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Polycyclic Aromatic Hydrocarbon Contamination in Three Tea Samples Collected in Two Different Areas of Vietnam

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Abstract The present work was undertaken to identify and determine the concentration of PAHs in forty samples of three different types of tea (green, oolong and black teas) collected in two provinces of Vietnam (Thai Nguyen province in the North and Lam Dong province in the South). For sample preparation, the quick and simple QuEChRES procedure was used and eighteen PAHs congeners were detected by gas chromatography–tandem mass spectrometry (GC-MS/MS), with the percentage recovery higher than 70%. The concentration of the total 18 PAHs in tea samples ranged from 154.2 to 709.0 µg/kg with the highest of total PAHs found in black tea samples. Nine PAHs congeners were found in all samples with wide ranges of concentrations as follows: 1-methylnaphthalene (3.6 – 73.1 µg/kg), Acenaphthene (1.6 – 45.0 µg/kg), Fluorene (4.2 – 49.5 µg/kg), Anthracene (2.1 – 40.0 µg/kg), Pyrene (19.3 – 224.9 µg/kg), Chrysene (4.6 – 233.0 µg/kg), Benzo (b) fluoranthene (0.6 – 23.7 µg/kg), Indeno (1,2,3,cd) pyrene (1.0 – 38.4 µg/kg), and Dibenzo (a,h) anthraxcene (1.1 – 25.0 µg/kg). 3–4 rings PAHs were dominant in all tea samples, with a contribution of 66.0 – 84.3% of the total 18 PAHs content. The average content of the indicated PAHs in oolong tea from the South (OS) was the lowest and that of black tea from the South (BS) was the highest. It was also observed that the toxic equivalent (TEQ) values of tea samples from the North higher than those from the South.

Keywords: *tea, food safety, polycyclic aromatic hydrocarbons (PAHs), QuEChERS, gas chromatography–tandem mass spectrometry (GC-MS/MS)*

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1. Introduction

Tea is one of the most consumed beverages with the global tea market was valued at around USD 49,456.52 million in 2017 and is expected to reach approximately USD 73,132.82 million by 2024 [1]. The health effects of tea leaves have been widely studied [2,3,4]. There are a lot of scientific reports indicating that tea consumption might have health promoting properties like cholesterol reduction, antioxidant features, and protection against cardiovascular disease [5,6,7,8,9]. But it has also been suggested that certain pollutants in tea leaves may pose a health threat to tea drinkers [10,11].

Polycyclic aromatic hydrocarbons (PAHs) are a group of organic compounds consisting of conjugated benzene rings arranged in different configurations. It has been shown that many of PAHs are toxic, and some of them have been proven carcinogenic and genotoxic [12,13,14,15,16]. The US Environmental Protection Agency (EPA) has identified 16 PAHs as priority environmental pollutants to include Acenaphthene (ACP), Acenaphthylene (ACNP), Anthracene (AN), Benzo(a)anthracene (B(a)A), Benzo(a)pyrene (B(a)P), Benzo(b)fluoranthene (B(b)F), Benzo(g,h,i)perylene (B(ghi)P), Benzo(k)fluoranthene (B(k)F), Chrysene (Chy), Dibenz(a,h)anthracene (DBA), Fluoranthene (FLA), Fluorene (FL), Indeno(1,2,3-cd)pyrene (IP), Naphthalene (NaP), Phenanthrene (PHN), Pyrene (Py) [17]. In particular, B(a)P is a well-known substance classified by the International Agency for Research on Cancer (IARC) into group 1 of carcinogens—i.e., factors with proven harmful (carcinogenic) effects on the human body—while B(b)F, Chy and B(a)A are classified into group 2B—i.e., group of compounds with a possible carcinogenic effect on the human body [18,19,20]. These compounds are commonly called PAH4 which can serve as an indicator for the occurrence of PAHs in food [21,22]. Following the scientific opinion of the European Food Safety Authority (EFSA), the European Commission fixed limits for the sum of PAH4 in different foodstuffs in the amendment 835/2011 of the regulation 1881/2006

[18,19,20]. A major route of PAHs exposure to the human beings is through consumption of food which can be contaminated with PAHs from environmental sources, industrial food processing and certain home-cooking practices [19,23].

Plants can adsorb PAHs especially from air. Therefore, tea can easily reach high level of PAHs accumulation due to a high surface area of leaves [5,18,19,24]. Moreover, another source of PAHs may come from the manufacturing process of tea leaves, like drying step by burning wood, oil, or coal [7,25,26,27]. Black tea is produced by fermenting the slightly withered leaves for many hours before being either smoke fired, flame fired, or steamed [5,6,19]. In contrast, green tea is not fermented, but the leaves are steamed or pan fired to inactivate the polyphenol oxidase, thus avoiding oxidation [24,28]. Oolong tea is prepared by withering the fresh leaves in the sun, then bruising them slightly, and partially fermenting. The color of oolong tea is intermediate between that of green and black tea [29,30]. Since, many of them, especially black tea, are dried may also be contaminated with PAHs. Otherwise, the presence of PAHs in tea may also come from water, sediments, and particulate air containing PAHs [19,25,31]. Thus, tea consumption can cause human exposure to PAHs with possible threat for human health.

The main objectives of the present study were to determine PAHs content in three brands of Vietnamese tea (green tea, oolong tea and black tea) collected in two different areas: Thai Nguyen province (in the north of Vietnam) and Lam Dong province (in south of Vietnam). PAHs analyses was done by using QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) technique. The main advantages of this technique are the speed and ease of sample preparation and environmental safety due to the low consumption of chemical reagents, as well as lower cost of analysis compared to other methods [32-37]. The analysis of the final extracts was made by Gas Chromatography-Triple Quadrupole Mass Spectrometer (*GC-MS/MS*). Further, we used a central composite design (CCD) and a response surface methodology (RSM) to develop a modeling to study the transfer of PAHs from tea into tea infusion. This modeling examines the main and/or interactive effects of a factorial combination of brewing times, V_{tea}/V_{water} ratio and temperature. Finally, we analyzed the health risk to the tea drinkers by calculating the toxic equivalents (TEQ) of tea samples.

2. Materials and Methods

2.1. Samples

Forty samples from three different types of tea (green tea, oolong tea and black tea) were of different manufacturers in the north (Thai Nguyen province) and the south (Lam Dong province) of Vietnam. Before analysis the samples were stored according to the recommendations given on packages, i.e., in a dry place away from light. Each sample was given a code number according to their type as shown in Table 1.

Table 1. Tea Type, Area, and Their Code Numbers

Sample #	Tea samples	Area	Code
1-7	Green tea	North	GN1 – GN7
8-14	Green tea	South	GS1 – GS7
15-19	Oolong tea	North	ON1 – ON5
20-24	Oolong tea	South	OS1 – OS5
25-35	Black tea	North	BN1 – BN11
36-40	Black tea	South	BS1 – BS5

2.2. Chemicals

The standard mixture of 18 PAHs in solvent acetonitrile: toluene (92:8) (EPA Method 8310 PAH Mixture, Restek, Bellefonte, PA, USA) includes acenaphthene, acenaphthylene, anthracene, benz(a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(g,h,i)perylene, benzo(k)fluoranthene, chrysene, dibenz(a,h)anthracene, fluoranthene, fluorene, indeno(1,2,3-cd)pyrene, 1-methylnaphthalene, 2-methylnaphthalene, naphthalene, phenanthrene, and pyrene. Isotope compounds were obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany), including the internal standards: benzo(a)anthracence-13C6, and benzo(g,h,i)pyrylene-13C12. All solvents (acetonitrile, *n*-hexane), which are of HPLC grade or pro analysis, were obtained from Fisher chemical (Pittsburgh, PA, USA). $MgSO_4$ and NaCl were obtained from Merck. The sorbent Primary Secondary Amin (PSA) and octadecylsilane (C18) were purchased from Agilent (Santa Clara, CA, USA).

2.3. Methods

2.3.1. Sample Preparation

Forty samples of three tea types collected in Thai Nguyen province (the North) and Lam Dong province (the South) were taken without grinding. The sample preparation was reported elsewhere [38,39,40]. Briefly, the procedure diagram for sample preparation for the determination of PAHs using GC-MS/MS is presented in Figure 1.

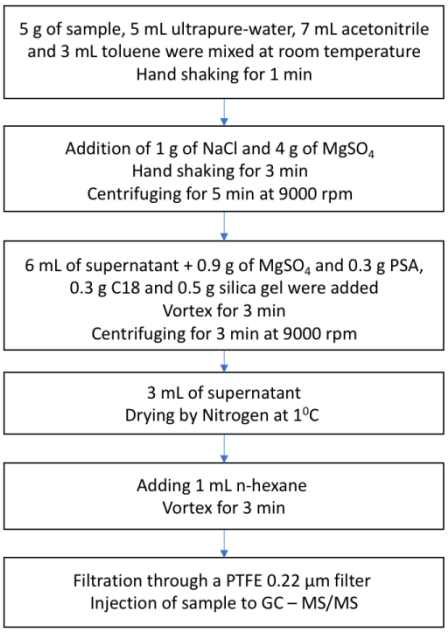

Figure 1. Procedure diagram for tea sample preparation for the determination of polycyclic aromatic hydrocarbons (PAHs) using GC-MS/MS

2.3.2. GC – MS/MS Analysis

GC–MS/MS analyses were performed on a Thermo Fisher Scientific (Waltham, MA, USA) system consisting of a Trace GC 1310 gas chromatograph, a TriPlus RSH Autosampler, and TSQ 8000 mass spectrometer (Thermo, Waltham, MA, USA). The TraceFinder software from Thermo Fisher Scientific was used for data processing. A DB5-MS (30 m × 0.25 mm, 0.25 μm) gas chromatography column was used to separate PAHs (Agilent, USA). Helium was used as a carrier gas at 1 mL/min. The following temperature program was used: isothermal at 70 °C for 1 min, then temperature was increased by 10 °C/min to 270 °C, then by 2 °C/min to 280 °C, then held for 3 min, then increased by 2 °C/min to 310 °C, and finally held for 1 min. The GC was interfaced by a heated transfer liner (310 °C) to the mass spectrometer in electron ionization mode with an electron energy of 70 eV. Nitrogen was used as the collision gas at the rate 1 mL/min. The criteria for the identification of PAHs were both the same retention times as the standard within ±2%, and correct relative abundance of two characteristic ions within ±15%. Identifying and quantifying ions, retention time, and collision energy are listed in **Supplemental Table S1.**

2.3.3. Validation

Linearity, specificity and matrix effect

Spiking was performed at 1 μg/kg with at least three series of duplicate blank sample to obtain the limit of detection as well as the repeatability. To achieve intermediate reproducibility, additional spiking at 8 and 12 of duplicate samples in three series was included. 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.

Recovery

The standard addition method was used to evaluate the recovery of PAHs. The standard of 18 PAHs and the isotopic standards of benzo(a)anthracence-13C6 and benzo(g,h,i)pyrylene-13C12 at concentration of 10 μg/mL were diluted by adding acetonitrile to 100 ng/mL, followed by adding water to provide the blank samples with a target concentration of 10 ng/mL. Accurate 5.00 mL of this standard solution was subjected into the sample treatment processing. The isotopic standards were used during the study to assess the recovery of the method and estimate the background effect as well as control the uncertainty of the GC–MS/MS analysis. The quantification of the compounds was done on the basis of peak areas normalized with the areas of the respective internal standards, and comparison with a matrix-matched calibration curve. Recovery for the method was based on spiking at 10 μg/kg with six replicates. For each spiked sample, the individual PAH concentration was determined.

Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection (LOD) was calculated as three times of the standard deviation on the calculated amount in each of the spiked samples. The limit of quantification (LOQ) was the lowest concentration where both the quantifying and the qualifying transition presented a signal-to-noise ratio of 10.

2.3.4. Release of PAHs from Tea Leaves into to the Infusion Modeling

PAHs can be transferred from dried tea leaves into hot water during infusion preparation. Therefore, contamination extent of this commodity should be controlled by a reliable method. In this study, three factors, utilized to determine the release of PAHs from tea leaves into the infusion, are brewing times (X_1), V_{tea}/V_{water} ratio (X_2) and temperature (X_3). A response surface methodology (RSM) was used to examine these factors on the release of PAHs. Independent variable and experiment level were summarized in the **Supplemental Table S2**. A total of 20 trials were conducted to optimize the releasing process for the quantitative analysis of PAHs into the infusion. The relationship between the response function Y and the coded variables (X1, X2, X3, and X4) is indicated in the following equation:

$$Y = \beta_0 + \beta_i \sum x_i + \beta_{ii} \sum x_i^2 + \beta_{ij} \sum x_i x_j \quad (1)$$

where Y is a response function; x_i and x_j are independent variables; β_0 is a constant; and β_i, β_{ii}, and β_{ij} are linear, quadratic, and interactive coefficients, respectively.

The appropriate fitting model for the response was selected based on the comparison of various statistical parameters such as R^2, Q^2, lack of fit and adequate precision.

2.3.5. Calculation of Toxic Equivalent of PAHs

Although PAHs themselves are not direct carcinogens, some of them can be converted into carcinogenic derivatives when metabolized. To estimate the overall carcinogenic potential of the PAHs, toxic equivalency factors (TEF) have been developed. The factor for each of the PAHs expresses its potency relative to B(a)P and the concentrations of each of the individual PAH compounds multiplied by its TEF are summed to yield B(a)P equivalent concentrations, as follows:

$$TEQ = \sum PAH_i \times TEF_i \quad (2)$$

where TEQ is the toxic equivalents of reference compound; PAH_i and TEF_i are the concentration and TEF, respectively, for individual PAH congeners. The list of TEF reported by Nisbet and LaGoy are shown in **Supplemental Table S3**.

By this means, the concentrations of a suite of PAHs can be represented by a single concentration, which reflects the overall carcinogenic potential of the PAHs within the sample for which TEFs have been assigned.

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3. Results and Discussion

3.1. Method Validation

The LOD of 18 PAHs and LOQ ranged from 0.01-0.20 μg L^{-1} and 0.03-0.60 μg L^{-1}, respectively. According to EU regulatory requirements for 18 PAHs analysis, LOD is 0.3 μg L^{-1}. Therefore, the current method is acceptable and appropriate for analysis of PAHs in team samples. Acceptable intermediate reproducibility of 5-20% relative standards deviations (RSD_r) were found to be within the criterion of $RSD_r < 23\%$. The repeatability relative standard deviation RSD_R was acceptable within the criterion of $RSD_R < 23\%$ (Supplemental Table S4)

Recovery

The recovery of 18 PAHs ranged from 70 to 101%. Some PAHs such as NaP, M2N and M1N had low recovery rates caused by the fact that they are volatile compounds. Despite efforts to minimize the loss by performing solvent removal under low temperature conditions, these substances were still partially evaporated. High molecular weight compounds such as IP, BDA and B(g,h,i)P gave low recovery, due to the results of using C18 to eliminate and absorb available fat in the sample which also absorbed high molecular weight and non-polar PAHs. The recovery of isotopic standards was more than 90% and did not show loss of sample. For the purpose of identifying multiple compounds simultaneously in one analytical process, the recovery of the substances had to be above 80% (except for NaP, which was recovered at 70%), the process was highly quantitative that enables to identify and quantify the concentration of PAHs in the food sample.

3.2. Levels of PAHs in the Samples

Following successful method validation, this study measured PAH levels of tea leaves (green, black and oolong tea, each from two different areas in Vietnam: Thai Nguyen province in the North of Vietnam and Lam Dong province in the South of Vietnam). A total of forty tea samples (7 green tea from the North, 7 green tea from the South, 5 oolong tea from the North, 5 oolong tea from the South, 11 black tea from the North and 5 black tea from the South) were collected at the Hanoi market in 2017; the aggregated data are shown in Table 2.

Table 2. PAHs Concentration in Three Different Types of Tea Collected in the North and the South of Vietnam

			Sample ID								
No.	PAHs	Abbr.	**GN1**	**GN2**	**GN3**	**GN4**	**GN5**	**GN6**	**GN7**	**Mean**	**Stdev**
1	Naphthalene	NaP	11.21		4.20	13.45	15.69	10.20	11.20	**10.99**	3.9
2	2-Methylnaphthalene	M2N	21.45	2.40	2.40	25.74	30.03	12.10	7.65	**14.54**	11.3
3	1-methylnapththalene	M1N	23.75	43.10	3.60	28.50	33.25	13.67	6.70	**21.80**	14.5
4	Acenaphthylene	ACNP	13.30	4.80	4.80	15.96	3.99	1.12		**7.33**	5.9
5	Acenaphthene	ACP	8.03	6.00	6.00	9.64	11.24	3.12	12.76	**8.11**	3.4
6	Fluorene	FL	14.52	7.20	7.20	17.42	20.33	11.56	12.60	**12.98**	4.9
7	Phenanthrene	PHN		8.40	8.40			19.92	3.27	**10.00**	7.0
8	Anthracene	AN	3.14	6.87	9.60	3.77	4.40	7.55	5.43	**5.82**	2.3
9	Fluoranthene	FLA	37.80	10.80	10.80	45.36	18.90	7.72	13.65	**20.72**	14.8
10	Pyrene	Py	66.25	82.00	102.20	79.50	92.75	124.34	59.80	**86.69**	22.0
11	Benzo (a) anthracene	B(a)A	2.17	13.20	13.20	2.60	3.04	1.14	0.56	**5.13**	5.6
12	Chrysene	CHR	73.52	103.20	135.30	101.01	62.71	42.95	111.53	**90.03**	31.7
13	Benzo (b) fluoranthene	B(b)F	3.23	23.70	15.60	3.88	4.52	3.51	2.56	**8.14**	8.2
14	Benzo (k) fluoranthene	B(k)F	2.68	16.80	16.80	3.22	3.75	2.57		**7.64**	7.1
15	Benzo (a) pyrene	B(a)P	7.92	2.51	6.63	1.04	2.20	6.63	1.45	**4.05**	2.9
16	Indeno (1,2,3,cd) pyrene	IP	27.42	20.40	20.40	32.90	38.39	10.01	17.60	**23.88**	9.7
17	Dibenzo (a,h) anthraxcene	BDA	23.57	19.20	19.20	28.28	16.50	3.37	1.11	**15.89**	10.1
18	Benzo (ghi) perylene	B(ghi)P	2.29	3.43	2.00	2.74	3.20	2.00	1.87	**2.50**	0.6
		SUM	**342.25**	**374.01**	**388.33**	**415.02**	**364.89**	**283.49**	**269.74**	**356.24**	53.8
			Sample ID								
No.	PAHs	Abbr.	**GS1**	**GS2**	**GS3**	**GS4**	**GS5**	**GS6**	**GS7**	**Mean**	**Stdev**
1	Naphthalene	NaP	1.18		0.71	0.94	1.42	1.65	1.89	**1.11**	0.4
2	2-Methylnaphthalene	M2N	24.77	9.91	22.18	19.82	29.72	34.68	39.63	**25.81**	9.9
3	1-methylnapththalene	M1N	29.57	19.50	17.74	23.66	35.48	41.40	31.60	**28.42**	8.6
4	Acenaphthylene	ACNP	9.45	3.78	5.67	7.56	11.34	13.23	15.12	**9.45**	4.1
5	Acenaphthene	ACP	12.72	5.09	7.63	10.17	15.26	17.80	20.34	**12.72**	5.5
6	Fluorene	FL	30.96	22.31	18.58	24.77	37.15	43.34	49.54	**32.38**	11.5
7	Phenanthrene	PHN	5.06	3.21	3.24	4.04	6.07	7.08	8.32	**5.29**	2.0
8	Anthracene	AN	7.86	2.08	4.72	6.29	9.43	11.00	12.58	**7.71**	3.7
9	Fluoranthene	FLA	21.16	8.46	12.69	16.93	25.39	29.62	33.85	**21.16**	9.1
10	Pyrene	Py	87.93	75.17	52.76	70.35	95.52	83.11	100.69	**80.79**	16.3
11	Benzo (a) anthracene	B(a)A	2.04		1.23	1.63	2.45	2.86	3.27	**1.93**	0.8
12	Chrysene	CHR	66.88	54.30	39.70	53.51	80.26	53.24	68.70	**59.51**	13.3
13	Benzo (b) fluoranthene	B(b)F	6.92	2.77	4.15	5.53	8.30	9.68	11.06	**6.92**	3.0
14	Benzo (k) fluoranthene	B(k)F	5.65	2.26	3.39	4.52	6.78	7.91	9.04	**5.65**	2.4
15	Benzo (a) pyrene	B(a)P	3.21	2.12	2.16	2.57	3.85	4.12	4.23	**3.18**	0.9
16	Indeno (1,2,3,cd) pyrene	IP	3.32	1.33	1.99	2.66	3.99	4.65	5.32	**3.32**	1.4
17	Dibenzo (a,h) anthraxcene	BDA	3.98	1.59	2.39	3.19	4.78	5.58	6.37	**3.98**	1.7
18	Benzo (ghi) perylene	B(ghi)P	2.86	1.14	1.71	2.29	3.43	4.00	4.57	**2.86**	1.2
		SUM	**325.52**	**215.02**	**202.64**	**260.41**	**380.62**	**374.95**	**426.12**	**312.18**	87.5

			Sample ID						
No.	PAHs	Abbr.	**ON1**	**ON2**	**ON3**	**ON4**	**ON5**	**Mean**	**Stdev**
1	Naphthalene	NaP	2.48	1.49	1.99	2.98	3.48	**2.48**	0.8
2	2-Methylnaphthalene	M2N	30.31	40.63	24.25	16.45		**27.91**	10.2
3	1-methylnapththalene	M1N	15.86	9.51	5.62	19.03	22.20	**14.44**	6.8
4	Acenaphthylene	ACNP	2.43	1.46	1.94	4.21	3.40	**2.69**	1.1
5	Acenaphthene	ACP	6.60	3.96	5.28	7.92	1.67	**5.09**	2.4
6	Fluorene	FL	14.30	8.58	11.44	4.21	20.02	**11.71**	6.0
7	Phenanthrene	PHN	9.45	5.67	7.56	11.34	13.23	**9.45**	3.0
8	Anthracene	AN	4.35	3.90	5.43	8.20	6.54	**5.68**	1.7
9	Fluoranthene	FLA	1.05	-	0.84	1.26	1.47	**1.15**	0.3
10	Pyrene	Py	56.92	79.61	63.35	74.34	46.70	**64.18**	13.2
11	Benzo (a) anthracene	B(a)A	0.57	0.76	1.14	1.14	2.13	**1.15**	0.6
12	Chrysene	CHR	41.01	38.08	61.05	22.95	30.30	**38.68**	14.4
13	Benzo (b) fluoranthene	B(b)F	0.69	1.10	0.55	0.83	0.96	**0.83**	0.2
14	Benzo (k) fluoranthene	B(k)F							
15	Benzo (a) pyrene	B(a)P	0.91		0.73	1.09	1.82	**1.14**	0.5
16	Indeno (1,2,3,cd) pyrene	IP	2.25	3.37	3.22	3.21	4.52	**3.31**	0.8
17	Dibenzo (a,h) anthraxcene	BDA	6.68	9.81	10.01	11.30	23.20	**12.20**	6.4
18	Benzo (ghi) perylene	B(ghi)P	2.29	12.48	2.00	1.57		**4.58**	5.3
		SUM	**198.14**	**220.42**	**206.40**	**192.03**	**181.64**	**206.68**	14.7

			Sample ID						
No.	PAHs	Abbr.	**OS1**	**OS2**	**OS3**	**OS4**	**OS5**	**Mean**	**stdev**
1	Naphthalene	NaP	1.60	0.96	1.28	1.91	2.20	**1.59**	0.5
2	2-Methylnaphthalene	M2N	20.09	12.05	16.07	24.10	24.10	**19.28**	5.2
3	1-methylnapththalene	M1N	11.40	36.08	23.75	33.10	13.67	**23.60**	11.1
4	Acenaphthylene	ACNP	0.93			1.12	1.12	**0.63**	0.1
5	Acenaphthene	ACP	2.60	1.56	45.00	3.12	3.12	**11.08**	19.0
6	Fluorene	FL	9.63	5.78	7.71	11.56	11.56	**9.25**	2.5
7	Phenanthrene	PHN	16.60	21.53	13.28	19.92	19.92	**18.25**	3.3
8	Anthracene	AN	32.96	38.91	33.14	26.87	39.55	**34.29**	5.2
9	Fluoranthene	FLA	6.43	3.86	5.15	7.72	7.72	**6.18**	1.7
10	Pyrene	Py	32.16	19.30	25.73	38.60	38.60	**30.88**	8.4
11	Benzo (a) anthracene	B(a)A	0.73			1.46	2.32	**0.90**	0.8
12	Chrysene	CHR	6.78	11.70	23.15	32.10	4.58	**15.66**	11.7
13	Benzo (b) fluoranthene	B(b)F	2.92	15.20	2.34	23.70	3.51	**9.53**	9.5
14	Benzo (k) fluoranthene	B(k)F	2.14	1.29	1.71	2.57	2.57	**2.06**	0.6
15	Benzo (a) pyrene	B(a)P	1.05		2.05	2.51	6.63	**2.45**	2.5
16	Indeno (1,2,3,cd) pyrene	IP	2.81	1.69	2.25	3.37	3.37	**2.70**	0.7
17	Dibenzo (a,h) anthraxcene	BDA	3.34	2.01	2.68	4.01	4.01	**3.21**	0.9
18	Benzo (ghi) perylene	B(ghi)P		1.14		1.24	3.43	**1.16**	1.3
		SUM	**154.18**	**173.05**	**205.27**	**238.99**	**191.99**	**192.70**	32.3

			Sample ID						
No.	PAHs	Abbr.	**BN1**	**BN2**	**BN3**	**BN4**	**BN5**	**BN6**	**BN7**
1	Naphthalene	NaP	2.60	1.56	2.08	5.19	4.20	3.54	13.67
2	2-Methylnaphthalene	M2N	10.09	6.05	8.07	12.10	12.10	1.26	17.42
3	1-methylnapththalene	M1N	21.40	36.08	23.75	73.10	25.67	14.23	43.20
4	Acenaphthylene	ACNP	8.93	23.23	7.15	37.52	10.72		
5	Acenaphthene	ACP	2.60	11.97	45.00	4.42	3.90	1.56	4.65
6	Fluorene	FL	9.63	28.90	7.71	11.56	11.56	28.30	46.20
7	Phenanthrene	PHN	16.60	21.53	13.28	19.92	19.92	4.53	8.65
8	Anthracene	AN	22.96	18.91	3.14	26.87	27.55	14.35	33.90
9	Fluoranthene	FLA	6.43	3.86	5.15	11.58	7.72	12.32	24.67
10	Pyrene	Py	40.16	224.92	152.62	208.86	168.69	46.70	56.70
11	Benzo (a) anthracene	B(a)A	1.95	3.12	7.41	6.24	2.34	2.13	3.21
12	Chrysene	CHR	210.10	121.01	168.08	211.05	123.00	180.30	238.70
13	Benzo (b) fluoranthene	B(b)F	2.92	5.20	2.34	23.70	3.51	7.64	12.45
14	Benzo (k) fluoranthene	B(k)F	2.14	3.43	1.71	8.57	4.71	1.23	2.54
15	Benzo (a) pyrene	B(a)P	5.05		2.05	6.51	6.63		6.89
16	Indeno (1,2,3,cd) pyrene	IP	2.81	1.69	2.25	3.37	9.00	3.21	4.52
17	Dibenzo (a,h) anthraxcene	BDA	8.34	5.01	6.68	25.03	10.01	11.30	23.20
18	Benzo (ghi) perylene	B(ghi)P	18.14	1.71	2.29	13.43	12.00	1.57	
		SUM	**392.87**	**518.17**	**460.74**	**709.03**	**463.24**	**334.17**	**540.57**

			Sample ID					
No.	PAHs	Abbr.	**BN8**	**BN9**	**BN10**	**BN11**	**Mean**	**stdev**
1	Naphthalene	NaP	11.20		14.57	23.90	**8.25**	7.3
2	2-Methylnaphthalene	M2N	7.65	2.34	6.75	6.54	**8.22**	4.6
3	1-methylnapththalene	M1N	6.70	13.65	13.20	25.30	**26.93**	18.6
4	Acenaphthylene	ACNP			1.34		**14.81**	13.3
5	Acenaphthene	ACP	2.76	10.54	3.65	6.12	**8.83**	12.4
6	Fluorene	FL	12.60	27.30	9.87	12.57	**18.75**	12.2
7	Phenanthrene	PHN	13.27	5.32	22.31	4.65	**13.63**	7.0
8	Anthracene	AN	5.43	11.20	16.54	37.32	**19.83**	11.1
9	Fluoranthene	FLA	13.65	19.80	5.32	7.98	**10.77**	6.6
10	Pyrene	Py	69.80	87.60	45.40	104.30	**109.61**	68.0
11	Benzo (a) anthracene	B(a)A		4.32	2.34	1.67	**3.47**	1.9
12	Chrysene	CHR	191.00	145.20	217.90	123.78	**175.47**	42.3
13	Benzo (b) fluoranthene	B(b)F	2.56	3.45	1.89	2.56	**6.20**	6.6
14	Benzo (k) fluoranthene	B(k)F		1.21	2.01	5.13	**3.27**	2.3
15	Benzo (a) pyrene	B(a)P	9.45	20.97	21.32	13.06	**10.21**	6.9
16	Indeno (1,2,3,cd) pyrene	IP	11.11	0.98	2.43	3.20	**4.05**	3.1
17	Dibenzo (a,h) anthraxcene	BDA	17.60	9.87	4.76	7.69	**11.77**	7.0
18	Benzo (ghi) perylene	B(ghi)P	1.87	10.87	7.11	6.93	**7.59**	5.8
		SUM	**376.65**	**374.62**	**398.71**	**392.70**	**461.69**	106.7

			Sample ID						
No.	PAHs	Abbr.	**BS1**	**BS2**	**BS3**	**BS4**	**BS5**	**Mean**	**stdev**
1	Naphthalene	NaP	11.60	6.96	9.28	13.91	3.20	**8.99**	4.1
2	2-Methylnaphthalene	M2N	20.09	12.05	16.07	24.10	24.10	**19.28**	5.2
3	1-methylnapththalene	M1N	11.40	36.08	23.75	73.10	13.67	**31.60**	25.2
4	Acenaphthylene	ACNP	10.93	6.56	8.75	13.12	13.12	**10.50**	2.9
5	Acenaphthene	ACP	2.60	1.56	45.00	3.12	3.12	**11.08**	19.0
6	Fluorene	FL	9.63	5.78	7.71	11.56	11.56	**9.25**	2.5
7	Phenanthrene	PHN	16.60	21.53	13.28	19.92	19.92	**18.25**	3.3
8	Anthracene	AN	32.96	38.91	33.14	26.87	39.55	**34.29**	5.2
9	Fluoranthene	FLA	6.43	3.86	5.15	7.72	7.72	**6.18**	1.7
10	Pyrene	Py	132.16	79.30	105.73	158.60	158.60	**126.88**	34.5
11	Benzo (a) anthracene	B(a)A	2.13	3.21	0.56	4.32	2.34	**2.51**	1.4
12	Chrysene	CHR	221.10	276.01	176.88	191.05	232.95	**219.60**	38.7
13	Benzo (b) fluoranthene	B(b)F	2.92	15.20	2.34	23.70	3.51	**9.53**	9.5
14	Benzo (k) fluoranthene	B(k)F	2.14	1.29	1.71	2.57	2.57	**2.06**	0.6
15	Benzo (a) pyrene	B(a)P	1.05		2.05	2.51	6.63	**2.45**	2.5
16	Indeno (1,2,3,cd) pyrene	IP	2.81	1.69	2.25	3.37	3.37	**2.70**	0.7
17	Dibenzo (a,h) anthraxcene	BDA	3.34	2.01	2.68	4.01	4.01	**3.21**	0.9
18	Benzo (ghi) perylene	B(ghi)P	2.29	2.48	2.00	1.57		**1.67**	0.4
		SUM	**492.19**	**514.47**	**458.31**	**585.14**	**549.95**	**520.01**	49.4

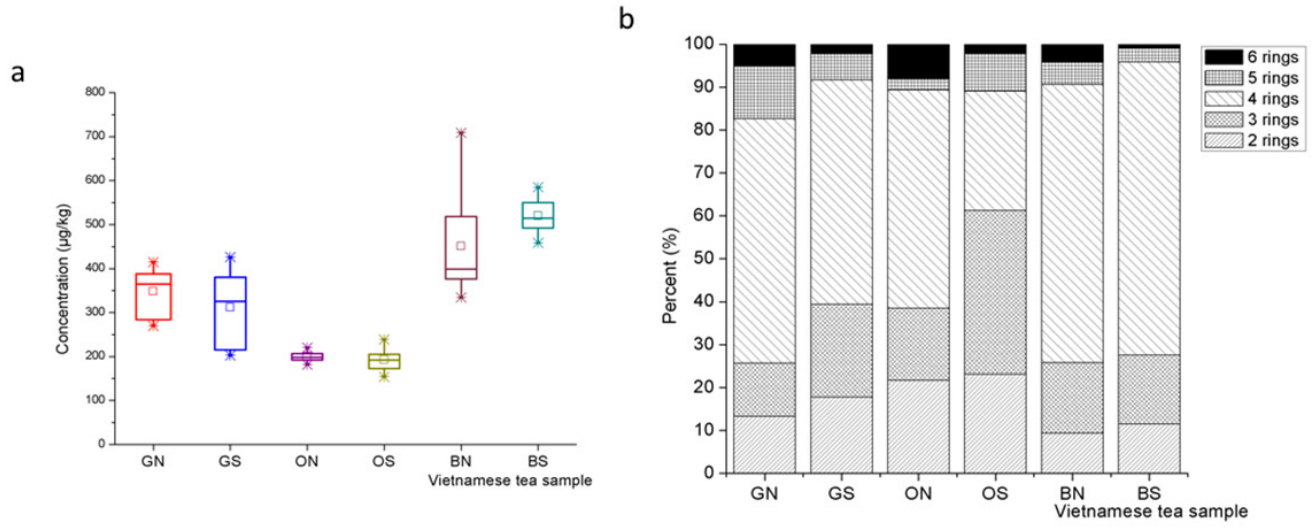

Figure 2. (Color online) (a) The average contents of total PAHs in the six varieties of Vietnamese made teas. The center horizontal line of the box is the median of the data; the top and bottom of the box are the 25th and 75th percentiles (quartiles); the ends of the whiskers are the 10th and 90th percentiles. Any points outside, labeled as X, are considered outliers. The small square of each box means the average of the data. (b) Distribution of PAHs in the six types of Vietnamese tea by number of rings. *Notes:* GN, green tea from the North, GS, green tea from the South, ON, oolong tea from the North, OS, oolong tea from the South, BN, black tea from the North and BS, black tea from the South

As shown in the Figure 2a, the total mean PAHs contents in the tea samples studied ranged from 154.2 to 709.0 µg/kg. Results of the study showed that black tea contained higher PAHs contents compared to green and oolong tea samples, probably due to drying processes employed during production. The average content of total PAHs reached at 520.0 µg/kg in black tea from Lam Dong province (BS), with a range of 458.3 – 585.1 µg/kg, which is the highest in this study. The similar results were detected in black tea [24,41] collected in the North of Vietnam (BN) with a range between 334.2 and 709.0 µg/kg. These results are consistent with other studies with concentrations of PAH in black teas varied from 4.9 to 103.6 µg/kg, from 9.0 to 44.6 µg/kg, from 6.4 to 70.0 µg/kg, and from 21.6 to 65.8 µg/kg [25,26,42,43,44]. However, the total PAHs content in oolong tea from both provinces were lower than 200 µg/kg. The total concentration of PAHs in green tea samples, collected in Thai Nguyen province (GN) were between 270.0 and 388.3 µg/kg, while those from Lam Dong province (GS) varied from 202.6 to 426.1 µg/kg. Many reports proved that the combustion of firewood will release PAHs [45,46,47]. In the production process, oolong tea is made by wilting the fresh leaves in the sun, then bruising them slightly and partially fermenting them. Green tea is made of young tea leaves and processed by several steps including withering, steaming or pan firing, drying and grading. The tea is sold to the market without fermentation. Meanwhile, black tea is made by fermentation for many hours, followed by drying by either smoke fired, flame fired or steamed. When the tea leaves absorb the scent of the smoke, they will also absorb the PAHs. This might be the main reason for the high level of PAHs in black tea.

The results of this study were subsequently compared with the PAHs content reported in similar studies conducted worldwide. In the study of Ziegenhals *et al.*, the mean PAHs content in black tea collected at the German market was 21.2 µg/kg, while this number was rather high in Czech samples [7,28].

Figure 2b showed the distribution of 2–6 rings PAHs in the tea samples. It can be seen that the 3–4 rings PAHs were predominant in all samples and account for 66.0 – 84.3% of the total PAHs. 2 rings PAHs occupied only 9.4% of total PAHs content in the North black tea samples (BN). These components were higher in oolong teas from both areas with 21.7% in samples collected in Thai Nguyen province and 23.1% in samples collected in Lam Dong province. More toxic PAHs with 5–6 rings were detected highest in the North green tea samples (GN) analyzed, accounting for 17.4%, while those in other samples contributed lower than 11%.

In detail of PAHs concentration of the individual compounds, at least 15 of 18 target PAHs exceeded limits of quantitation (LOQs) in all analyzed samples (Figure 3). B(k)F was absent in the oolong tea samples collected in the North of Vietnam. 9 of 18 PAHs including M1N, ACP, FL, AN, Py, Chy, B(b)F, IP, and DBA were found in all brands of tea samples. Indeed, pyrene and chrysene were the most abundant in all samples, except the South oolong teas. The amount of Py in various brands was found to be 86.7, 80.8, 64.2, 30.9, 109.6 and 126.9 µg/kg in green tea North, green tea South, oolong tea North, oolong tea South, black tea North and black tea South, respectively.

3.3. PAH4 analysis in Tea Leaves

PAH is a general name of a very large class of compounds. Therefore, it is impossible to monitor all of them simultaneously. In order to overcome this problem, the European Food Safety Authority (EFSA) identified four PAHs (Chy, B(a)A, B(b)F and B(a)P), commonly called PAH4, as an indicator of food contamination by carcinogenic/genotoxic PAHs [25]. According to a European Union Directive (98/83/CE), the European Commission fixed the maximum level allowed for B(a)P is 10 µg/kg and the maximum limit for the sum of PAH4 is 50 µg/kg in the amendment 835/2015 of the regulation 1881/2006 [48]. Given these parameters, three out of forty samples in this study exceeded the guideline value proposed for the concentration of B(a)P and 78% samples were higher than the limit value of the sum of the PAH4

B(b)F and Chy were detected in all samples with concentrations ranged from 0.6 to 23.7 µg/kg and from 4.6 to 233.0 µg/kg, respectively. B(a)A was detected in 13 out of 14 samples for the green tea, in 8 out of 10 samples for the oolong tea and in 15 out of 16 samples for the black tea, and B(a)P was detected all samples for the green tea, in 8 out of 10 samples for the oolong tea and in 13 out of 16 samples for the black tea. The concentrations in green tea in the North ranged from 0.6 to 13.2 µg/kg for B(a)A and from 1.0 to 7.9 µg/kg for B(a)P, while those from the South ranged from 0 to 3.3 µg/kg for B(a)A and from 2.1 to 4.2 µg/kg for B(a)P. The concentrations of B(a)A in oolong tea samples collected in both Thai Nguyen province and Lam Dong province were low, ranging from 0 to 2.3 µg/kg.

Due to manufacture process, it seems that the concentrations of PAH4 on the black tea are considerably higher than on the green tea and the oolong tea. The summed concentrations of PAH4 were approximately 195.4 and 234.1 µg/kg for the black tea collected in the North and the South of Vietnam, respectively. In contrast, the total concentrations of PAH4 were 107.4 µg/kg for the green tea collected in the North, 71.5 µg/kg for the green tea collected in the South, 41.8 µg/kg for the oolong tea collected in the North and 28.6 µg/kg for the oolong tea collected in the South.

The concentrations measured in green tea cannot be compared to previous results as no such results have been published so far. However, the measured concentrations for black tea are in line with previous findings of other research groups. Concentrations of PAH4 in black teas measured in previous studies varied from 4.9 to 103.6 µg/kg [49], from 6.4 to 700 µg/kg [28], from 9.0 to 44.6 µg/kg [50], and from 21.6 to 65.8 µg/kg [51]. Schlemitz and Pfannhauser measured concentrations ranging from 0.4 µg/kg (for B(a)P) to 45.4 µg/kg (for Chy) [49]; Ziegenhals *et al.* measured concentrations ranging from 0.8 µg/kg (for B(a)P) to 18.1 µg/kg (for Chy) [50]; Ishizaki *et al.* measured concentrations ranging from 4.3 µg/kg (for B(a)A) to 73.2 µg/kg (for B(a)P) [51]; Li *et al.* measured a mean B(a)P concentration of 9.4 µg/kg in black tea [52]. Dabrova *et al.* measured higher maximum concentrations, with values ranging from 0.2 (for B(a)P) to 229.0 µg/kg (for Chy) [28], though median values from 1.4 µg/kg (B(a)A) to 10.4 µg/kg (Chy) and mean values from 20.7 µg/kg (B(b)F) to 41.9 µg/kg (Chy) were similar to the concentrations measured in the present study. Lin,

Tu, and Zhu also measured slightly higher concentrations, with values ranging from 37.6 µg/kg (for B(b)F) to 241.0 µg/kg (for Chy), although their median and mean values were not presented [27].

Figure 3. (Color online) Concentrations of PAHs in ppm for all of the tested samples. Notes: GN, green tea from the North, GS, green tea from the South, ON, oolong tea from the North, OS, oolong tea from the South, BN, black tea from the North and BS, black tea from the South

3.4. Modeling of PAHs Release into Tea Fusion

Twenty experiments were conducted to optimize the release of PAHs from green tea leaves into tea infusion for the quantitative analysis (Table 3). Total 20 trials were done for an investigation (Supplemental Table S5). MODDE 12.1 software was used to design experimental matrices, calculate regression values and analytical variance. The regression coefficients for the encoded variables of the polynomial function (3) are shown in Table 3. Student statistic tests were used to evaluate the significance of the regression coefficients.

The regression of recombinant PAHs was obtained after the elimination of non-significant coefficients.

$$Y = 38.7249 + 3.6495X_1 + 4.9290X_2 + 6.6177X_3 - 3.3261X_1^2 - 3.0698X_2^2 - 5.6925X_3^2. \quad (3)$$

Analysis of variance by using ANOVA was also used to predict the suitability of a model with experiment results. The obtained results (Supplemental Table S6) indicated that the predicted values of the model were not conflict with the experiments. The coefficient of determination of R^2 was 0.890 and the coefficient of determination adjustment R^2_{adj} was 0.791. The suitability of the model was also shown in P values and Fisher test. $P_{regression}$ value was 0.001 (<0.05), and $P_{Lack\ of\ fit}$ was 0.970 (> 0.005), which showed that the obtained model was consistent with the experiment.

Table 3. Regression Coefficient

PAHs	Coeff. SC	Std. Err.	P	Conf. int(±)
Constant	38,7249	1,9375	2,15987e-09	4,3170
Time (X_1)	3,6495	1,2854	0,0175733	2,8641
Water/Tea ratio (X_2)	4,9290	1,2854	0,00329402	2,8641
Temperature (X_3)	6,6177	1,2854	0,000432523	2,8641
X_1*X_1	-3,3261	1,2512	0,0239643	2,7877
X_2*X_2	-3,0698	1,2512	0,0340477	2,7877
X_3*X_3	-5,6925	1,2512	0,00105856	2,7877
X_1*X_2	-2,0962	1,6796	0,240431	3,7423
X_1*X_3	-1,0962	1,6796	0,528669	3,7423
X_2*X_3	-1,3112	1,6796	0,45306	3,7423
N = 20	Q^2 =	0,741	Cond. no. =	3,591
DF = 10	R^2 =	0,890	RSD =	4,751
	R^2 adj. =	0,791		
			Confidence =	0,95

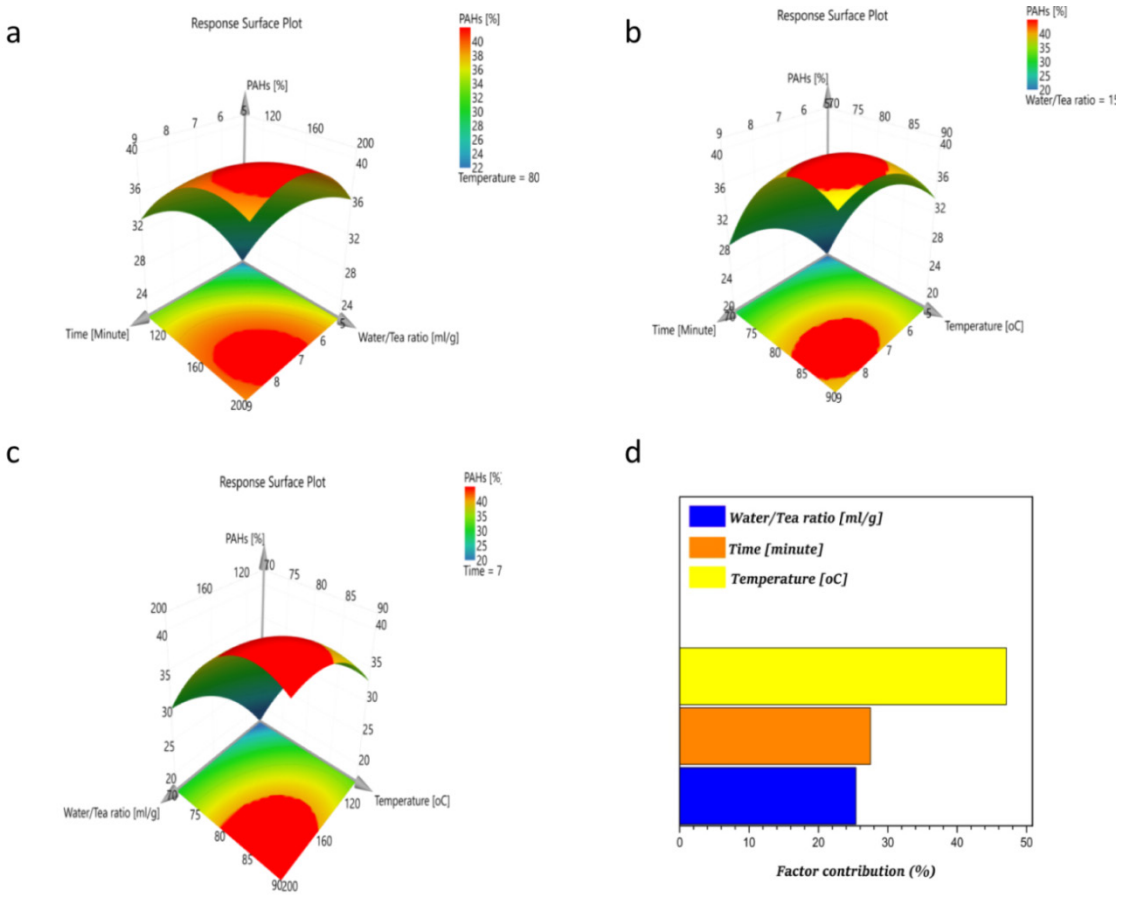

Figure 4. (color online) Response surface plots for the central composite design (CCD). Note*:* (a) Brewing times vs. $V_{solvent}/V_{sample}$ ratio at constant temperature (80^0C); (b) Brewing time vs. temperature with constant $V_{solvent}/V_{sample}$ ratio = 150; (c) $V_{solvent}/V_{sample}$ ratio vs. temperature after 7 minutes; (d) The PAHs concentration vs. eventually reaches equilibrium

The three-dimensional response surface shows the effect and interaction of the three factors on the target function. Figure 4a shows the combined effect of the V_{water}/V_{tea} ratio and brewing time at constant temperature (80°C) while Figure 4b shows the image effect of brewing time vs. temperature with constant $V_{solvent}/V_{sample}$ ratio = 150. Interaction between the V_{water}/V_{tea} ratio and temperature after 7 minutes is shown in Figure 4c. In general, temperature is the most important factor affect the efficiency of the PAHs release (approximately 47%), followed by extraction time (27%), and the V_{water}/V_{tea} ratio (26%) (Figure 4d).

3.5. Health Risk of PAHs from Tea

Figure 5 shows that tea samples collected in the North of Vietnam were found to have the average Toxic Equivalent (TEQ) higher than those in the South of Vietnam. The TEQ values of green tea (North) and black tea (North) were 25.60 and 25.93, respectively, followed by oolong tea (North): 14.49. Oolong tea sample (South) was found to have the least toxic equivalent of 8.49; while green tea (South) and black tea (South) had toxic equivalents of 9.87 and 10.75, respectively. 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). It was found that the average TEQ values of green tea and black tea were difference significantly when collected in two areas ($P < 0.05$). Up to date, there is no regulatory standard using the TEQ values have been established yet. However, these values enable us to make comparisons of the toxic equivalent amongst these tea samples.

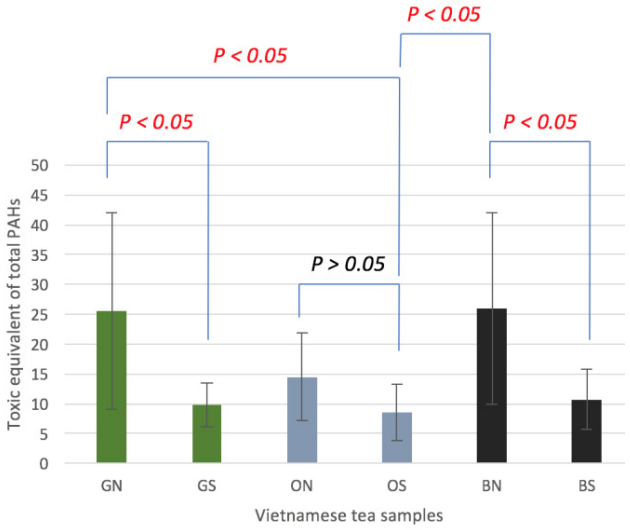

Figure 5. (Color online) Average toxic equivalent (TEQ) of total PAHs in Vietnamese tea samples. *Notes:* GN, green tea from the North, GS, green tea from the South, ON, oolong tea from the North, OS, oolong tea from the South, BN, black tea from the North and BS, black tea from the South.

4. Conclusion

The content of the 18 PAHs in the three types of Vietnamese tea collected in two different area (North and South of Vietnam) ranged from 154.2 to 709.0 µg/kg. All samples of tea showed the presence of 15 to 18 PAHs out of 18 PAHs. 3–4 rings PAHs were dominant in all tea samples, with a contribution of 66.0 – 84.3% of the total 18 PAHs content. Pyrene and chrysene were the major PAHs in the tea samples analyzed in this study. Oolong tea (OS) samples, collected in Lam Dong province were the lowest in their content of the indicated PAHs and black tea (BS) samples were the highest. PAHs contents show that the PAH contamination depends on the drying process of tea leaves and special procedures during the manufacturing of different types of tea. It was also observed that tea samples from the North has TEQ values higher than those from the South.

Abbreviations

Acenaphthene (ACP), Acenaphthylene (ACNP), Anthracene (AN), Benzo(a)anthracene (B(a)A), Benzo(a)pyrene (B(a)P), Benzo(b)fluoranthene (B(b)F), Benzo(g,h,i)perylene (B(ghi)P), Benzo(k)fluoranthene (B(k)F), Chrysene (Chy), Dibenz(a,h)anthracene (DBA), Fluoranthene (FLA), Fluorene (FL), Indeno(1,2,3-cd)pyrene (IP), 1-Methylnaphthalene (M1N), 2-Methylnaphthalene (M2N), Naphthalene (NaP), Phenanthrene (PHN), Pyrene (Py).

Funding

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Conflicts of Interest

The authors declare no conflict of interest.

References

[1] Research, Z.M., Tea Market By Product Type (Green Tea, Black Tea, Oolong Tea, Herbal Tea, and Others), By Application (Household and Commercial), By Packaging (Plastic Container, Loose Tea Packets, Tea Bags, and Aluminum Tins), and By Distribution Channel (Supermarket/Hypermarket, Convenience Store, Specialty Store, Online Retail and Others): Global Industry Perspective, Comprehensive Analysis and Forecast, 2017-2024. 2018.

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Supplemental documents

Supplemental Table S1. Identifying, Quantifying Ions, Retention Time and Collision Energy of 18 PAHs and Two Radiolabeled Compounds

No	Abbr.	Compound	Retention time (t_R)	Precursor (m/z)	Fragment (m/z)	Collision energy (eV)	Note
1	NaP	Naphthalene	7.1	128.2	127.2	15	Quantitation
					102.1	20	Confirm
2	M2N	2-Methylnaphthalene	8.63	141.1	89.1	16	Quantitation
					115.1	14	Confirm
3	M1N	1-methylnapththalene	8.87	141.1	89.1	32	Quantitation
					115.1	15	Confirm
4	ACNP	Acenaphthylene	10.69	154.1	153.0	15	Quantitation
					152.0	20	Confirm
5	ACP	Acenaphthene	11.14	152.1	151.0	15	Quantitation
					150.0	20	Confirm
6	FL	Fluorene	12.32	166.1	154.0	15	Quantitation
					164.0	20	Confirm
7	PHN	Phenalthrene	14.55	178.2	176.0	15	Quantitation
					172.0	20	Confirm
8	AN	Anthracene	14.65	178.1	176.1	15	Quantitation
					152.1	20	Confirm
9	FLA	Floranthene	17.38	202.1	200.1	15	Quantitation
					152.1	20	Confirm
10	Py	Pyrene	17.88	202.1	200.2	15	Quantitation
					152.1	20	Confirm
11	B(a)A	Benzo(a)athracene	20.76	228.1	226.1	30	Quantitation
					202.2	35	Confirm
12	Chy	Chrysene	20.84	228.1	226.2	30	Quantitation
					202.1	35	Confirm
13	B(b)F	Benzo(b)fluoranthene	23.6	252.2	250.0	25	Quantitation
					226.0	30	Confirm
14	B(k)F	Benzo(k)fluoranthene	23.6	252.2	250.1	25	Quantitation
					226.1	30	Confirm
15	B(a)P	Benzo(a)pyrene	24.41	252.0	250.1	25	Quantitation
					226.1	30	Confirm
16	IP	Indeno(1,2,3-cd)pyrene	28.77	276.0	274.1	30	Quantitation
					250.0	40	Confirm
17	BDA	Dibenz(a,h)anthracene	28.99	278.2	276.1	30	Quantitation
					252.1	40	Confirm
18	B(ghi)P	Benzo(g,h,i)perylence	29.91	276.1	274.1	30	Quantitation
					250.1	40	Confirm
19	B(a)A 13C6	Benzo(a)anthracence-$^{13}C_6$	20.76	234.1	232.1	30	Quantitation
					208.1	35	Confirm
20	B(ghi)P 13C12	Benzo(g,h,i)pyrylene-$^{13}C_{12}$	29.91	288.2	286.2	30	Quantitation
					261.2	40	Confirm

Supplemental Table S2. The List of Toxic Equivalency Factors (TEF) Reported by Nisbet and LaGoy[1]

No.	PAHs	Abbreviations	TEF
1	Naphthalene	NaP	0.001
2	2-Methylnaphthalene	M2N	0.001
3	1-methylnapththalene	M1N	0.001
4	Acenaphthylene	ACNP	0.001
5	Acenaphthene	ACP	0.001
6	Fluorene	FL	0.001
7	Phenanthrene	PHN	0.001
8	Anthracene	AN	0.01
9	Fluoranthene	FLA	0.001
10	Pyrene	Py	0.001
11	Benzo (a) anthracene	B(a)A	0.1
12	Chrysene	Chy	0.01
13	Benzo (b) fluoranthene	B(b)F	0.1
14	Benzo (k) fluoranthene	B(k)F	0.1
15	Benzo (a) pyrene	B(a)P	1
16	Indeno (1,2,3,cd) pyrene	IP	0.1
17	Dibenzo (a,h) anthraxcene	BDA	1
18	Benzo (ghi) perylene	B(ghi)P	0.01

[1]Nisbet, I.C. and P.K. LaGoy, *Toxic equivalency factors (TEFs) for polycyclic aromatic hydrocarbons (PAHs).* Regul Toxicol Pharmacol, 1992. **16**(3): p. 290-300.

Supplemental Table S3. Independent Variable and Experiment Level

Coded	Independent variable	Level independent variable				
		-α	-1	0	+1	+α
X_1	Time	3.6	5.0	7.0	9.0	10.4
X_2	Water/tea ratio (ml/g)	65.9	100.0	150.0	200.0	234.1
X_3	Temperature	63.2	70.0	80.0	90.0	96.8

Supplemental Table S4. Limit of Detection (LOD; μg/kg), Limit of Quantification (LOQ; μg/kg), Repeatability Relative Standard Deviation (RSD_r; n = 8), and Reproducibility Relative Standard Deviation (RSD_R; n = 12)

Abbr.	Compounds	LOD (μg/kg)	LOQ (μg/kg)	RSD_r (%)	RSD_R (%)
NaP	Naphthalene	0.10	0.30	20	17, 10, 20
M2N	2-Methylnaphthalene	0.20	0.60	18	16, 9, 11
M1N	1-Methylnapththalene	0.05	0.15	16	19, 13, 18
ACNP	Acenaphthylene	0.05	0.15	12	18, 6, 14
ACP	Acenaphthene	0.05	0.15	10	16, 5, 13
FL	Fluorene	0.01	0.03	14	8, 9, 15
PHN	Phenalthrene	0.05	0.15	8	13, 9, 18
AN	Anthracene	0.05	0.15	13	16, 7, 17
FLA	Floranthene	0.01	0.03	18	10, 6, 12
Py	Pyrene	0.05	0.15	7	11, 6, 15
B(a)A	Benzo(a)athracene	0.05	0.15	9	6, 8, 13
Chy	Chrysene	0.05	0.15	10	12, 7, 16
B(b)F	Benzo(b)fluoranthene	0.05	0.15	12	15, 5, 20
B(k)F	Benzo(k)fluoranthene	0.05	0.15	19	11, 8, 18
B(a)P	Benzo(a)pyrene	0.05	0.15	11	11, 5, 16
IP	Indeno(1,2,3-cd)pyrene	0.05	0.15	20	13, 8, 18
BDA	Dibenz(a,h)anthracene	0.10	0.30	15	10, 6, 15
B(ghi)P	Benzo(g,h,i)perylence	0.10	0.30	8	9, 8, 14

Reproducibility relative standard deviation (RSD_R; n = 12) of 1 μg/kg, 5 μg/kg, and 10 μg/kg standard value.

Supplemental Table S5. Experiment Results

No	Run Order	Time	Water /Tea ratio	Temperature	PAHs
					Exp
1	6	-1	-1	-1	9.26
2	18	1	-1	-1	19,46
3	9	-1	1	-1	23,54
4	3	1	1	-1	30,79
5	2	-1	-1	1	24,64
6	11	1	-1	1	35,89
7	17	-1	1	1	39,11
8	4	1	1	1	36,54
9	10	-1,68	0	0	21,18
10	20	1,68	0	0	35,28
11	1	0	-1,68	0	21,05
12	15	0	1,68	0	36,86
13	13	0	0	-1,68	10,46
14	12	0	0	1,68	32,61
15	14	0	0	0	37,5
16	7	0	0	0	48,54
17	5	0	0	0	35,89
18	19	0	0	0	30,04
19	8	0	0	0	42,5
20	16	0	0	0	38,25

Supplemental Table S6. Analysis of Variance (ANOVA)

PAHs	DF	SS	MS (variance)	F	p	SD
Total	20	20616,6	1030,83			
Constant	1	18567,8	18567,8			
Total corrected	19	2048,82	107,832			10,3842
Regression	9	1823,14	202,571	8,9762	**0,001**	14,2327
Residual	10	225,676	22,5676			4,75054
Lack of Fit (Model error)	5	29,9211	5,98423	0,15285	**0,970**	2,44627
Pure error (Replicate error)	5	195,755	39,1509			6,25707
	N = 20	Q^2 =	0,741	Cond. no. =	3,591	
	DF = 10	R^2 =	0,890	RSD =	4,751	
		R^2 adj. =	0,791			

Note: degrees of freedom, DF; sum of squares, SS; mean square, MS; Fisher, F; probability value, P; and standard deviation, SD.