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Polycyclic aromatic hydrocarbons (PAH) in chocolate on the German market

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Abbreviations: 5MC = 5-methylchrysene; ASE = accelerated solvent extraction; BaA = benzo[a]anthracene; BaP = benzo[a]pyrene; BbF = benzo[b]fluoranthene; BcL = benzo[c]-fluorene; BgP = benzo[g,h,i]perylene; BkF = benzo[k]fluoranthene; BjF = benzo[j]fluoranthene; CHR = chrysene; CPP = cyclopenta[c,d]pyrene; DhA = dibenzo[a,h]anthracene; DeP = dibenzo[a,e]pyrene; DhP = dibenzo[a,h]pyrene; DiP = dibenzo[a,i]pyrene; DlP = dibenzo[a,l]pyrene; EFSA = European Food Safety Authority; GPC = gel permeation chromatography; HRMS = high resolution mass spectrometry; IcP = indeno[1,2,3-cd]pyrene; PAH = polycyclic aromatic hydrocarbons; SEC = size exclusion chromatography.

Abstract: Benzo[a]anthracene (BaA), chrysene (CHR), cyclopenta[c,d]pyrene (CPP), 5-methylchrysene (5MC), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[j]fluoranthene (BjF), benzo[a]pyrene (BaP), dibenzo[a,h]anthracene (DhA), indeno[1,2,3-cd]pyrene (IcP), benzo[g,h,i]perylene (BgP), dibenzo[a,l]pyrene (DlP), dibenzo[a,e]pyrene (DeP), dibenzo[a,i]pyrene (DiP) and dibenzo[a,h]pyrene (DhP), the 15 SCF-PAH, assessed to be relevant as well as benzo[c]fluorene (BcL) recommended by the European Food Safety Authority (EFSA), were analysed in different types of chocolate. The sample preparation included accelerated solvent extraction (ASE), size exclusion chromatography (SEC) and solid phase chromatography using small silica gel columns. The individual PAH were separated by gas chromatography using a VF-17ms GC column and detected by high resolution mass spectrometry (HRMS). The investigation of 40 samples of various types of chocolate with different cocoa contents resulted in a median benzo[a]pyrene (BaP) content of 0.22 μg/kg. Furthermore, the results showed a linear correlation between the content of BaP and the sum content of the 16 priority PAH. Therefore, the analysis of BaP as a leading substance seems to be suitable to estimate the PAH contamination in chocolate.

Zusammenfassung: Für die Bestimmung der 15+1 von der EU als prioritär eingestuften PAK in verschiedenen Schokoladen wurde eine Analysenmethode bestehend aus beschleunigter Lösungsmittelextraktion (ASE), Gelpermeationschromatographie (GPC) und Nachreinigung an einer Minikieselgelsäule verwendet. Die Identifizierung und Quantifizierung der einzelnen Verbindungen erfolgte nach gaschromatographischer Trennung mit dem hochauflösenden Massenspektrometer unter Verwendung einer VF-17ms GC-Säule. Die Untersuchung von 40 Schokoladenproben mit verschiedenen Kakaogehalten erbrachte im Median einen Gehalt an Benzo[a]pyren (BaP) von 0,22 μg/kg. Es zeigte sich weiterhin eine enge Korrelation des BaP-Gehalts vom Gesamtgehalt der 15+1 EU-PAK. Über die Leitsubstanz BAP lässt sich somit der Gesamtgehalt der 15+1 EU-PAK durch die alleinige Bestimmung von BaP in Schokolade abschätzen.

1. Introduction

According to Directive 2000/36/EC (Directive 2000/36/EC) of the European Union chocolate is defined as a product obtained from cocoa products and sugars which contains no less than 35% total dry cocoa solids, including no less than 18% cocoa butter and no less than 14% of dry non-fat cocoa solids. Chocolates are classified in different groups (e.g. milk chocolate and white chocolate) with specified minimum requirements for the amounts of the different ingredients (Directive 2000/36/EC).

The estimated consumption of chocolate and chocolate confectionery in Germany in the year 2007 was 9.1 kg per capita (Association of the German Confectionery Industry, 2007a). The most important component of chocolate is cocoa.

The main suppliers of cocoa for German chocolate in the year 2006 were the Ivory Coast (52%), Nigeria (13%), Ghana (13%) and Togo (9%) in Africa (Association of the German Confectionery Industry, 2007b). Chocolates are made from nonalkalized cocoa liquor by incorporating sucrose, cocoa butter, aroma or flavoring substances, and, occasionally, other constituents (Belitz et al., 2004). A number of manufacturing steps are necessary for the production of a homogenous chocolate of high quality in respect to flavor, consistency and homogeneity. Harvesting cocoa and cocoa processing include the following steps: plucking and opening the pods and fermenting and drying the cocoa seeds. During the manufacturing procedure of chocolate, cocoa is roasted and winnowed, and the cocoa nibs are ground. Then the resulting cocoa liquor with the other ingredients is blended and, finally, the chocolate is molded (The World Atlas of Chocolate, 2003).

Within this manufacturing procedure there are some critical steps during which cocoa, and as a consequence chocolate may be contaminated with polycyclic aromatic hydrocarbons (PAH). PAH are generally a result of the incomplete combustion of organic material (Smith, 1984) and consist of two or more condensed aromatic carbon rings. About 660 different compounds belong to the PAH group (Sanders and Wise, 1997) of which some show carcinogenic properties (IARC, 1987). The most critical step is drying the cocoa seeds in their respective country of origin.

The contamination of cocoa seeds can occur by drying cocoa on asphalt, on bitumen in the sun, or by using direct drying processes. Furthermore, cocoa beans can be contaminated with PAH during storage and transport in jute or sisal bags that had been treated with batching oil (Grob et al., 1993). However, since 1998, international standards recommend that ingredients of batching oils must be non-toxic and approved for use in packaging materials (IJO, 1998). Moret et al. (1997) postulated a possible contamination of cocoa beans by lube and hydraulic oils.

There exists a maximum level of 2 μg/kg benzo[a]pyrene in oils and fats intended for direct human consumption or use as an ingredient in foods throughout the European Union (European Commission, 2006). Cocoa butter is excluded from this category. Therefore, the Commission recommends the member states to analyse the contents of BaP in cocoa butter and, in addition, the contents of the 15 PAH compounds, classified as priority (15 SCF-PAH), to check the suitability of benzo[a]pyrene as a marker (European Commission, 2005).

These 15 priority SCF-PAH are benzo[a]anthracene (BaA), chrysene (CHR), cyclopenta[c,d]pyrene (CPP), 5-methylchrysene (5MC), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[j]fluoranthene (BjF), benzo[a]pyrene (BaP), dibenzo[a,h]anthracene (DhA), indeno[1,2,3-cd]pyrene (IcP), benzo[g,h,i]perylene (BgP), dibenzo[a,l]pyrene (DlP), dibenzo[a,e]pyrene (DeP), dibenzo[a,i]pyrene (DiP) and dibenzo[a,h]pyrene (DhP). Additionally, the European Food Safety Authority (EFSA) recommends analysing benzo[c]fluorene (BcL) assessed to be relevant by the Joint FAO/WHO Experts Committee on Food Additives (JECFA, 2005).

Until now, there exist only a few data on the PAH contents in chocolate. Lodovici et al. (1995) analysed a mixed sample of three different types of chocolate and found a BaP content of 0.33 μg/kg. The BaP contents in six chocolate samples determined by Dennis et al. (1991) ranged between 0.13 and 0.32 μg/kg. In one chocolate candy, 0.18 μg BaP/kg were detected (Kazerouni et al., 2001). In a study by the Food Safety Authority of Ireland (2006) into levels of 15 SCF-PAH in food on the Irish market, the BaP contents of 16 analysed chocolate samples ranged from 0.06 to 0.30 μg/kg. Dibenzpyrenes (DeP, DhP, DiP and DlP) were not detectable (LOD: 0.1 μg/kg). In the year 2007, 25 samples of bitter chocolate sold on the German market were analysed. One of these samples showed a BaP content of 10 μg/kg (Stiftung Warentest, 2007).

In the present study, the contents of the 16 priority PAH in 40 samples of various types of chocolates were determined using a GC/HRMS method. The chromatographic conditions chosen (VF-17ms) did not allow the separation of CHR and triphenylene (TP). Therefore, both CHR+TP were determined in sum (Jira et al., 2008).

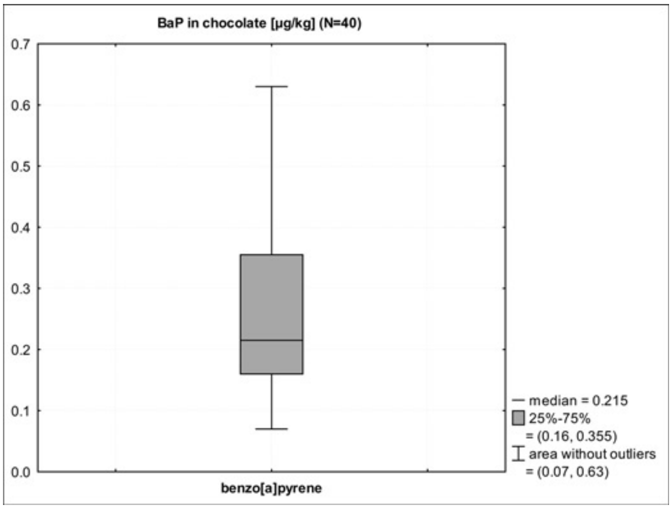

Fig. 1 Box Plots of the BaP contents in chocolate (N = 40).

2. Materials and methods

2.1 Materials

The native PAH standard solutions (BcL, BaA, CHR, CPP, BbF, BkF, BjF, BaP, IcP, DhA, BgP, DlP, DeP, DiP, DhP, 5MC) were obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany). The isotope labelled PAH standard solutions were purchased from Promochem (Wesel, Germany) and the fluorinated PAH standards from Biochemical Institute for Environmental Carcinogens Prof. Dr. Gernot Grimmer-Foundation (Grosshansdorf, Germany).

All solvents were obtained in picograde quality from Promochem (Wesel, Germany). The drying material (poly(acrylic-acid), partial sodium salt-graft-poly(ethylene oxide)) was purchased from Sigma Aldrich (Munich, Germany), Bio Beads S-X3 (200–400 mesh) from Bio-Rad Laboratories (Munich, Germany) and silica gel from Merck (Darmstadt, Germany).

Glass microfibre filters (18 mm i.d.) were obtained from Dionex (Idstein, Germany). The PTFE-Filters (1 µm pore size, 25 mm i.d.) and the SPE-Cartridges (12 mm i.d.) were purchased from Alltech (Unterhaching, Germany).

2.2 Methods

2.2.1 Sample preparation

Accelerated solvent extraction (ASE): A bar of chocolate was minced with a rasp and about 3 g were levigated with the same amount of the drying material poly(acrylic acid), partial sodium salt-graft-poly(ethylene oxide). The resulting material was poured into 33-mL cells, which were locked with glass microfiber filters at the outlet end of the extraction cells. Afterwards, 50 µL of a PAH standard mixture containing isotope labelled (^{13}C and ^{2}H) and fluorinated PAH compounds (5-fluorobenzo[c]fluorene, benzo[a]anthracene-$^{13}C_6$, chrysene-$^{13}C_6$, 5-methylchrysene-d_3, benzo[b]fluoranthene-$^{13}C_6$, benzo[k]fluoranthene-$^{13}C_6$, benzo[a]pyrene-$^{13}C_4$, benzo[g,h,i]perylene-$^{13}C_{12}$, dibenzo[a,h]anthracene-d_{14}, indeno(1,2,3-cd)pyrene-d_{12}, dibenzo[a,e]pyrene-$^{13}C_6$, dibenzo[a,i]pyrene-$^{13}C_{12}$ and 13-fluorodibenzo[a,l]pyrene in isooctane) were added. The extraction was performed with an ASE 200 from Dionex (Sunnyvale, USA) and carried out with n-hexane at 100 °C and 100 bar at a preheat time of 5 min and a static time of 10 min. The flush volume was 60% and the purge time 120 s. Two static cycles were accomplished. The solvent was evaporated using a nitrogen stream (water bath, 40 °C).

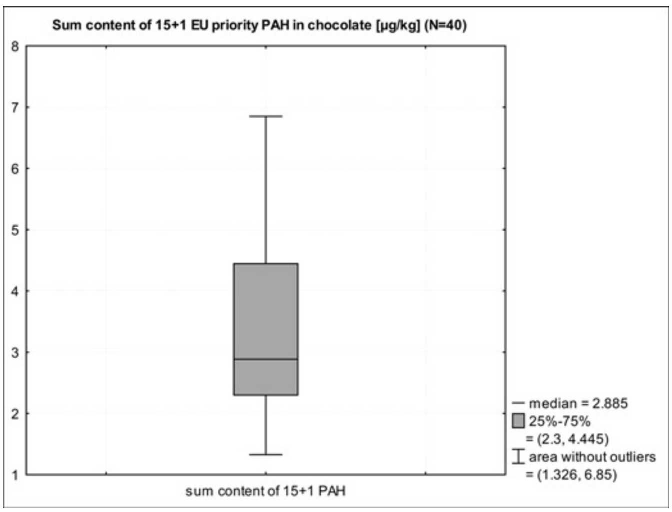

Fig. 2 Box Plots of the sum contents of the 15+1 EU priority PAH in chocolate (n = 40).

Gel permeation chromatography (GPC): The evaporated ASE-extract was dissolved in 4.5 mL cyclohexane/ethylacetate (50:50 v/v) and filtered through a PTFE filter with a pore size of 1 µm. The GPC column (25 mm i.d.) was filled with Bio-Beads S-X3 (weight of filling 60 g). Samples were eluted at a flow rate of 5 mL/min applying cyclohexane/ethylacetate (50:50 v/v) (dump time 0–36 min, collect time 36–65 min). The solvent was removed by first employing a rotary evaporator, and, finally, a nitrogen stream.

Solid phase extraction (SPE): This clean-up step to remove more polar substances was performed automatically with a modified ASPEC Xli (**A**utomatic **S**ample **P**reparation with **E**xtraction **C**olumns) (Kleinhenz et al., 2006) from Gilson (Bad Camberg, Germany). This system was modified with a fitting rack, teflon funnels and teflon tubes. Silica dried for 12 h at 550 °C was deactivated with 15% bidestilled water. 1 g dried deactivated silica was filled into commercial 8-mL SPE columns (12 mm i.d.). After conditioning the columns with 3 mL cyclohexane the samples were applied and eluted with 10 mL cyclohexane.

Preparation for GC/HRMS analysis: The dried eluate of SPE was dissolved in 1 mL isooctane and 50 µL of the PAH-recovery standard mixture (benzo[a]anthracene-d_{12} and benzo[a]-pyrene-d_{12} in isooctane) and transferred to a 1 mL tapered vial. The remaining sample was carefully concentrated in a nitrogen stream to a volume of about 50 µL.

Reagent and procedural blanks were simultaneously analysed to detect present PAH in parallel to each series of samples passing the extraction and cleanup procedures using drying material instead of real samples.

2.2.2 GC/HRMS analysis

The GC/HRMS analysis of PAH was performed on a HP 5890 II gas chromatograph with a split/splitless injection port. The GC was equipped with a VF-17ms capillary column (60 m x 0.25 mm i.d., 0.25 µm film thickness) purchased from Varian (Darmstadt, Germany). Helium was used as carrier gas at a constant pressure of 27 psi. The injection temperature was 300 °C and the injection volume was 1 µL (splitless). The following temperature program was used: isothermal at 50 °C for 1 min, at 25 °C/min to 280 °C, at 1 °C/min to 330 °C, and isothermal at 330 °C for 30 min.

The quantification of PAH by GC/HRMS was performed by using a VG Autospec (Waters, Manchester, UK) working in the EI positive ion mode using an electron energy of 35 eV. The transfer line temperature and the ion source temperature were maintained at 280 °C and 250 °C, respectively. The resolution of the MS was tuned to 8,000 (10% valley definition). The PAH were analysed in a 4-function Selected Ion Registration (SIR) experiment.

3. Results and Discussion

In this investigation 40 samples of various types of chocolate with different cocoa contents were analysed (Tab. 1). These chocolate samples were analysed according to their contents of the 16 priority PAH (Tab. 2).

The BaP contents in chocolate ranged between 0.07 and 0.63 µg/kg (median: 0.22 µg/kg). The highest PAH contents were found for CHR+TP (0.83 to 2.09 µg/kg). The contents of BcL and BaA were in the same order of magnitude. Within the group of the three benzofluoranthenes BbF dominated followed by BjF and BkF. The contents of DhA were below the limit of detection in more than 90% of the analysed samples. The three dibenzpyrenes DlP, DiP and DhP were in analysed samples not detectable, DeP was detected in a single sample in

Tab. 1 Overview of analysed chocolate samples.

Chocolate number	Type of chocolate	Cocoa content [%]	Country of origin
1	White chocolate	ca. 30	Unknown
2	Whole milk chocolate	30	Unknown
3	Dark chocolate	52	Unknown
4	Dark chocolate	70	Unknown
5	Dark chocolate	85	Unknown
6	no declaration	99	Unknown
7	Whole milk chocolate	37	Java
8	Whole milk chocolate	46	Madagascar
9	Dark chocolate	56	Venezuela
10	Dark chocolate	60	Amazon
11	Dark chocolate	70	Ecuador
12	Dark chocolate	75	Trinidad
13	Dark chocolate	60	Unknown
14	Dark chocolate	72	Unknown
15	Dark chocolate	85	Unknown
16	Dark chocolate	63	Unknown
17	Dark chocolate	75	Unknown
18	Dark chocolate	85	Unknown
19	no declaration	70	Unknown
20	Dark chocolate	86	Unknown
21	Dark chocolate	60	Unknown
22	Whole milk chocolate	35	Nouméa
23	Whole milk chocolate	37	Java
24	Whole milk chocolate	39	Madagascar
25	Whole milk chocolate	43	Puerto Cabello
26	no declaration	33	Java
27	no declaration	37	Costa Rica
28	no declaration	42	Venezuela
29	no declaration	51	Ecuador
30	no declaration	70	Santo Domingo
31	no declaration	84	Ghana
32	White chocolate	ca. 30	Unknown
33	White chocolate	ca. 30	Unknown
34	Dark chocolate	77	San Martin Peru
35	Dark chocolate	78	Papua Neuguinea
36	Dark chocolate	45	Unknown
37	Dark chocolate	50	Unknown
38	Dark chocolate	50	Unknown
39	Bittersweet chocolate	50	Unknown
40	Dark chocolate	60	Amazon

a low amount (0.07 µg/kg). The sum contents of the analysed 15+1 EU priority PAH compounds (upper bound) were between 1.3 – 6.9 µg/kg (median 2.9 µg/kg). The sum contents of the 15 SCF-PAH (upper bound) ranged from 1.1 to 6.3 µg/kg and were, therefore, in the same range as the sum content found in the chocolates in Ireland (1.1 – 3.9 µg/kg). In addition, the median sum contents of the 15 SCF-PAH were very similar: 2.6 µg/kg in the present study and 2.3 µg/kg in the Irish study.

The BaP contents in chocolate of 0.33 µg/kg (Lodovici et al., 1995) and 0.13 – 0.32 µg/kg (Dennis et al., 1991) found in the literature were in the same order of magnitude as the contents analysed in the present study. The Box Plots of the BaP- and PAH-sum contents in chocolate are shown in Fig. 1 and 2.

A linear correlation between the BaP contents and the sum contents of the 15+1 EU priority PAH was detectable (Fig. 3A). Considering all of the chocolate samples analysed in this study, the following formula can be calculated: $y = 9.8\,x + 0.9$ (y = sum of PAH; x = BaP concentration), with a correlation coefficient of $R = 0.94$ ($N = 40$).

In contrast, EFSA concluded that BaP is not a suitable indicator for the occurrence of PAH in food and assessed that the sum content of the four PAH compounds BaP, CHR, BaA and BbF ("PAH4") is the most suitable indicator of PAH in food (EFSA, 2008). A correlation between the sum content of the PAH compounds BaP, CHR+TP, BaA and BbF and the sum content of the 15+1 EU priority PAH is shown in Fig. 3B. The

Tab. 2 PAH contents in various types of chocolate [µg/kg] (N = 40).

Sample	BcL	BaA	CHR + TP	CPP	5MC	BbF	BkF	BjF	BaP	IcP	DhA	BgP	DIP	DeP	DiP	DhP	total PAH	Sum of 15 SCF-PAH
1	0.55	0.88	1.26	0.69	0.05	0.16	0.19	0.37	0.53	0.31	<0.06	0.49	<0.02	<0.06	<0.06	<0.02	5.70	5.15
2	0.51	1.15	1.80	0.34	0.09	0.31	0.31	0.41	0.60	0.43	0.08	0.62	<0.06	<0.06	<0.06	<0.02	6.85	6.34
3	0.36	0.32	0.66	0.08	0.03	0.23	0.07	0.14	0.13	0.07	<0.02	0.10	<0.02	<0.06	<0.02	<0.02	2.33	1.97
4	0.33	0.54	0.99	0.13	<0.02	0.67	0.21	0.25	0.42	0.27	0.07	0.39	<0.06	<0.06	<0.02	<0.02	4.45	4.12
5	0.47	0.33	0.71	0.07	<0.02	0.30	0.08	0.20	0.16	0.13	<0.02	0.20	<0.06	<0.06	<0.02	<0.02	2.85	2.38
6	0.80	1.06	1.86	0.45	0.06	0.64	0.20	0.36	0.42	0.19	<0.02	0.23	<0.02	<0.06	<0.02	<0.02	6.41	5.61
7	0.49	0.84	1.40	0.51	<0.006	0.60	0.22	0.37	0.55	0.23	<0.06	0.35	<0.02	<0.06	<0.02	<0.02	5.75	5.26
8	0.40	0.45	0.86	0.18	0.03	0.32	0.09	0.19	0.20	0.11	<0.02	0.15	<0.02	<0.06	<0.02	<0.02	3.12	2.72
9	0.39	0.36	0.76	0.13	<0.006	0.32	0.10	0.16	0.16	0.11	<0.02	0.18	<0.02	<0.06	<0.02	<0.02	2.82	2.43
10	0.28	0.39	0.78	0.14	0.04	0.29	0.09	0.17	0.23	0.07	<0.02	0.13	<0.02	<0.02	<0.02	<0.02	2.71	2.43
11	0.19	0.24	0.59	0.12	0.03	0.21	0.05	0.13	0.17	<0.06	<0.02	0.14	<0.02	<0.02	<0.02	<0.02	2.03	1.84
12	0.22	0.31	0.73	0.12	<0.006	0.25	0.08	0.17	0.16	<0.06	<0.02	0.06	<0.02	<0.02	<0.02	<0.02	2.27	2.05
13	0.31	0.50	0.88	0.13	0.04	0.32	0.12	0.20	0.24	0.08	<0.02	0.09	<0.02	<0.02	<0.02	<0.02	3.01	2.70
14	0.32	0.25	0.49	0.21	0.03	0.22	0.05	0.13	0.12	<0.06	<0.02	0.07	<0.02	<0.02	<0.02	<0.02	2.05	1.73
15	0.27	0.24	0.48	0.28	0.03	0.22	0.09	0.15	0.17	0.06	<0.06	0.10	<0.06	<0.02	<0.02	<0.02	2.27	2.00
16	0.24	0.31	0.66	0.18	<0.006	0.31	0.10	0.17	0.24	0.09	<0.06	0.12	<0.06	<0.02	<0.02	<0.02	2.61	2.37
17	0.36	0.45	0.92	0.43	0.05	0.39	0.13	0.25	0.33	0.10	<0.02	0.14	<0.02	<0.02	<0.02	<0.02	3.65	3.29
18	0.44	0.47	1.07	0.23	0.07	0.46	0.16	0.29	0.31	0.18	<0.06	0.20	<0.02	<0.02	<0.02	<0.02	4.02	3.58
19	0.51	1.14	1.98	0.48	0.10	0.70	0.25	0.39	0.63	0.22	<0.06	0.31	<0.02	<0.02	<0.02	<0.02	6.85	6.34
20	0.52	0.64	1.34	0.35	0.07	0.51	0.17	0.32	0.38	0.13	<0.06	0.22	<0.02	<0.02	<0.02	<0.02	4.79	4.27
21	0.19	0.12	0.32	0.06	<0.006	0.16	0.05	0.12	0.09	<0.06	<0.02	0.05	<0.02	<0.02	<0.02	<0.02	1.33	1.14
22	0.42	0.64	1.25	0.25	0.07	0.47	0.16	0.27	0.35	0.11	<0.06	0.16	<0.02	<0.02	<0.02	<0.02	4.29	3.87
23	0.30	0.52	0.95	0.21	0.04	0.39	0.13	0.23	0.32	0.08	<0.02	0.11	<0.02	<0.02	<0.02	<0.02	3.38	3.08
24	0.21	0.27	0.55	0.12	<0.006	0.23	0.09	0.16	0.16	0.07	<0.06	0.08	<0.06	<0.02	<0.02	<0.02	2.13	1.92
25	0.23	0.43	0.76	0.10	0.04	0.36	0.13	0.21	0.30	0.08	<0.06	0.14	<0.02	<0.02	<0.02	<0.02	2.92	2.69
26	0.34	0.75	1.42	0.32	0.06	0.55	0.18	0.38	0.44	0.22	0.06	0.26	<0.02	<0.02	<0.02	<0.02	5.06	4.72
27	0.50	0.65	1.31	0.27	0.08	0.45	0.15	0.31	0.32	0.17	<0.06	0.09	<0.02	<0.02	<0.02	<0.02	4.44	3.94
28	0.56	0.72	1.51	0.31	0.14	0.60	0.19	0.44	0.40	0.19	<0.06	0.32	<0.02	0.07	<0.02	<0.02	5.57	5.01
29	0.22	0.11	0.40	0.27	<0.02	0.14	<0.02	0.14	0.09	0.07	<0.06	0.11	<0.02	<0.06	<0.02	<0.02	1.77	1.55
30	0.40	0.24	0.74	0.11	0.02	0.26	0.07	0.24	0.18	0.13	<0.06	0.17	<0.06	<0.06	<0.02	<0.02	2.78	2.38
31	0.30	0.23	0.64	0.11	0.03	0.22	0.02	0.18	0.15	0.08	<0.06	0.10	<0.02	<0.02	<0.02	<0.02	2.20	1.90
32	0.29	0.41	0.89	0.24	0.03	0.26	0.09	0.25	0.20	0.11	<0.06	0.12	<0.06	<0.06	<0.02	<0.02	3.11	2.82
33	0.26	0.32	0.80	0.22	0.03	0.22	0.05	0.21	0.14	0.09	<0.02	0.12	<0.02	<0.02	<0.02	<0.02	2.56	2.30
34	0.39	0.34	0.88	0.21	0.03	0.32	0.08	0.27	0.18	0.10	<0.06	0.14	<0.02	<0.06	<0.02	<0.02	3.12	2.73
35	0.59	0.96	2.09	0.43	0.08	0.51	0.17	0.39	0.36	0.14	<0.06	0.19	<0.02	<0.02	<0.02	<0.02	6.05	5.46
36	0.23	0.39	0.76	0.19	0.05	0.24	0.05	0.23	0.18	0.11	<0.02	0.17	<0.02	<0.02	<0.02	<0.02	2.70	2.47
37	0.26	0.14	0.49	0.05	<0.006	0.18	0.03	0.16	0.08	0.08	<0.02	<0.006	<0.02	<0.02	<0.02	<0.02	1.58	1.32
38	0.22	0.14	0.38	0.17	<0.006	0.15	0.03	0.14	0.07	0.08	<0.06	0.05	<0.02	<0.02	<0.02	<0.02	1.58	1.36
39	0.36	0.39	0.88	0.11	0.04	0.25	0.05	0.18	0.26	0.10	<0.06	0.02	<0.02	<0.02	<0.02	<0.02	2.78	2.42
40	0.41	0.32	0.76	0.15	0.04	0.23	0.05	0.19	0.12	0.09	<0.06	0.02	<0.02	<0.02	<0.02	<0.02	2.52	2.11

Tab. 2 *(continued)*

Sample	BcL	BaA	CHR +TP	CPP	5MC	BbF	BkF	BjF	BaP	IcP	DhA	BgP	DlP	DeP	DiP	DhP	total PAH	Sum of 15 SCF-PAH
Median	0.35	0.39	0.83	0.2	0.03	0.31	0.09	0.21	0.22	0.1	<0.06	0.14	<0.02	<0.02	<0.02	<0.02	2.89	2.58
Min.	0.19	0.11	0.32	0.06	<0.006	0.14	<0.02	0.12	0.07	<0.06	<0.02	0.01	<0.02	<0.02	<0.02	<0.02	1.33	1.14
Max.	0.8	1.15	2.09	0.69	0.14	0.7	0.31	0.44	0.63	0.43	0.08	0.62	<0.06	0.07	<0.06	<0.02	6.85	6.34

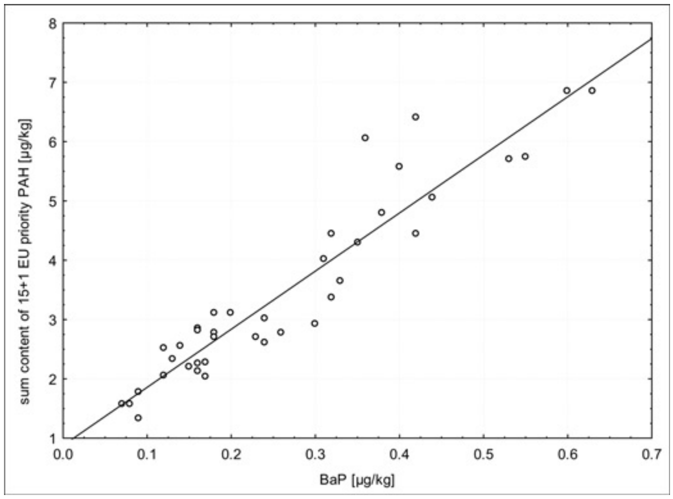

Fig. 3a Correlation between BaP contents and sum contents of 15+1 PAH in chocolate (N = 40), (R = 0.94).

correlation coefficient was 0.99. The mean of the PAH4-concentration (upper bound) in chocolate samples (n = 148) collected by EFSA (EFSA, 2008) was 1.8 µg/kg. In comparison, a mean PAH4-concentration of 2.0 µg/kg was found in the present study.

It was shown that a correlation between the PAH contents in chocolate and the labelled cocoa contents does not exist. Following the German "Lebensmittel-Kennzeichnungsverordnung", the total content of cocoa dry mass, given as "cocoa: x % minimum" represents the sum content of fat free cocoa dry mass and cocoa fat.

Depending on the respective quality level, chocolate is produced with different proportions of cocoa and cocoa butter. For example, whole milk chocolate (not less than 30% cocoa) contains 10–15% cocoa mass and about 20% cocoa butter, dark chocolate (no less than 50% cocoa) about 45% cocoa mass and 5–10% cocoa butter and bitter chocolate (no less than 60% cocoa), about 55% cocoa mass and 5–10% cocoa butter. As a consequence, the following percentages of cocoa butter can be calculated (including the contingents of cocoa butter and cocoa mass (52–58%): whole milk chocolate (26%), bittersweet chocolate (30%), dark chocolate (33%) whereas white chocolate contains already 26% cocoa butter (Römpp, 1995).

The above calculations show only slight differences in the contents of cocoa butter and are also reflected in the PAH compounds and their amounts in the different types of chocolate.

It was not possible to evaluate the influence of the origin of the cocoa on the PAH contents in chocolate due to the lack of information on the labels of some of the chocolates. The percentage contributions of the single PAH compound to the sum content of the 15+1 EU priority PAH (mean values, upper bound) in the analysed chocolate samples were as follows: CHR+TP 27%, BaA 13%, BcL 11%, BbF 10%, BaP 7%, BjF 7%, CPP 7%, BgP 5%, IcP 4%, BkF 3%, DhA 1%, DeP 1%, DhP 1%, DlP 1%, 5MC 1% and DiP 1%.

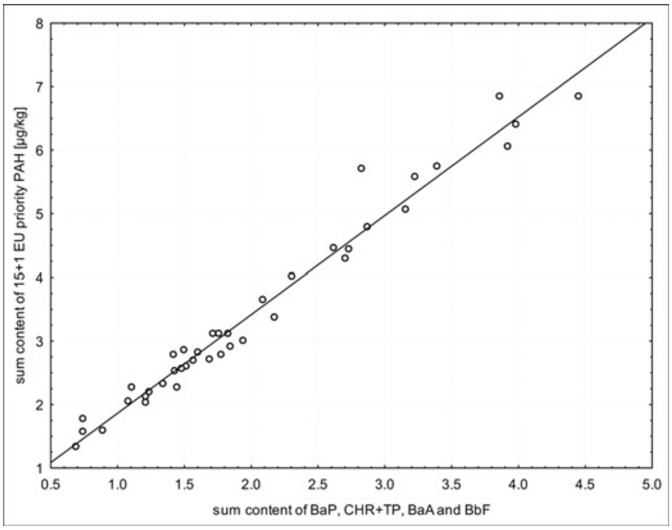

Fig. 3b Correlation between sum contents of BaP, CHR+TP, BaA and BbF and sum contents of 15+1 EU priority PAH in chocolate (N = 40), (R = 0.99).

4. Conclusions

Investigations of different types of chocolate showed a median BaP content of 0.22 µg/kg. A correlation between BaP contents and the contents of cocoa butter was not observed for the analysed chocolate samples. The determined PAH contents in chocolate show that BaP is a suitable leading substance for estimating the total content of the 15+1 EU priority PAH in chocolate, since a significant correlation between the BaP contents and the sum contents of the 15+1 PAH was observed. With the help of the following formula it is possible to estimate the sum content of the 15+1 EU PAH:

$y = 9.8\ x + 0.9$ (y = sum of the PAH; x = BaP concentration)

Furthermore, a good correlation between the individual PAH compounds was detectable. Considering the analytical aspects of determining the PAH compounds, BaP is the most suitable marker substance as, on the one hand, the chromatographic separation from other possibly interfering substances is easy to manage, and, on the other hand, as the limits of quantification are lower than the LOQ for PAH with a higher molecular weight. The consideration of the four PAH compounds favoured by EFSA (PAH4) results in a slightly better correlation to the sum content of the 15+1 EU priority PAH and consequently, allows a better estimation of the PAH contamination level. Nevertheless, the consideration of PAH4 comprises the disadvantage that the determination of CHR is critical due to the fact that the chromatographic separation of CHR and TP by the commonly used GC methods is rather difficult.

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