzo(a)pyrene	Sum of PAH4
A	11.3	12.3	<0.3	3.8	27.4
B	125.0	93.0	<0.3	<0.3	218.0
C	13.7	15.7	9.4	<0.3	38.8
D	39.3	46.3	<0.3	5.6	91.2
E	47.3	56.3	<0.3	6.6	110.2
F	34.6	38.8	6.4	5.4	85.2
G	22.8	24.6	4.2	2.8	54.4
H	86.3	92.2	1.2	<0.3	179.7
I	76.0	67.0	9.6	9.2	161.8
J	58.3	62.5	34.4	21.9	177.1
K	0.9	1.3	1.4	<0.3	3.6
L	1.7	1.9	2.1	0.6	6.3
M	9.5	9.1	10.8	5.6	35.0
N	1.5	1.6	1.8	0.6	5.5
O	0.6	0.9	<0.3	<0.3	1.5

Table 4
PAH concentrations (in μg/L) in infusions of smoked (A–J) tea samples, the sum of PAH4 (in μg/L), and the release of PAH4 from leaves into infusion.

Sample	Benzo(a)anthracene	Chrysene	Benzo(b)fluoranthene	Benzo(a)pyrene	Sum of PAH4	Release of PAHs from tea leaves
A	<0.3	<0.3	<0.1	<0.1	<0.8	–
B	0.6	0.8	1.2	<0.1	2.7	123%
C	<0.1	<0.1	<0.1	<0.1	<0.4	–
D	<0.3	<0.3	<0.1	<0.1	<0.8	–
E	<0.3	<0.3	<0.1	<0.1	<0.8	–
F	<0.1	<0.07	<0.1	<0.1	<0.4	–
G	<0.3	<0.3	<0.1	<0.1	<0.8	–
H	0.6	0.8	<0.1	<0.1	1.5	82%
I	0.7	0.9	<0.1	<0.1	1.6	97%
J	0.6	0.8	<0.1	<0.1	1.5	82%

It was found that the concentrations of BaA ($p = 0.0064$) and Chr ($p = 0.0004$) are higher on the smoked tea than on the non-smoked tea, though no difference was observed for BbF ($p = 0.3950$) and BaP ($p = 0.0979$). The difference between the concentrations of PAHs on smoked and non-smoked teas becomes more obvious when the sum of BaA, Chr, BbF and BaP (sum of PAH4, Table 3) is considered; the summed concentrations range from 27.4 to 218.0 μg/kg for smoked tea and from 3.6 to 35.0 μg/kg for non-smoked tea.

Although higher PAH concentrations were measured on smoked than on non-smoked tea, the smoked tea concentrations measured in this study are similar to the concentrations reported for Mate tea, which is also smoked over wood fire and where the following concentrations were measured: from 1.4 to 50.9 μg/kg (both times BaA) with mean concentrations of 10.6 μg/kg (BaA), 11.5 μg/kg (Chr), 12.8 μg/kg (BbF) and 13.6 μg/kg (BaP) in a study of Zuin et al. (2005); and from 24.8 μg/kg (BaP) to 746.3 μg/kg (Chr) with mean concentrations of 147.0 μg/kg (BaA), 276.2 μg/kg (Chr), 105.2 μg/kg (BbF) and 106.6 μg/kg (BaP) in a study of Ziegenhals et al. (2008). Schlemitz and Pfannhauser (1997) measured partially higher concentrations of PAHs on Mate tea: 1.7 and 306.6 μg/kg (BaA), 450.7 and 579.0 μg/kg (Chr), 1742.2 and 387.7 μg/kg (BbF) and 542.3 and 224.8 μg/kg (BaP).

3.3. PAH4 analysis in infusions of smoked tea leaves

Previous papers reported that the PAH concentrations in tea infusions are low compared to the concentrations measured in tea leaves: Lin et al. (2005) measured PAH4 concentrations of 0.07 μg/L for BaA and 0.06 μg/L for Chr after an extraction time of 10 min at >90 °C. BbF and BaP were not detected. Slightly higher concentrations were measured by Bishnoi, Mehta, Sain, and Pandit (2005) with concentrations ranging from 0.1 μg/L for BaA to 6.5 μg/L for BbF. BaP was not detected and Chr was not monitored. In a recent study, Loh, Sanagi, Ibrahim, and Hasan (2013) monitored BaP in 8 samples of green tea and they also did not detect any BaP. Therefore the release of PAHs from non-smoked tea leaves into tea infusions was not studied again and infusions in this study were only made for smoked tea samples.

Infusions of the 10 samples of smoked tea (A–J) were prepared and analysed, as described above. The results are presented in Table 4. BaA and Chr were detected in 8 out of 10 samples, though only 4 times above the LOQ (concentrations ranging from 0.6 to 0.7 μg/L for BaA and from 0.8 to 0.9 μg/L for Chr). BbF was detected only once (1.2 μg/L in sample E) and BaP was never detected. These concentrations are in a comparable range to the concentrations measured by Bishnoi et al. (2005) in infusions of non-smoked tea leaves. The sum of PAH4 ranged from 1.4 to 2.7 μg/kg. This shows that the concentrations of PAHs in tea infusion are quite low in comparison to the limits set for other foodstuffs in the regulation 835/2011/EC, though much higher than the limits set in the regulation 98/83/EC for drinking water. According to Lin et al. (2005) PAH release from tea increases with infusion time and therefore this time should be kept to a minimum for teas with high amounts of PAHs, like smoked tea or Mate tea.

3.4. Release of PAHs from tea leaves into the infusion

The concentrations of PAHs in tea infusions appear to be very low compared to those in tea leaves, though only 2 g of tea leaves were used to prepare 200 mL of tea infusion. Therefore, the release of PAHs from the leaves into the infusion was calculated for those infusion samples where PAHs > LOQ were detected. For reasons of simplicity it was assumed that the density of the tea infusion is 1 g/cm^3 and the release calculations and comparisons are based on the sum of PAH4. The release calculations are presented in Table 4.

The release of PAH4 from the leaves into the infusion ranged from 82% to 123%, suggesting that most of the PAHs deposited on the tea leaves migrate into the infusion. This is interesting because PAHs are non-polar and have a very low affinity for water, and such high release rates were not expected. However, Grover, Singh, and Pal (2013), and Lin and Zhu (2004) showed that the largest amounts of PAHs are not absorbed on the tea leaves during the growing of the plant but accumulate on it during the drying step where warm air is blown through the leaves, and are therefore deposed on the surface of the leaves where they might be washed away during the infusion. Secondly, tea contains essential oils and may be flavoured with oils (e.g., bergamot oil in Earl Grey tea or jasmine oil in jasmine tea) that are partially released into the infusion, too. This might change the physical and chemical properties of the water used for tea making and increase the affinity of PAHs for the aqueous infusion.

4. Conclusion

This study describes for the first time the analysis of PAHs in smoked tea. The concentrations are quite high compared to the limits fixed in the Regulation 835/2011/EC for other foodstuffs, and at least for BaA and Chr the measured concentrations were higher than in the comparative group of five non-smoked teas. The results of this study also suggest that the release rates of PAHs from the tea leaves into the infusions are high, even though the final PAH concentrations remain low. However, as the consumption of tea can be high for individuals, PAH intake from tea consumption might be an issue for those persons and tea should be included in official monitoring programs in order to assess potential risks to human health originating from smoked and non-smoked teas.

References

Bi, X., Sheng, G., Peng, P., Chen, Y., & Fu, J. (2005). Size distribution of n-alkanes and polycyclic aromatic hydrocarbons (PAHs) in urban and rural atmospheres of Gangzhou, China. *Atmospheric Environment, 39*(3), 477–487.

Bishnoi, N. R., Mehta, U., Sain, U., & Pandit, G. G. (2005). Quantification of polycyclic aromatic hydrocarbons in tea and coffee samples of Mumbai city (India) by high performance liquid chromatography. *Environmental Monitoring and Assessment, 107*, 399–406.

Chen, G., Cao, P., & Liu, R. (2011). A multi-residue method for fast determination of pesticides in tea by ultra performance liquid chromatography–electrospray tandem mass spectrometry combined with modified QuEChERS sample preparation procedure. *Food Chemistry, 2011*, 1406–1411.

Da Silva Pinto, M. (2013). Tea: A new perspective on health benefits. *Food Research International, 53*, 558–567.

Dabrova, L., Pulkrabova, J., Kalachova, K., Tomaniova, M., Kocourek, V., & Hajslova, J. (2012). Rapid determination of polycyclic aromatic hydrocarbons (PAHs) in tea using two-dimensional gas chromatography coupled with time of flight mass spectrometry. *Talanta, 100*, 207–216.

Danyi, S., Brose, F., Brasseur, C., Schneider, Y. J., Larondelle, Y., Pussemier, L., et al. (2009). Analysis of EU priority polycyclic aromatic hydrocarbons in food supplements using high performance liquid chromatography coupled to an ultraviolet, diode array or fluorescence detector. *Analytica Chimica Acta, 633*, 293–299.

EFSA, European Food Safety Agency, (2008). Polycyclic aromatic hydrocarbons in food. *The EFSA Journal, 724*, 96–114.

Fiedler, H., Cheung, C. K., & Wong, M. H. (2002). PCDD/PCDF, chlorinated pesticides and PAH in Chinese teas. *Chemosphere, 46*, 1429–1433.

Forsberg, N. D., Wilson, G. R., & Anderson, K. A. (2011). Determination of parent and substituted polycyclic aromatic hydrocarbons in high-fat salmon using a modified QuEChERS extraction, dispersive SPA and GC–MS. *Journal of Agricultural and Food Chemistry, 59*, 8108–8116.

Grover, I. S., Singh, S., & Pal, B. (2013). Priority PAHs in orthodox black tea during manufacturing process. *Environmental Monitoring and Assessment, 185*, 6291–6294.

Hu, J., Liu, C. Q., Zhang, G. P., & Zhang, Y. L. (2012). Seasonal variation and source appointment of PAHs in TSP in the atmosphere of Guiyang, Southwest China. *Atmospheric Research, 118*, 271–279.

Ishizaki, A., Saito, K., Hanioka, N., Narimatsu, S., & Kataoka, H. (2010). Determination of polycyclic aromatic hydrocarbons in food samples by automated on-line in-tube solid-phase microextraction coupled with high-performance liquid chromatography-fluorescence detection. *Journal of Chromatography A, 1217*, 5555–5563.

ISO 15753, 2006. Animal and vegetable fats and oils – Determination of Polycyclic Aromatic Hydrocarbons.

Jain, A., Manghani, C., Kohli, S., Nigam, D., & Rani, V. (2013). Tea and human health: The dark shadow. *Toxicology Letters, 220*, 82–87.

Li, X. Y., Li, N., Luo, H. D., Lin, L. R., Zou, Z. X., Jia, Y. Z., et al. (2011). A novel synchronous fluorescence spectroscopic approach for the rapid determination of three polycyclic aromatic hydrocarbons intea with simple microwave-assisted pretreatment of sample. *Journal of Agricultural Food and Chemistry, 59*, 5899–5905.

Lin, D., Tu, Y., & Zhu, L. (2005). Concentrations and health risk of polycyclic aromatic hydrocarobons in tea. *Food Chemistry and Toxicology, 43*, 41–48.

Lin, D., & Zhu, L. (2004). Polycyclic aromatic hydrocarbons: pollution and source analysis of a black tea. *Journal of Agricultural Food and Chemistry, 52*, 8268–8271.

Liu, J., Li, J., Lin, T., Liu, D., Xu, Y., Caemfa, C., et al. (2013). Diurnal and nocturnal variations of PAHs in the Lhasa atmosphere, Tibetan Plateau: Implication for local sources and the impact of atmospheric degradation processing. *Atmospheric Research, 124*, 34–43.

Loh, S. H., Sanagi, M. M., Ibrahim, W. A. W., & Hasan, M. N. (2013). Multi-walled carbon nanotube-impregnated agarose film microextraction of polycyclic aromatic hydrocarbons in green tea beverage. *Talanta, 106*, 200–205.

Lozano, A., Rajski, L., Belmonte-Valles, N., Uclés, A., Uclés, S., Mezcua, M., et al. (2012). Pesticide analysis in tea and chamomile by liquid chromatography and gas chromatography tandem mass spectrometry using a modified QuEChERS method: Validation and pilot survey in real samples. *Journal of Chromatography A, 1268*, 109–122.

Masih, J., Singhvi, R., Taneja, A., Kumar, K., & Masih, H. (2012). Gaseous/particulate bound polycyclic aromatic hydrocarbons (PAHs), seasonal variations in North central part of rural India. *Studies of Changing Societies, 3*, 30–36.

Schlemitz, S., & Pfannhauser, W. (1997). Supercritical fluid extraction of mononitrated polycyclic aromatic hydrocarbons from tea – correlation with the PAH concentration. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung, 205*, 305–310.

Sevastyanova, O., Binkova, B., Topinka, J., Sram, R. J., Kalina, I., Popov, T., et al. (2007). In vitro genotoxicity of PAH mixtures and organic extract from urban air particles: Part II: Human cell lines. *Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis, 620*, 123–134.

Spink, D. C., Wu, S. J., Spink, B. C., Hussain, M. M., Vakharia, D. D., Pentecost, B. T., et al. (2008). Induction of CYP1A1 and CYP1B1 by benzo(k)fluoranthene and benzo(a)pyrene in T-47D human breast cancer cells: Roles of PAH interactions and PAH metabolites. *Toxicology and Applied Pharmacology, 226*, 213–224.

Wickramasinghe, A. P., Karunaratne, D. G. G. P., & Sivakanesan, R. (2011). PM10-bound polycyclic aromatic hydrocarbons: Concentrations, source characterization and estimating their risks in urban, suburban and rural areas in Kandy, Sri Lanka. *Atmospheric Environment, 45*(16), 2642–2650.

Ziegenhals, K., Jira, W., & Speer, K. (2008). Polycyclic aromatic hydrocarbons (PAHs) in various types of tea. *European Food Research and Technologies, 228*, 83–91.

Zuin, V. G., Montero, L., Bauer, C., & Popp, P. (2005). Stir bar sorptive extraction and high-performance liquid chromatography-fluorescence detection for the determination of polycyclic aromatic hydrocarbons in Mate teas. *Journal of Chromatography A, 1091*, 2–10.