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Cocoa beans of different origins and varieties and their derived products contamination with polycyclic aromatic hydrocarbons

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Dibenzo[*a,e*]pyrene (PubChem CID: 9126)
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ABSTRACT

Levels of polycyclic aromatic hydrocarbons (PAHs) in cocoa beans of several varieties originating from different countries and their derived products from one technological line were examined. PAHs analysis was performed using HPLC–FLD/DAD and confirmed by GC–MS. Significant differences in total 19 PAHs contents between raw cocoa beans of different varieties and origins were observed. The highest sums of 19 PAHs were determined in roasted cocoa beans, cocoa mass and cocoa butter (16.69–74.15 μg kg^{-1} of fat). The roasting temperature of 160 °C led to PAHs formation, though not the heavy ones. Lowering temperature to 140 °C while extending the time minimized the total contamination but to a small extent. In all samples relatively low levels of total contamination were noted, with light PAHs being predominant and the sum of 4 heavy and marker PAHs much lower than the maximum legal limit. Therefore, analysed products, especially chocolate, do not threaten consumers' health.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a diverse group of toxic chemicals, ubiquitous in the environment. The incomplete combustion of organic matter or its pyrolysis is the source of their formation in the industry and various human activities (Abdel-Shafy & Mansour, 2016; EFSA, 2008; Lawal, 2017; Singh, Varshney, & Agarwal, 2016). The occurrence of PAHs in different elements of the environment and consequently also in foodstuffs has been confirmed in many scientific reports (Bansal & Kim, 2015; Ciecierska et al., 2019; Molle, Abballe, Gomes, Furlani, & Tfouni, 2017; Plaza-Bolaños, Garrido Frenich, & Martínez Vidal, 2010; Rozentale, Zacs, Perkons, & Bartkevics, 2017; Surma, Sadowska-Rociek, & Cieślik, 2014; Zhai et al., 2018). Environmental deposition, as well as thermal processing of food, constitute two main sources of food contamination with PAHs. Among these thermal treatment processes, in particular, smoking, grilling, roasting and direct drying can contribute to a high level of PAHs contamination (De Lima et al., 2017; Esposito et al., 2015; Ledesma, Rendueles, & Díaz, 2016; Lee et al., 2016; Olatunji, Fatoki, Opeolu, & Ximba, 2014; Rose et al., 2015; SCF, 2002; Singh et al., 2016).

According to the opinion of the European Union Scientific Committee on Food (SCF), expressed on 4 December 2002, 15 heavy PAHs are genotoxic carcinogens (SCF, 2002). It should be mentioned that heavy PAHs are much more stable and more toxic than light PAHs (EFSA, 2008; Kuppusamy, Thavamani, Megharaj, & Naidu, 2016; Lawal, 2017; Raters & Matissek, 2014). The European Commission Recommendation, dated 4 February 2005, indicated that further analyses of the above-mentioned heavy PAHs in food are necessary (Commission of the European Communities, 2005). In accordance with the opinion of the European Food Safety Authority (EFSA) from 2008, as well as Commission Regulation (EU) No. 835/2011, 4 specific, heavy PAHs (benzo[*a*]pyrene (B[*a*]P), benzo[*a*]anthracene (B[*a*]A), benzo[*b*]fluoranthene (B[*b*]F) and chrysene (Chr)) are the most suitable markers for PAH occurrence in foodstuffs. For that reason, these compounds

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should be controlled in food (Commission of the European Communities, 2011a; EFSA, 2008).

Cocoa butter and products derived from cocoa bean processing, including chocolate, can be contaminated by PAHs mainly as a consequence of inappropriate drying and roasting practices of the cocoa beans and the fact that cocoa butter cannot be refined in the same way as other vegetable oils and fats. Furthermore, they could be also contaminated as a result of environmental deposition (Belo et al., 2017; Commission of the European Communities, 2011a; Lowor et al., 2012; Misnawi, 2012; Ziegenhals, Speer, & Jira, 2009). It thereby can contribute to human exposure to PAHs, and especially to child exposure, due to the relatively high consumption of these products among children (Commission of the European Communities, 2011a).

Since cocoa beans and derived products may be a source of PAH intake for consumers, maximum permissible levels for PAHs (the sum of 4 heavy PAHs mentioned above and B[*a*]P alone) have been established in the Commission Regulation (EU) No. 835/2011. These limits for PAHs have been set at levels as low as reasonably achievable, considering the current technological possibilities of countries producing cocoa beans. Furthermore, according to this regulation, PAH levels in cocoa beans and derived products should be regularly monitored to assess the possibility for further reducing the maximum levels in future (Commission of the European Communities, 2011a).

Studies concerning the level of PAHs occurrence, the possibility of their formation and possible interventions for prevention and reduction of PAHs contamination in cocoa beans and their derived products are needed. In scientific reports, data on PAHs content in cocoa beans of different origins and varieties, and products of their processing, derived from one technological line, are very limited. The main aim of this work was to determine PAHs contamination levels in cocoa beans of several varieties derived from different countries (Forastero, Trinitario, Criollo and Nacional) and their processed products. In the case of cocoa and coffee beans, roasting conditions, especially temperature and time, are undoubtedly important factors in PAHs generation (Guatemala-Morales et al., 2016; Singh et al., 2016; Żyżelewicz, Oracz, Krysiak, Budryn, & Nebesny, 2017). Therefore, they should be tested and monitored to reduce or minimise PAHs formation. Publications on PAHs concentration in cocoa beans depending on various roasting conditions are restricted to the work published by Żyżelewicz et al. (2017). However, the varietal diversity of beans was not considered by the authors. Thereby an important aspect of this study was to assess if, for selected cocoa bean varieties, applying a lower roasting temperature, than typically used in the industry, for a longer time, would result in reduced contamination in the beans and their derived products.

PAHs analysis included determination of 19 compounds using high-performance liquid chromatography with fluorescence and diode array detectors (HPLC–FLD/DAD). This technique is commonly used for PAHs determinations (Santonicola, Albrizio, Murru, Ferrante, & Mercogliano, 2017; Veiga et al., 2014; Zachara, Gałkowska, & Juszczak, 2017). Among compounds analysed, the above-mentioned 15 heavy PAHs from the SCF list, including 4 marker PAHs, and 4 light PAHs (phenanthrene (Phen), anthracene (Anthr), fluoranthene (F) and pyrene (Pyr)), from the United States Environmental Protection Agency (EPA) list, were determined. The extension of the analysed 15 heavy PAHs by an additional 4 light PAHs resulted from the fact, that in previous studies on PAHs contamination of food products, these compounds had always dominated in the qualitative and quantitative profiles of PAHs (Ciecierska et al., 2019; Murkovic, Pedreschi, & Ciesarova, 2019; Sadowska-Rociek, Cieślik, & Sieja, 2015; Żyżelewicz et al., 2017).

2. Materials and methods

2.1. Samples

2.1.1. General

The materials investigated were raw cocoa beans of different origins and varieties and their derived products (roasted cocoa beans, cocoa mass, cocoa butter and the final products of their processing, i.e. dark chocolate) collected from the production line of a chocolate factory situated in central Poland. The cocoa beans of different varieties, as fermented and sun dried beans packed in jute bags (with initial moisture content of 5–7%), were imported from Dominican Republic (Trinitario), Ecuador (Nacional), Ghana and Ivory Coast (Forastero), Nicaragua (Trinitario) and Venezuela (Criollo). Nine samples of every kind of product, from three different batches, were analysed.

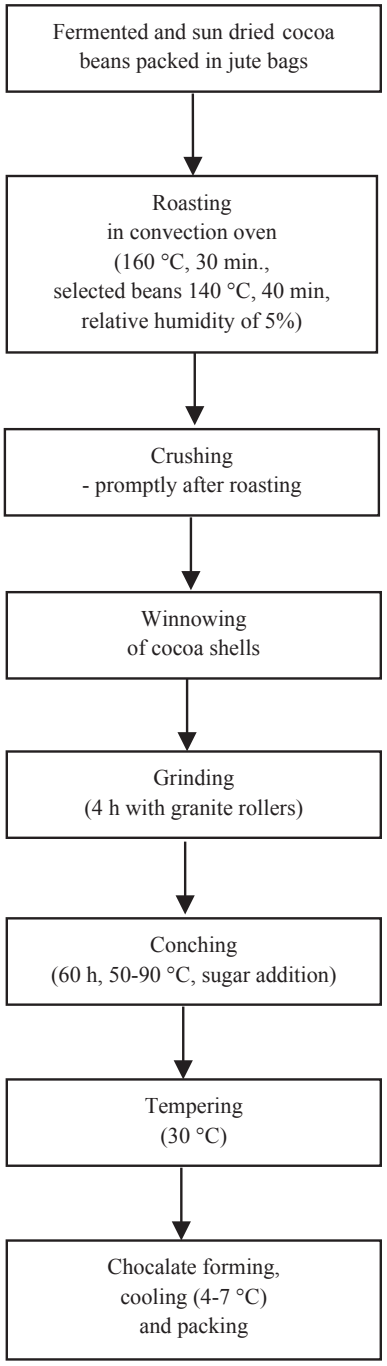

Fig. 1. Scheme of chocolate manufacturing with processing parameters.

2.1.2. Roasting and chocolate manufacturing

The most important process in terms of PAHs formation, i.e. roasting of cocoa beans, was carried out in a convection oven (Honest HT-HB100 brand, Henan, China) at 160 °C for 30 min. Selected cocoa

beans of two different varieties and origins (Trinitario from Nicaragua and Criollo from Venezuela) were additionally roasted at a lower temperature than typically used (160 °C), and for a longer time, that is at 140 °C for 40 min. The applied relative humidity of air during roasting was 5.0%. After roasting, the water content of the beans was approximately 2%. The scheme of chocolate manufacturing, with processing parameters applied, is presented in Fig. 1.

For the analysed products, the declared fat contents for cocoa beans, cocoa mass and chocolate were respectively 28–33%, 50–55% and 32–43%. All investigated chocolate consisted of 70% cocoa solids.

2.2. Chemicals and materials

All solvents used in the study (acetone, acetonitrile, dichloromethane, hexane – all HPLC grade) and anhydrous sodium sulfate (of analytical purity > 99.0%) were provided by POCH S.A. (Gliwice, Poland). Deionised water was collected from a Millipore Milli-Q water purification device. Polytetrafluoroethylene (PTFE) filters (25 mm i.d., 1 μm pore size) were supplied by Bio Analytic (Gdańsk, Poland).

Two standard mixtures of 15 PAHs listed by the SCF (PAH-Mix 183, Dr Ehrenstorfer) and 16 PAHs listed by the US EPA (PAH-Mix 9, Dr Ehrenstorfer) were purchased from Witko (Łódź, Poland). The first mixture consisted of cyclopenta[*c,d*]pyrene (C[*cd*]P), benzo[*a*]anthracene (B[*a*]A), chrysene (Chr), 5-metylchrysene (5-MChr), benzo[*j*]fluoranthene (B[*j*]F), benzo[*b*]fluoranthene (B[*b*]F), benzo[*k*]fluoranthene (B[*k*]F), benzo[*a*]pyrene (B[*a*]P), dibenzo[*a,h*]anthracene (D[*ah*]A), benzo[*g,h,i*]perylene (B[*ghi*]P), indeno[*c,d*]pyrene (I[*cd*]P) and 4 dibenzopyrenes such as dibenzo[*a,l*]pyrene (D[*al*]P), dibenzo[*a,e*]pyrene (D[*ae*]P), dibenzo[*a,i*]pyrene (D[*ai*]P) and dibenzo[*a,h*]pyrene (D[*ah*]P). The second mixture served only for 4 light PAHs (Phen, Anthr, F, Pyr) determination. Furthermore, deuterated PAH Mixture 918 (Dr Ehrenstorfer) was also supplied by Witko (Łódź, Poland) and served for quantitative confirmation of results by gas chromatography with mass spectrometry.

2.3. Extraction and clean-up

Extraction and purification were performed using the method described by Ciecierska and Obiedziński (2013a) with some modifications. Approximately 3 g of ground cocoa beans, cocoa mass or chocolate, dried with anhydrous sodium sulfate, were added with 20 mL of hexane/acetone (60:40, v/v) into an Erlenmeyer flask and placed for 30 min into an ultrasonic bath. The obtained extract was centrifuged for 10 min at 5000 rpm and then poured into heart flasks and concentrated under nitrogen to evaporate the solvent. During the evaporation, it was important to avoid evaporation to dryness, and to minimise splashes on the walls of the flask. After weighing the amount of fat extracted, dichloromethane was added to it, so that 0.250 g of fat was dissolved in 1 mL of solvent. Subsequently, the extract was filtered with the use of PTFE filter.

In order to purify the extract and isolate the PAHs fraction, gel permeation chromatography (GPC) using a TSK Gel G1000HXL column (300 × 7.8 mm, 5 μm), supplied by Polygen (Gliwice, Poland), was used. A 0.5-mL aliquot of the extract prepared previously was separated by an isocratic method with a flow of 0.75 mL min^{-1} of dichloromethane. The dump time of the separation was 0–20 min and collection time of the PAH fraction was 20–35 min. A UV–Vis detector working at 254 nm was applied. Subsequently, approximately 11 mL of the PAH fraction in dichloromethane were subjected to solvent evaporation. The extract was dissolved in 1 mL of acetonitrile and subjected to chromatographic analysis.

In the case of PAHs determination in cocoa butter, 1.5 g sample dissolved in 15 mL of dichloromethane was filtered through the PTFE filter; 1 mL of the above-mentioned solution (100 mg mL^{-1}) was subjected to separation and PAHs isolation using GPC as described above. Further treatment, as in the case of the other kinds of samples described above, including evaporation of dichloromethane from PAHs fraction and dissolution in acetonitrile, was performed.

2.4. HPLC-FLD/DAd

PAHs analysis was performed according to the method described by Ciecierska et al. (2019), and Ciecierska and Obiedziński (2013) using a Shimadzu (Kyoto, Japan) HPLC, equipped with liquid chromatograph LC-10ATVP, degasser DGU-14A, auto injector SIL-10ADVP, detectors: fluorescence detector RF-10AXL, diode array detector SPD-M10AVP and system controller SCL-10AVP. Data were collected and analysed by LabSolution 2.1 programme. PAHs were separated on a BAKERBOND PAH-16 Plus column (250 × 3 mm, 5 μm, Witko, Łódź, Poland), which was thermostated at 30 °C. A gradient elution with flow rate 0.5 mL min^{-1} and acetonitrile/water (50:50) and acetonitrile as mobile phase was applied. The gradient elution programme was as follows: 0–25 min 30% B, 25–50 min 30% B to 100% B and 50–68.5 min 100% B. For PAHs detection with the use of fluorescence detector, different excitation and emission wavelengths were applied: 256/370 nm (for Phen, Anthr), 270/420 nm (for F, Pyr, B[*a*]A, Chr, 5-MChr, B[*b*]F, B[*k*]F, B[*a*]P, D[*ah*]A, D[*al*]P, B[*ghi*]P, D[*ae*]P), 270/500 nm (for B[*j*]F, I[*cd*]P) and 270/470 nm (for D[*ai*]P, D[*ah*]P). In order to detect C[*cd*]P, the diode array detector and 254 nm wavelength were used.

2.5. Quantification and validation of method

Quantification using an external standard method and validation of the method were performed according to the procedure described by Ciecierska et al. (2019), and Ciecierska and Obiedziński (2013). For this purpose, the above-mentioned two standard mixtures of PAHs (PAH-Mix 183 and PAH-Mix 9, Dr Ehrenstorfer) were used. Six concentration levels of PAHs working standards solutions (in the range 1–50 μg L^{-1}) were used to build the calibration curves for individual PAHs and three replicates for each point were done. Briefly, the method linearity was proven for almost all PAHs in the analysed range of concentrations (1–50 μg L^{-1}). For all analysed PAHs, the validation parameters, such as the limit of detection (LOD), the limit of quantification (LOQ), the mean values of recovery with relative standard deviation (RSD) and $HORRAT_R$ (indicator of the method precision), are shown in Table 1. Therefore, to validate the method all parameters, for which specific performance criteria had been set according to Commission Regulation (EU) No. 836/2011 (Commission of the European Communities, 2011b), were calculated. Recovery experiments were conducted by spiking one of the analysed chocolate samples (chocolate derived from Criollo cocoa beans roasted at 160 °C) with three different concentrations of PAHs standard solution at the levels of 1, 10 and 50 μg kg^{-1}. The fortified samples, as well as unfortified ones were analysed in triplicate. The LOD and the LOQ were calculated respectively as equal to three and six times the standard deviation of the mean of blank determinations ($n > 20$). Recovery means the percentage of the true concentration of a substance recovered during the analytical procedure. Therefore, the values of recovery were computed as differences in PAHs content in fortified and un-fortified samples relative to the fortification level. The relative standard deviations for the recovery experiments were determined dividing the standard deviation for the mean recovery of an individual compound by this respective value of recovery and expressed in %. Furthermore, the $HORRAT_R$ values, which are considered as the method's precision indicator, for all PAHs analysed at three different concentrations were calculated, dividing RSD by RSD_R from the Horwitz equation ($RSD_R = 2^{(1-0,5\log C)}$, where C = the concentration given as a dimensionless fraction).

2.6. Results confirmation

Gas chromatography with mass spectrometry (GC–MS) using a

Table 1
Performance of the HPLC–FLD/DAD method for the PAHs analysis in one of the analysed chocolate samples.

Method performance	Phen	Anthr	F	Pyr	C[*cd*]P	B[*a*]A	Chr	5-MChr	B[*j*]F	B[*b*]F	B[*k*]F	B[*a*]P	D[*ah*]A	D[*al*]P	B[*ghi*]P	I[*cd*]P	D[*ae*]P	D[*ai*]P	D[*ah*]P
LOD (μg kg^{-1})	0.06	0.07	0.13	0.08	0.47	0.05	0.08	0.07	0.32	0.10	0.10	0.12	0.13	0.30	0.15	0.28	0.29	0.13	0.16
LOQ (μg kg^{-1})	0.11	0.14	0.26	0.16	0.94	0.10	0.16	0.15	0.64	0.20	0.19	0.24	0.26	0.60	0.30	0.56	0.59	0.25	0.33
Recovery for 50 μg kg^{-1} of sample fortification	79.1	83.9	89.5	91.1	104.4	88.6	86.1	88.3	84.2	87.7	88.3	91.9	85.5	84.3	93.0	88.4	80.1	83.2	77.0
Recovery for 10 μg kg^{-1} of sample fortification	77.6	78.8	86.7	88.9	106.1	85.9	83.1	83.2	79.2	85.9	86.3	89.9	78.1	78.2	83.6	86.2	78.2	78.9	72.6
Recovery for 1 μg kg^{-1} of sample fortification	75.9	76.2	82.9	82.1	111.2	82.4	81.8	81.2	76.5	80.0	81.0	84.3	75.8	73.3	81.8	76.1	73.1	72.8	69.9
Recovery[a] (%)	77.5	79.6	86.4	87.4	107.2	85.7	83.7	84.2	79.9	84.6	85.2	88.7	79.8	78.6	86.2	83.5	77.1	78.3	73.2
RSD[a] (%)	9.4	9.7	10.1	9.8	7.9	7.1	7.4	7.3	7.8	7.8	7.3	6.7	7.6	8.8	7.9	6.8	9.6	10.1	9.7
HORRAT$_R$ value[a]	0.8	0.8	0.8	0.8	0.7	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.7	0.7	0.6	0.8	0.8	0.8

[a] Mean values of recovery, RSD, and HORRAT$_R$ of three different levels of sample fortification.

Shimadzu QP2010, based on details published by Ciecierska and Obiedziński (2013), was performed to confirm the results from HPLC–FLD/DAD analysis. The following conditions were applied: ZB-5ms capillary column (Phenomenex, Torrance, CA), 30 m × 0.25 mm i.d. × 0.25 μm film thickness, a helium carrier gas flow of 0.74 mL min^{-1}, injector temperature 265 °C, injection volume 1 μL in splitless injection mode; the ion source and interface temperature: 230 and 270 °C, respectively. Temperature programme used for separation was as follows: 92 °C (1.5 min), 92–140 °C (15 °C/min), 140 °C (1 min), 140–315 °C (5 °C/min) and 315 °C (5 min). The abundance of ions with *m/z* from 100 to 400 was analysed. Detector voltage of 1.5 kV and electron ionization (70 eV) were applied. For each analysed PAH selected ion monitoring mode was applied with the use of two most abundant and characteristic ions, which were measured. PAHs identification was conducted based on the comparison of GC retention times in real samples with PAHs standard solutions as well as with the use of characteristic ions monitored for every PAH. Quantitative confirmation of results from the HPLC method was performed using the above-mentioned deuterated PAH mixture.

2.7. Statistical analysis

Statistica 10.0 programme was applied to perform statistical analysis of the results obtained. In order to estimate the significance of the differences in the mean PAHs contents, between analysed cocoa beans and their derived products, multiple comparisons method based on Tukey's test (at significance level $\alpha = 0.05$) was used. Cocoa-derived products after roasting at two different temperatures were also included in this analysis.

3. Results and discussion

According to requirements of the Commission Regulation (EU) No. 836/2011, performance criteria for methods of analysis for 4 heavy and marker PAHs (B[*a*]P, B[*a*]A, B[*b*]F and Chr) are as follows: LOD and LOQ, respectively, less than 0.3 μg kg^{-1} and 0.9 μg kg^{-1}, recovery ranging from 50% to 120% and HORRAT$_R$ values less than 2 (Commission of the European Communities, 2011b). On the basis of the obtained validation parameters for these 4 heavy and marker PAHs, such as LOD from 0.05 to 0.12 μg kg^{-1}, LOQ from 0.10 to 0.24 μg kg^{-1}, mean values of recovery from 83.7 to 88.7%, and HORRAT$_R$ values of 0.6, it was confirmed that the applied method, HPLC–FLD/DAD, fulfilled all requirements of the Commission Regulation (EU) No. 836/2011 in terms of the methods of analysis of these compounds in foodstuffs. For the remaining 15 PAHs analysed, both for the light ones from the EPA list and the heavy PAHs listed by SCF, the performance results were also satisfactory (Table 1). The chromatograms of the 15 PAHs from the SCF list and 16 US EPA PAHs, as well as one of the real samples (Criollo cocoa beans roasted at 160 °C), are presented in Fig. 2. Furthermore, comparison of results from the HPLC and GC–MS method confirmed the lack of statistically significant differences between the obtained data.

Other studies have also confirmed that HPLC–FLD method meets the requirements of the Commission Regulation (EU) No. 836/2011 regarding analysis methods criteria for determining 4 heavy PAHs and is hence suitable for monitoring the observance of the maximum levels applicable under Commission Regulation (EU) No. 835/2011 (Raters & Matissek, 2014; Santonicola et al., 2017; Zachara et al., 2017).

Data on mean contents of PAHs, individual compounds, a total sum of 19 PAHs and the sum of selected groups of PAHs (4 light EPA PAHs and 4 heavy and marker SCF PAHs) in analysed cocoa beans of different origins and varieties and their derived products, including roasted cocoa beans, cocoa mass, cocoa butter and chocolate, are shown in Table 2. Correspondingly, analogous data for cocoa beans of two selected varieties and origins (Trinitario from Nicaragua and Criollo from Venezuela), after roasting at two different temperatures, and their

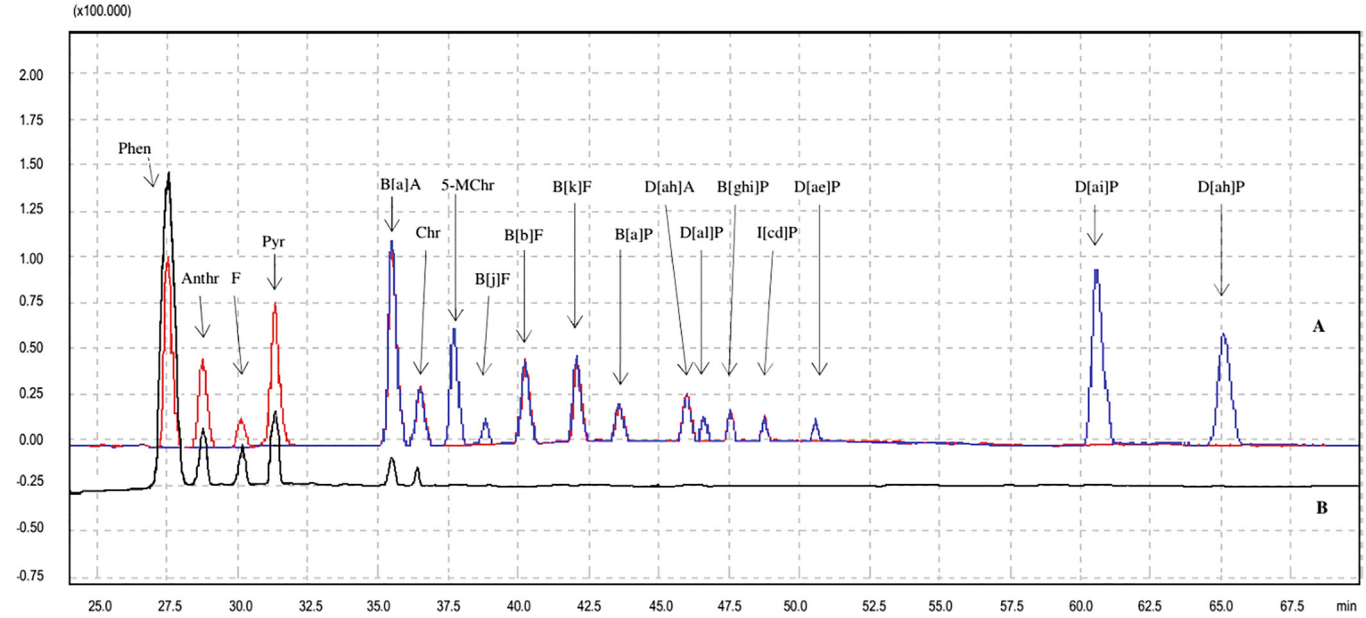

Fig. 2. Chromatograms of: (A) 15 PAHs listed by SCF (PAH–Mix 183, Dr. Ehrenstorfer, 10 pg μL^{-1}; blue colour) and 16 US EPA PAHs (PAH–Mix 9, Dr. Ehrenstorfer, 10 pg μL^{-1}; red colour) and (B) Criollo cocoa beans roasted at 160 °C. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

processed products, are presented in Table 3. Additionally, Fig. 3 shows the mean total contents of 19 PAHs and 4 heavy and marker PAHs in individual groups of products from cocoa beans processing.

Describing PAHs qualitative profiles, it can be stated that they are very similar for all raw materials analysed and their derived products. Four light PAHs were predominant in these profiles. Both in the group of raw and roasted cocoa beans, they constituted from 95 to 100% of all PAHs under investigation. In the case of cocoa mass and cocoa butter, 4 light PAHs ranged from 94 to 100% in the total PAHs contamination, and in chocolate from 96 to 100%. Summarizing, in all analysed products, 4 light PAHs averaged 98% of all PAH content. Among heavy PAHs, only B[*a*]A and Chr, belonging to the group of 15 heavy PAHs and simultaneously to 4 marker PAHs, were detected in the vast majority of analysed samples, however in small amounts. Only in the Trinitario cocoa bean variety, both from the Dominican Republic and Nicaragua, and their derived products, B[*a*]A and Chr were not detected. Therefore, 4 heavy and marker PAHs averaged only 2% of the total 19 PAHs content. The remaining heavy PAHs, including C[*cd*]P, 5-MChr, B[*j*]F, B[*b*]F, B[*k*]F, B[*a*]P, D[*ah*]A, B[*ghi*]P, I[*cd*]P and 4 dibenzopyrenes, were not found in any of samples under investigation, both in raw cocoa beans and their processed products. Other studies, regarding the content of PAHs in cocoa and chocolate samples, also confirmed that compounds belonging to light PAHs were mostly identified in them (Sadowska-Rociek, Cieślik, et al., 2015; Sadowska-Rociek, Surma, & Cieślik, 2015; Żyżelewicz et al., 2017).

Maximum permissible level for the sum of 4 heavy and marker PAHs, established in the Commission Regulation (EU) No. 835/2011, was given per fat of the products since PAHs concentrate in the fat fraction, the cocoa butter (Commission of the European Communities, 2011a). Therefore, to compare the concentration levels of 4 marker PAHs obtained in this work with those established by law, they were also expressed per fat fraction. Analogously, contents of the sum of 19 PAHs were calculated and expressed also per fat, apart from the contents per kg of product.

Taking into consideration the level of total 19 PAHs contamination in the group of raw cocoa beans statistically significant differences were stated between individual cocoa beans of different origins and varieties. Statistically the highest levels of 19 PAHs content, being equal to 11.11 and 10.17 μg kg^{-1} (respectively 41.15 and 36.32 μg kg^{-1} of fat), were found in the Nacional variety from Ecuador and Forastero from Ivory Coast. Statistically insignificantly lower level of contamination than in the Forastero variety from Ivory Coast was found in the same variety of beans originating from Ghana, at a level of 9.47 μg kg^{-1} (31.57 μg kg^{-1} of fat). Another, statistically lower sum of 19 PAHs content, being equal to 5.94 μg kg^{-1} (19.80 μg kg^{-1} of fat), was determined in cocoa beans of the Trinitario variety from Nicaragua. Significantly the lowest levels of total PAHs contamination were noted in the Criollo variety from Venezuela and the Trinitario from the Dominican Republic, respectively 4.90 and 3.58 μg kg^{-1} (14.70 and 12.79 μg kg^{-1} fat). Analogous statistical differentiation in the levels of total PAHs concentration was observed both in the group of roasted cocoa beans and in all other groups of derived products. Therefore, the most contaminated, in terms of 19 PAHs content, were processed products of cocoa beans of the Nacional variety from Ecuador. The derived products of the Forastero variety, regardless of the country of origin, were also relatively highly contaminated with 19 PAHs in comparison with the others. However, the processed products of the Trinitario variety from the Dominican Republic and Nicaragua and Criollo from Venezuela were the least contaminated.

The results described above showed a significant differentiation in levels of total contamination between raw cocoa beans under investigation. The country of beans origin, and more precisely the level of environmental pollution of the place of their cultivation and the methods of its drying may contribute to such a large differentiation in PAHs contamination. Scientists stated that the contamination of cocoa beans can also occur by drying cocoa on asphalt, on bitumen in the sun, or by using direct drying processes (Misnawi, 2012; Ziegenhals et al., 2009).

Regarding the main aim of this work, based on the statistical analysis carried out, for each production chain, regardless of the country of origin and beans variety, significant differentiation in the sum of 19 PAHs between raw cocoa beans and their processed products were confirmed. Therefore, for every variety of cocoa beans originated from different countries and their derived products, statistically the highest levels of total 19 PAHs contamination were recorded in cocoa butter, in a range from 16.76 to 65.06 μg kg^{-1}. The mean total 19 PAHs content in the group of cocoa butter was equal to 35.96 μg kg^{-1}. Statistically lower levels of contamination were found in cocoa mass, in a range

Table 2
Mean content of PAHs in cocoa beans of different origins and varieties and their derived products (μg kg^{-1} ± SD).

PAH	Dominican Republic – Trinitario					Ecuador – Nacional				
	Raw cocoa beans	Roasted cocoa beans (160 °C)	Cocoa mass	Cocoa butter	Chocolate	Raw cocoa beans	Roasted cocoa beans (160 °C)	Cocoa mass	Cocoa butter	Chocolate
Phen	2.06 ± 0.18	3.02 ± 0.22	4.41 ± 0.35	8.52 ± 0.66	2.61 ± 0.19	5.10 ± 0.60	7.93 ± 0.77	13.23 ± 1.85	27.56 ± 2.75	7.21 ± 0.66
Anthr	0.27 ± 0.05	0.50 ± 0.10	0.81 ± 0.10	1.52 ± 0.13	0.41 ± 0.07	0.99 ± 0.11	1.48 ± 0.15	2.64 ± 0.36	4.73 ± 0.59	1.30 ± 0.12
F	0.48 ± 0.07	0.80 ± 0.13	1.50 ± 0.12	2.93 ± 0.23	0.65 ± 0.10	2.30 ± 0.44	5.01 ± 0.49	7.09 ± 0.86	15.02 ± 1.43	4.59 ± 0.42
Pyr	0.77 ± 0.09	1.13 ± 0.23	1.96 ± 0.16	3.79 ± 0.31	0.92 ± 0.17	2.45 ± 0.38	5.15 ± 0.53	7.61 ± 0.95	16.09 ± 1.88	4.71 ± 0.44
B[*a*]A	nd[a]	nd	nd	nd	nd	0.12 ± 0.05	0.21 ± 0.05	0.33 ± 0.07	0.67 ± 0.09	0.19 ± 0.04
Chr	nd	nd	nd	nd	nd	0.15 ± 0.06	0.24 ± 0.07	0.40 ± 0.08	0.89 ± 0.10	0.23 ± 0.04
Σ 19 PAHs [b]	3.58 ± 0.37^{d1}	5.45 ± 0.57^{c1}	8.68 ± 0.66^{b1}	16.76 ± 1.13^{a1}	4.59 ± 0.48^{c1}	11.11 ± 1.10^{d2}	20.02 ± 1.94^{c2}	31.3 ± 3.12^{b2}	65.06 ± 4.96^{a2}	18.23 ± 1.70^{c2}
Σ 4 heavy PAHs [c]	nd	nd	nd	nd	nd	0.27 ± 0.05^{d5}	0.45 ± 0.06^{c5}	0.73 ± 0.08^{b5}	1.56 ± 0.10^{a5}	0.42 ± 0.04^{c5}
Σ 4 light PAHs [d]	3.58 ± 0.37^{d8}	5.45 ± 0.57^{c8}	8.68 ± 0.66^{b8}	16.76 ± 1.13^{a8}	4.59 ± 0.48^{c8}	10.84 ± 1.04^{d9}	19.57 ± 1.85^{c9}	30.57 ± 3.06^{b9}	63.90 ± 4.88^{a9}	17.81 ± 1.64^{c9}
Σ 19 PAHs (per fat)	12.79 ± 1.32^{b12}	19.46 ± 2.04^{a12}	16.69 ± 1.32^{a12}	16.76 ± 1.13^{a12}	12.75 ± 1.50^{b12}	41.15 ± 4.07^{c13}	74.15 ± 7.19^{a13}	62.60 ± 6.24^{a13}	65.06 ± 4.96^{a13}	53.62 ± 5.00^{b13}
Σ 4 heavy PAHs (per fat)	nd	nd	nd	nd	nd	1.00 ± 0.19^{bc16}	1.67 ± 0.22^{a16}	1.46 ± 0.16^{ab16}	1.56 ± 0.10^{a16}	1.24 ± 0.12^{b16}

PAH	Ghana – Forastero					Ivory Coast – Forastero				
	Raw cocoa beans	Roasted cocoa beans (160 °C)	Cocoa mass	Cocoa butter	Chocolate	Raw cocoa beans	Roasted cocoa beans (160 °C)	Cocoa mass	Cocoa butter	Chocolate
Phen	4.11 ± 0.42	6.10 ± 0.45	10.12 ± 0.99	18.36 ± 1.55	5.22 ± 0.41	4.06 ± 0.30	6.03 ± 0.41	9.08 ± 0.77	17.95 ± 1.21	5.01 ± 0.36
Anthr	0.73 ± 0.10	1.14 ± 0.16	1.86 ± 0.16	3.45 ± 0.31	1.02 ± 0.12	1.11 ± 0.12	1.45 ± 0.10	2.85 ± 0.20	5.54 ± 0.23	1.33 ± 0.11
F	2.04 ± 0.19	3.02 ± 0.15	4.92 ± 0.37	8.99 ± 0.76	2.31 ± 0.15	2.06 ± 0.15	2.83 ± 0.18	5.01 ± 0.15	10.76 ± 0.88	2.28 ± 0.17
Pyr	2.32 ± 0.23	3.32 ± 0.26	5.76 ± 0.49	10.27 ± 0.95	2.73 ± 0.22	2.84 ± 0.44	4.12 ± 0.67	6.04 ± 0.56	11.32 ± 0.96	3.43 ± 0.49
B[*a*]A	0.12 ± 0.03	0.22 ± 0.04	0.33 ± 0.06	0.62 ± 0.07	0.21 ± 0.04	0.10 ± 0.03	0.20 ± 0.05	0.29 ± 0.06	0.57 ± 0.10	0.15 ± 0.05
Chr	0.15 ± 0.04	0.25 ± 0.05	0.37 ± 0.05	0.69 ± 0.09	0.24 ± 0.05	nd[a]	nd	nd	nd	nd
Σ 19 PAHs [b]	9.47 ± 0.91^{d3}	14.05 ± 1.05^{c3}	23.36 ± 2.06^{b3}	42.38 ± 3.51^{a3}	11.73 ± 0.97^{c3}	10.17 ± 0.89^{d4}	14.63 ± 0.86^{c4}	23.27 ± 1.23^{b4}	46.14 ± 2.89^{a4}	12.20 ± 0.96^{d4}
Σ 4 heavy PAHs [c]	0.27 ± 0.03^{d6}	0.47 ± 0.05^{c6}	0.70 ± 0.06^{b6}	1.31 ± 0.08^{a6}	0.45 ± 0.05^{c6}	0.10 ± 0.03^{c7}	0.20 ± 0.05^{bc7}	0.29 ± 0.06^{b7}	0.57 ± 0.10^{a7}	0.15 ± 0.05^{c7}
Σ 4 light PAHs [d]	9.20 ± 0.88^{d10}	13.58 ± 1.01^{c10}	22.66 ± 2.01^{b10}	41.07 ± 3.42^{a10}	11.28 ± 0.91^{c10}	10.07 ± 0.81^{d11}	14.43 ± 0.81^{c11}	22.98 ± 1.16^{b11}	45.57 ± 2.79^{a11}	12.05 ± 0.91^{d11}
Σ 19 PAHs (per fat)	31.57 ± 3.03^{b14}	46.83 ± 3.50^{a14}	42.47 ± 3.75^{a14}	42.38 ± 3.91^{a14}	33.51 ± 2.77^{b14}	36.32 ± 3.18^{b15}	52.25 ± 3.07^{a15}	46.54 ± 2.46^{a15}	46.14 ± 2.79^{a15}	38.13 ± 3.00^{b15}
Σ 4 heavy PAHs (per fat)	0.90 ± 0.08^{b17}	1.57 ± 0.17^{a17}	1.27 ± 0.11^{a17}	1.31 ± 0.08^{a17}	1.29 ± 0.14^{a17}	0.36 ± 0.11^{b18}	0.71 ± 0.18^{a18}	0.58 ± 0.12^{a18}	0.57 ± 0.10^{a18}	0.47 ± 0.16^{ab18}

n = 9 (nine samples of every kind of products were analysed).
C[*cd*]P, 5-MChr, B[*j*]F, B[*b*]F, B[*k*]F, B[*a*]P, D[*ah*]A, D[*al*]P, B[*ghi*]P, I[*cd*]P, D[*ae*]P, D[*ai*]P, D[*ah*]P were not detected in any of analysed samples.

[a] nd – not detected

[b] Different letters by the same number (meaning one comparison) below the mean values of sum of 19 PAHs, 4 light PAHs or 4 heavy PAHs indicate statistically significant difference between means at α = 0.05 level.

[c] 4 heavy PAHs: B[*a*]A, Chr, B[*b*]F, B[*a*]P.

[d] 4 light PAHs: Phen, Anthr, F, Pyr.

Table 3
Mean content of PAHs in selected cocoa beans of different origins and varieties and their derived products after roasting at two different temperatures (μg kg^{-1} ± SD).

	Nicaragua – Trinitario								
	Raw cocoa beans	Roasted cocoa beans (160 °C)	Cocoa mass	Cocoa butter	Chocolate	Roasted cocoa beans (140 °C)	Cocoa mass	Cocoa butter	Chocolate
Phen	3.28 ± 0.28	4.89 ± 0.44	7.39 ± 0.48	13.96 ± 0.70	4.07 ± 0.20	4.75 ± 0.31	7.15 ± 0.56	13.13 ± 0.57	3.85 ± 0.33
Anthr	0.26 ± 0.06	0.45 ± 0.09	0.71 ± 0.13	1.47 ± 0.18	0.36 ± 0.08	0.40 ± 0.08	0.62 ± 0.10	1.35 ± 0.12	0.29 ± 0.08
F	1.17 ± 0.11	1.57 ± 0.15	2.36 ± 0.22	4.58 ± 0.44	1.22 ± 0.11	1.46 ± 0.12	2.18 ± 0.21	4.43 ± 0.38	1.14 ± 0.15
Pyr	1.23 ± 0.13	1.99 ± 0.16	3.29 ± 0.29	5.92 ± 0.53	1.44 ± 0.12	1.85 ± 0.14	3.13 ± 0.24	5.64 ± 0.46	1.32 ± 0.14
B[*a*]A	nd[a]	nd	nd	nd	nd	nd	nd	nd	nd
Chr	nd	nd	nd	nd	nd	nd	nd	nd	nd
Σ 19 PAHs [b]	5.94 ± 0.57^{d1}	8.90 ± 0.83^{c1}	13.75 ± 0.99^{b1}	25.93 ± 1.70^{a1}	7.09 ± 0.52^{d1}	8.46 ± 0.64^{c1}	13.08 ± 1.01^{b1}	24.55 ± 1.51^{a1}	6.29 ± 0.70^{d1}
Σ 4 heavy PAHs [c]	nd	nd	nd	nd	nd	nd	nd	nd	nd
Σ 4 light PAHs [d]	5.94 ± 0.57^{d4}	8.90 ± 0.83^{c4}	13.75 ± 0.99^{b4}	25.93 ± 1.70^{a4}	7.09 ± 0.52^{d4}	8.46 ± 0.64^{c4}	13.08 ± 1.01^{b4}	24.55 ± 1.51^{a4}	6.29 ± 0.70^{d4}
Σ 19 PAHs (per fat)	19.80 ± 1.90^{b6}	29.67 ± 2.77^{a6}	25.00 ± 1.80^{a6}	25.93 ± 1.70^{a6}	19.69 ± 1.44^{b6}	28.20 ± 2.13^{a6}	23.78 ± 1.84^{a6}	24.55 ± 1.51^{a6}	17.47 ± 1.94^{b6}
Σ 4 heavy PAHs (per fat)	nd	nd	nd	nd	nd	nd	nd	nd	nd
	Venezuela – Criollo								
	Raw cocoa beans	Roasted cocoa beans (160 °C)	Cocoa mass	Cocoa butter	Chocolate	Roasted cocoa beans (140 °C)	Cocoa mass	Cocoa butter	Chocolate
Phen	2.07 ± 0.22	3.26 ± 0.22	4.15 ± 0.36	7.97 ± 0.66	3.03 ± 0.20	3.14 ± 0.18	3.99 ± 0.31	7.53 ± 0.52	2.85 ± 0.18
Anthr	0.41 ± 0.05	0.81 ± 0.15	0.95 ± 0.09	1.95 ± 0.16	0.72 ± 0.13	0.73 ± 0.10	0.81 ± 0.08	1.65 ± 0.14	0.66 ± 0.11
F	1.08 ± 0.13	1.77 ± 0.24	2.17 ± 0.17	4.19 ± 0.32	1.63 ± 0.20	1.66 ± 0.19	2.09 ± 0.15	4.01 ± 0.36	1.47 ± 0.15
Pyr	1.10 ± 0.15	1.94 ± 0.27	2.33 ± 0.19	4.35 ± 0.35	1.70 ± 0.18	1.85 ± 0.24	2.18 ± 0.20	4.12 ± 0.28	1.55 ± 0.16
B[*a*]A	0.11 ± 0.04	0.19 ± 0.05	0.28 ± 0.05	0.52 ± 0.08	0.15 ± 0.06	0.15 ± 0.06	0.25 ± 0.06	0.42 ± 0.07	0.13 ± 0.05
Chr	0.13 ± 0.04	0.23 ± 0.07	0.33 ± 0.06	0.59 ± 0.10	0.18 ± 0.07	0.19 ± 0.06	0.30 ± 0.06	0.48 ± 0.09	0.16 ± 0.08
Σ 19 PAHs[b]	4.90 ± 0.45^{e2}	8.20 ± 0.86^{cd2}	10.41 ± 0.75^{b2}	19.57 ± 1.55^{a2}	7.41 ± 0.56^{cd2}	7.72 ± 0.77 ^{cd2}	9.62 ± 0.81^{bc2}	18.21 ± 1.41^{a2}	6.82 ± 0.68^{d2}
Σ 4 heavy PAHs[c]	0.24 ± 0.06^{d3}	0.42 ± 0.06^{c3}	0.61 ± 0.09^{b3}	1.11 ± 0.12^{a3}	0.33 ± 0.07^{cd3}	0.34 ± 0.07^{cd3}	0.55 ± 0.08^{b3}	0.90 ± 0.11^{a3}	0.29 ± 0.08^{cd3}
Σ 4 light PAHs[d]	4.66 ± 0.41^{e5}	7.78 ± 0.81^{cd5}	9.80 ± 0.67^{b5}	18.46 ± 1.44^{a5}	7.08 ± 0.49^{cd5}	7.38 ± 0.71^{cd5}	9.07 ± 0.74^{bc5}	17.31 ± 1.30^{a5}	6.53 ± 0.60^{d5}
Σ 19 PAHs (per fat)	14.70 ± 1.35^{c7}	24.62 ± 2.58^{a7}	19.99 ± 1.44^{ab7}	19.57 ± 1.55^{ab7}	17.11 ± 1.29^{bc7}	23.18 ± 2.31^{a7}	18.50 ± 1.56^{ab7}	18.21 ± 1.41^{ab7}	15.75 ± 1.57^{bc7}
Σ 4 heavy PAHs (per fat)	0.72 ± 0.13^{b8}	1.26 ± 0.18^{a8}	1.17 ± 0.17^{a8}	1.11 ± 0.12^{a8}	1.03 ± 0.16^{a8}	1.02 ± 0.21^{a8}	1.06 ± 0.15^{a8}	0.90 ± 0.11^{a8}	0.91 ± 0.18^{ab8}

n = 9 (nine samples of every kind of products were analysed).
C[*cd*]P, 5-MChr, B[*j*]F, B[*b*]F, B[*k*]F, B[*a*]P, D[*ah*]A, D[*al*]P, B[*ghi*]P, I[*cd*]P, D[*ae*]P, D[*ai*]P, D[*ah*]P were not detected in any of analysed samples.

[a] nd – not detected.

[b] Different letters by the same number (meaning one comparison) below the mean values of sum of 19 PAHs, 4 light PAHs or 4 heavy PAHs indicate statistically significant difference between means at α = 0.05 level.

[c] 4 heavy PAHs: B[*a*]A, Chr, B[*b*]F, B[*a*]P.

[d] 4 light PAHs: Phen, Anthr, F, Pyr.

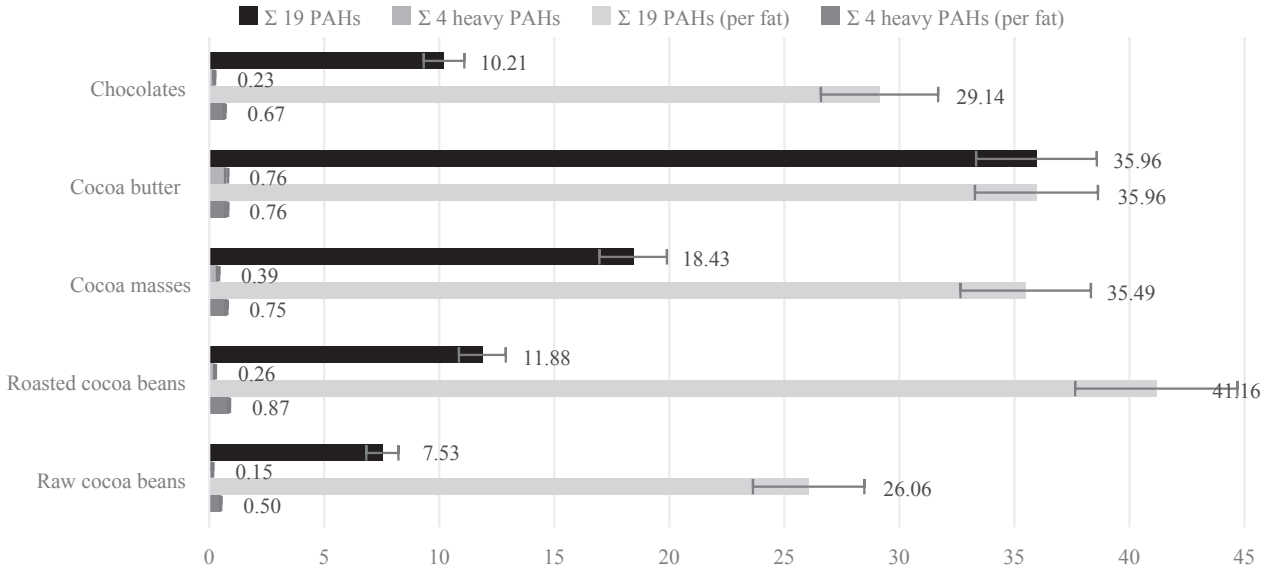

Fig. 3. Mean total 19 PAHs and 4 heavy and marker PAHs content in individual groups of products from cocoa beans processing (μg kg^{-1}).

from 8.68 to 31.30 μg kg^{-1}, with the mean content at a level of 18.43 μg kg^{-1}. Another statistically lower level of contamination was noted both in chocolate and roasted cocoa beans, respectively in the range from 4.59 to 18.23 μg kg^{-1} and 5.45 to 20.02 μg kg^{-1}. The mean contents of 19 PAHs in these groups of products were equal to, respectively, 10.21 and 11.88 μg kg^{-1}. Significantly the lowest concentrations of the sum of 19 PAHs were determined in raw cocoa beans and they varied from 3.58 to 11.11 μg kg^{-1}, with the mean content at a level of 7.53 μg kg^{-1}. Because PAHs always accumulate in fat, the above results confirmed that high-fat products are the most contaminated. This justifies the highest levels of cocoa butter or cocoa mass contamination. Other researchers also emphasised that among the chocolate ingredients, cocoa butter is the component considered as the main source of PAHs (Sadowska-Rociek, Cieślik, et al., 2015).

As a result of expressing the content of 19 PAHs on a fat basis, for every production chain, statistically the highest levels of contamination were confirmed in roasted cocoa beans and varied from 19.46 to 74.15 μg kg^{-1} of fat. Furthermore, statistically insignificantly lower levels of total contamination were found in cocoa butter (as stated above, from 16.76 to 65.06 μg kg^{-1}) and cocoa mass (from 16.69 to 62.60 μg kg^{-1}), in comparison with those found for roasted beans. However, statistically the lowest 19 PAHs concentrations per fat were found in raw cocoa beans and chocolate, respectively in ranges of 12.79–41.15 μg kg^{-1} and 12.75–53.62 μg kg^{-1}. The above results showed that the fat fractions of roasted cocoa beans, cocoa mass and cocoa butter itself are the most contaminated, and this may indicate that the roasting applied (at 160 °C for 30 min) is the source of their contamination. Other reports confirmed that roasting leads to the formation of PAHs while its conditions are key factors determining the level of contamination (Ciecierska et al., 2019; Houessou et al., 2007; Żyżelewicz et al., 2017). Researchers also proved that lipid compounds are important precursors for the PAHs formation, hence the higher the fat content in the raw products, the higher the amounts of PAHs that are produced during thermal processing (Saito, Tanaka, Miyazak, & Tsuzaki, 2014).

Similar differentiation between samples, as observed for the levels of 19 PAHs contamination, was also proved for the contents of 4 heavy PAHs. For every production chain, the highest levels of 4 heavy PAHs concentrations were also noted in roasted cocoa beans, cocoa mass and cocoa butter, respectively in the range 0.71–1.67, 0.58–1.46 and 0.57–1.56 μg kg^{-1} of fat. The mean sums of 4 PAHs contents for the above-mentioned groups of products were, respectively, 0.87, 0.75 and 0.76 μg kg^{-1}. Statistically less contaminated with 4 heavy and marker PAHs were raw cocoa beans and chocolate, with mean concentrations of the sum of 4 PAHs at levels of 0.50 and 0.67 μg kg^{-1}. According to Commission Regulation (EU) No. 835/2011, the maximum permissible level for 4 heavy and marker PAHs in cocoa beans and derived products is 30.0 μg kg^{-1} of fat (Commission of the European Communities, 2011a). Thus, the summary contents of 4 heavy PAHs determined in all products under investigation are much lower than the maximum tolerable limit set by law. Besides that, all tested samples met legal requirements regarding the maximum B[*a*]P limit of 5 μg kg^{-1} of fat for this group of foodstuffs, since as described above, B[*a*]P was not detected in any of the samples.

Summing up, the obtained results confirmed the relatively low level of analysed products contamination with PAHs. Raters and Matissek (2014) also stated that the PAHs contamination level of cocoa and chocolate samples under investigation was very slight overall. Ziegenhals et al. (2009) noted that the sum contents of the 15 SCF-PAHs in chocolate samples from the German market ranged from 1.1 to 6.3 μg kg^{-1}. These values significantly exceed the levels of 4 heavy and marker PAHs from this study, and simultaneously the sum of 15 SCF-PAHs, as other heavy PAHs were not detected in the samples analysed in this work. However, they are of the same order of magnitude, in general. Other studies showed that among the eight samples of Brazilian cocoa beans, only one sample had PAHs content (both B[*a*]P and the sum of 4 marker PAHs) above the maximum limits established by the European Union regulation. However, the PAHs contents for the other samples were below the LOD and LOQ (Belo et al., 2017). In another work, among 20 samples of chocolate from the Polish market, the sum of 4 marker PAHs slightly exceeded the limit mentioned above in 2 samples (Sadowska-Rociek, Cieślik, et al., 2015).

The obtained results also proved that for two selected cocoa beans varieties (Trinitario from Nicaragua and Criollo from Venezuela), applying a lower roasting temperature, 140 °C, than typically used, for a longer time, resulted in insignificant lower levels of contamination of roasted beans and their derived products compared to beans roasted at 160 °C and products of their processing, respectively. This was confirmed both for values expressed per product and fat. For example, for the Criollo variety, the mean level of total 19 PAHs contamination per fat in beans roasted at 160 °C was equal to 24.62 μg kg^{-1}, whereas in beans roasted at 140 °C levels reached 23.18 μg kg^{-1}. In the case of cocoa butter derived from beans roasted at 160 °C and 140 °C, the levels of 19 PAHs were 19.57 and 18.21 μg kg^{-1}, respectively. In chocolate

derived from beans roasted at 160 °C, the contamination level reached a value of 17.11 μg kg^{-1}, while in those from beans roasted at 140 °C were equal to 15.75 μg kg^{-1} (on a fat basis).

In another research, which has so far described the impact of roasting conditions on the content of PAHs in cocoa beans (without considering the varietal diversity of beans) and chocolate derived from them, it was found that beans roasted at 150 °C contained more PAHs than those roasted at 135 °C (Żyżelewicz et al., 2017). However, differences in the values of total PAHs content between these samples were not significant. The total sum of 12 PAHs in all samples of roasted cocoa beans, regardless of the roasting temperature, ranged from 0.38 to 0.76 μg kg^{-1} of the dry weight. Therefore, these values together with the standard deviations (also for the derived chocolate samples) were very low and estimated in a narrow range. It is worth noting that 7 light PAHs from the EPA list constituted almost 100% of all 12 PAHs under investigation. Furthermore, most compounds of the 12 PAHs analysed were present below LODs, which are typical for the applied method of PAHs analysis. Undoubtedly, the levels of contamination of cocoa beans and derived products determined in this paper and other works (Belo et al., 2017; Sadowska-Rociek, Cieślik, et al., 2015; Sadowska-Rociek, Surma, et al., 2015; Ziegenhals et al., 2009) are much higher than those described above by Żyżelewicz et al. (2017) and after converting the PAHs content to dry weight of the analysed products they would be even higher.

Roasting method, its conditions and degree of roasting, as well as the geographical origin and the level of contamination of raw products, are the most important determinants influencing PAHs content in roasted beans (Ciecierska et al., 2019; Houessou et al., 2007; Żyżelewicz et al., 2017). The level of contamination of raw cocoa beans may be strongly affected by their drying method in the respective country of origin (Misnawi, 2012). This may justify the relatively large variation, stated in this study, in the level of contamination of raw cocoa beans originating from different countries. According to the other scientific reports, pyrolysis reactions, which are associated with the formation of PAHs during roasting, occur at temperatures above 170 °C (Franca, Mendonça, & Oliveira, 2005; Yeretzian, Jordan, & Badoud, 2002). However, according to Houessou et al. (2007), the formation of light PAHs occurs at temperatures above 220 °C, while temperatures of 250–260 °C contribute to the formation of heavy PAHs. Therefore, it could be stated that the roasting conditions applied in this study led to the relatively low level of contamination of both roasted cocoa beans and their derived products with PAHs.

4. Conclusions

The levels of PAHs contamination in cocoa beans of several varieties (Forastero, Trinitario, Criollo and Nacional) originating from different countries and their processed products, derived from the production line of a Polish chocolate factory, were studied. The results proved statistically significant differences in total 19 PAHs contents between raw cocoa beans of different varieties and origins. Statistically the most contaminated with 19 PAHs were raw cocoa beans of the Nacional variety from Ecuador and their derived products, whereas the least contaminated were raw and processed products of the Trinitario and Criollo varieties. However, considering the relatively low level of contamination of all raw cocoa beans with a negligible percentage of heavy PAHs in their profile, it can be summarised, that the level of environmental pollution of the place of their origin was low, and the sun drying of all grains had been carried out safely in terms of potential contamination by PAHs. Furthermore, for each production chain, regardless of the country of origin and bean variety, the highest total contents of both 19 PAHs and 4 heavy and marker PAHs were stated in roasted cocoa beans, cocoa mass and cocoa butter. Based on the results obtained, it could be concluded that even a low roasting temperature as 160 °C leads to the formation of PAHs, though not the heavy ones. Furthermore, an attempt to lower the temperature of roasting to 140 °C while extending the time proves to be a way to minimize the overall level of contamination, but to a small extent. To sum up, the relatively low levels of total contamination with light PAHs being predominant and the sum of 4 heavy and marker PAHs much lower than the maximum permissible level of the Commission Regulation (EU) No. 835/2011, were observed in all samples under investigation. Therefore, it could be deduced that analysed cocoa beans and derived products, particularly chocolate, do not pose a threat to their consumers' health. However, since chocolate is often consumed especially by children and cocoa butter is the main chocolate ingredient considered as the main source of PAHs, which can be contaminated depending on various factors, further studies on PAHs contamination levels in cocoa-derived products are still needed.

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CRediT authorship contribution statement

M. Ciecierska: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing - original draft, Writing - review & editing, Visualization, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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