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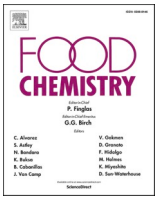

Concentration and potential health risk of polycyclic aromatic hydrocarbons for consumers of chocolate in Brazil

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ABSTRACT

Chocolate is widely consumed worldwide and its market grows every year, with emerging demands for new high-quality products. However, this product is susceptible to contamination with polycyclic aromatic hydrocarbons (PAHs), representing a risk for humans. In this study, a methodology for the evaluation of benzo[*a*]pyrene, benzo[*a*]anthracene, benzo[*b*]fluoranthene and chrysene in chocolate by high performance liquid chromatography was validated. The occurrence, dietary exposure, and health risks of 4 PAHs in 38 commercial chocolate samples was investigated. The methodology demonstrated adequate accuracy and precision, with recovery (95.25 %–108.12 %) and relative standard deviation (0.14 %–5.83 %). Benzo[*a*]pyrene and the ∑4 PAHs concentrations varied between 1.09 and 10.42 μg/kg and 8.38–41.58 μg/kg, respectively. The results of risk assessment suggest low potential health risk for chocolate consumers, considering the margin of exposure (MOE) and the incremental life cancer risk (ILCR) values.

1. Introduction

Chocolate is the most consumed confectionery product in the world and the most profitable as well (Lindt & Sprüngli, 2018). Although widely distributed on all continents, the European continent is the region with the highest consumption of chocolate in the world. In 2017, Switzerland, Austria, and Germany consumed an average of 8.80, 8.10, and 7.90 kg of chocolate per capita, respectively (Lindt & Sprüngli, 2018).

The chocolate market grows every year, with demands for new high-quality products. The production of chocolate involves the use of high temperatures (from 95 to 160 °C during roasting to 60–80 °C during drying and concoction). Moreover, high temperatures can result in the formation of harmful compounds to human health, such as polycyclic aromatic hydrocarbons (PAHs) (Kumari et al., 2012; Predan et al., 2019). The PAHs – such as benzo[*a*]pyrene (B*a*P), benz[*a*]anthracene (B*a*A), benzo[b] fluoranthene (B*b*F), and chrysene (Chr) are organic contaminants widely distributed in the environment and they are formed during incomplete combustion or pyrolysis of organic material, such as during cooking and food processing (IARC, 2012, Singh, Varshney and Agarwal, 2016; Cheng et al., 2019).

Data about the presence of PAHs in commercial chocolates are scarce. The presence of PAHs in raw materials used for the production of chocolate, such as cocoa beans, cocoa powder, cocoa butter and cocoa liquor confirms the need for further studies aimed at investigating PAHs in chocolates (Kumari et al., 2012; Raters and Matissek, 2014; Belo et al., 2017; Abballe et al., 2020). Studies such as those by Ziegenhals, Speer and Jira (2009) and Raters and Matissek (2014) quantified the concentrations of PAHs in chocolates. However, the conditions of human exposure and risk associated with frequent consumption of these foods due to the presence of PAHs have not been explored, demonstrating another gap in the literature as to the study of PAHs.

Levels of PAHs in foods should be restricted due to their mutagenic and carcinogenic effects. Considered carcinogenic to humans by the International Agency for Research on Cancer (IARC), the carcinogenic effect of B*a*P in different animal species is extensively described in the literature (Cavalieri et al., 1991, IARC, 2012). Furthermore, in humans, occupational exposure to B*a*P containing mixtures have been associated

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with several cancers, including those of lung, bladder, skin, haematolymphatic system, mouth, esophagus, and larynx (IARC, 2012).

Food is an important source of human exposure to B*a*P and other PAHs (Cheng et al., 2019). Contaminated ingredients, high temperatures and smoking involved in the processing of cocoa beans, among other factors, may increase PAHs concentration in various types of chocolate products. These factors support the importance of monitoring commercial samples of chocolate products; moreover, it is essential to assess the level of exposure associated with the consumption of popular foods susceptible to PAHs contamination, such as chocolate. Furthermore, this study aimed to quantify the four priority PAHs (B*a*P, B*a*A, B*b*F, and Chr) in commercial chocolates and to assess the degree of exposure to PAHs associated with chocolate consumption.

2. Materials and methods

2.1. Sample collection

In total, 38 chocolate bars from 11 different brands were obtained from local trade in the city of São Paulo, Brazil between 2019 and 2020. The samples were grouped according to type and percentage of cocoa solids (mass, paste, and/or liquor) declared on the label, resulting in 14 samples of milk chocolate, 10 samples of white chocolate, 11 samples of dark chocolate with varied cocoa contents (3 samples of dark chocolate with undeclared cocoa contents. Samples were stored in their original packaging at −20 °C. All analyses were performed at the Laboratory of Food Components and Health, School of Public Health (São Paulo, Brazil).

2.2. Chemical reagents and solvents

Standard solutions of individual PAHs were purchased from Supelco (Bellefonte, PA), including B*a*A (1000 μg/mL in methanol), Chr (1000 μg/mL in acetone), B*b*F (1000 μg/mL in acetone), and B*a*P (1000 μg/mL in acetone). A standard solution was prepared in acetonitrile (MeCN) for each compound (40 μg/mL for B*b*F, Chr, and B*a*P; 36 μg/mL for B*a*A). A work solution containing the four PAHs was prepared in MeCN (200 ng/mL) and maintained at −18 °C until the use.

The reagents and solvents were analytical-grade including potassium hydroxide-KOH (>95 %) and ethanol (>99.5 %) by Merck (Darmstadt, Germany), anhydrous sodium sulfate-Na_2SO_4 (>99 %) and diethyl ether (100 %) by LabSynth (Diadema, Brazil).

N-hexane (Merck, Darmstadt, Germany) and MeCN (Sigma-Aldrich, Burlington, MA) were HPLC-grade, and deionized water was obtained with the Milli-Q purification system (Millipore, Bedford, MA).

Silica cartridges (500 mg, 6 mL, Discovery DSC-SI; Sigma-Aldrich, Palo Alto, CA) were used for solid-phase extraction (SPE), whereas polytetrafluoroethylene filters (0.22 μm, 15 mm; Macherey-Nagel, Düren, Germany) were used for filtration.

The glassware was washed with detergent and water, dried, and sequentially rinsed with ethanol, acetone, and *n*-hexane before analysis.

2.3. PAHs extraction and clean-up

The methodology for extraction and clean-up was based on the studies of Kumari et al. (2012), Raters and Matissek (2014), Bogdanović et al. (2019), with modifications. One gram of ground chocolate sample and 5 mL of 2 Molar methanolic KOH solutions were homogenized and heated at 42 °C for 3 h in water-bath under continuous stirring and away from light. After cooling, 5 mL of deionized water and 10 mL of *n*-hexane were added, mixed and the organic phase was transferred into a 125 mL separating funnel; the extraction was performed twice with 10 mL of *n*-hexane. The hexane phases were combined and filtered in Na_2SO_4 to remove traces of water and concentrated until complete evaporation (water-bath at 37 °C and N_2 flow).

The residue was dissolved in 6 mL of MeCN and forwarded to the clean-up step. Using a vacuum manifold, the SPE silica cartridge was preconditioned with 3 mL of MeCN, and the sample solution was passed through the SPE with a maximum flow of 2 mL/min, followed by washing with 3 mL of MeCN. The eluate was completely evaporated (water-bath at 37 °C and nitrogen gas). The residue was dissolved in 1 mL of MeCN, filtered through a 0.22 μm membrane, and stored in an amber vial for chromatographic analysis.

2.4. Chromatographic conditions

The conditions were based on previous studies (Camargo, Antoniolli, and Vicente, 2011; Silva, Sampaio, and Torres, 2017). The HPLC system (Shimadzu, Tokyo, Japan) was equipped with a pump (LC-20 AT), auto sampler (SIL-20AC), controller (CBM-20A), column oven (CTO-20A), and fluorescence detector (RF-10AXL). A C18 column (250 × 4.6 mm, 5 μm, Shim Pack VP-ODS, Shimadzu) and guard column (C18; 50 × 4.0 mm, Shim Pack ODS, Shimadzu) were used. A gradient mobile phase with water and MeCN (flow rate of 1.5 mL/min) was used: 50 % MeCN from 0 to 20 min, 70 % MeCN from 20.01 to 50 min, 100 % MeCN from 50.01 to 55 min, and returned to initial condition. The column temperature was set at 35 °C, and the injection volume was 30 μL. PAHs were detected by fluorescence with excitation and emission wavelengths of 270/390 nm for B*a*A and Chr, and 290/430 nm for B*b*F and B*a*P. Data processing and quantification, by external standardization, were performed using LabSolutions analysis software (Shimadzu, Kyoto, Japan).

2.5. Validation

The methodology was validated according to the guidelines of the National Institute of Metrology, Quality, and Technology (INMETRO) (INMETRO, 2020) and the Commission Regulation N^o 836/2011 (EC, 2011b). The parameters used were linearity, selectivity, accuracy, precision (repeatability), limits of detection (LOD) and quantification (LOQ). A blank sample of milk chocolate, which presented concentrations of PAHs below the limits of detection, was selected.

The selectivity was evaluated using five levels of PAHs concentrations in two different groups: solvent (MeCN) and blank chocolate sample. The linearity was determined using five levels for B*a*A, Chr, and B*b*F (1.00, 2.00, 3.00, 4.00, and 5.00 ng/mL) in the chocolate matrix and six levels for B*a*P (0.05, 1.00, 2.00, 3.00, 4.00, and 5.00 μg/kg) in the MeCN solvent. All analyses were performed in triplicate.

The accuracy consisted of spiking a chocolate blank sample, before the saponification step, with different concentration levels, in triplicate. The precision, as repeatability, was studied by spiking chocolate blank samples with different concentrations of 4 PAHs. The relative standard deviation (RSD) was evaluated. The limits of detection (LOD) and limits of quantification (LOQ) were calculated, respectively, as three times and six times the standard deviation (SD) obtained from the concentrations of six replicates at the lowest levels of the selectivity assay (INMETRO, 2020).

2.6. Determination of total fat content

The total fat content of chocolates was determined by gravimetry using the study by Hawthorne et al. (2000), with modifications, employing Soxhlet extraction with ethyl ether. The analysis was performed in duplicate using 3 g of sample extraction device for 6 h at a fixed temperature of 56 °C.

2.7. Assessment of dietary exposure to PAHs

The estimated daily intake (EDI) of B*a*P and $\sum$4 PAHs attributed to the consumption of chocolate was calculated according to the principles and methods for assessing the risk of chemical substances in foods proposed by the World Health Organization in the International Program on Chemical Safety (WHO, 2010) as follows:

EDI = (C_{BaP} × IR) / BW.

Or.

EDI = ($C_{\sum 4PAHs}$ × IR) / BW.

where

C_{BaP} = concentration of B*a*P in chocolate (μg/kg) or $C_{\sum 4PAHs}$ is the concentration sum of B*a*A, Chr, B*b*F, and B*a*P.

IRis = daily dietary intake of chocolate (g/day) of a given population or group (for female: 4.2 g/day; for male: 2.7 g/day; for adolescents: 6.2 g/day; for adults: 3.2 g/day; for the elderly: 1.4 g/day).

BW = average body weight (kg) of a given population or group (for adolescents: female BW: 53.3 kg; male BW: 60.1 kg; for adults: female BW: 62.0 kg; male BW: 73.1 kg; for the elderly: female BW: 63.4 kg; male BW: 70.3 kg) (IBGE, 2010).

Data from the Consumer Expenditure Survey were used to determine chocolate consumption and average body weight (IBGE, 2010). We also stratified chocolate consumption according to three categories: sex (female and male) and age (adolescents, 14 – 18 years old; adults, 19 – 59 years old; and elderly, ≥60 years old).

2.8. Health risk assessment

The health risk assessment was calculated according to previous studies by Li et al. (2016), Wang et al. (2018), and Wang et al. (2021) with risk assessment method by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (2005), and the European Food Safety Authority (EFSA) (2008), using the bench mark dose lower confidence limit for a 10 % (BMDL10) value according to the European Food Safety Authority (EFSA, 2008). Data such as consumption, age, body weight and life expectancy were obtained from the IBGE (2010), (2020).

The Margin of Exposure (MOE) was calculated using the equation:

MOE = BMDL10 × BW / ID.

Where:

BMDL10 = 0.07 and 0.34 mg/kg/bw/day for B*a*P and ∑4 PAHs.

BW = average body weight (for adolescents: BW = 50.5 kg; for adults: BW = 67.5 kg; for the elderly: BW = 64.9 kg) (POF, 2010).

ID = Dietary Daily Intake (ng/day).

The Incremental Lifetime Cancer Risk (ILCR) was calculated using the equation:

ILCR = (TEQ × IR × EF × ED) / (BW × AT) × SF × CF.

Where:

TEQ = toxic equivalency quotients (μg/kg).

IR = ingestion of chocolate (g/day) (IBGE, 2010).

EF = Exposure Frequency = 365 days/year.

ED = Exposure Duration (for adolescents: ED = 10 years; for adults: ED = 40; for elderly: ED = 17) (IBGE, 2010).

SF = carcinogenic slope factor for ingestion of B*a*P (7.3 mg/kg/day).

CF = conversion factor (10^{-6} mg/ng).

BW = average body weight (for adolescents: BW = 50.5 kg; for adults: BW = 67.5 kg; for elderly: BW = 64.9 kg) (IBGE, 2010).

AT = average life span (76.6 years in Brazil) (IBGE, 2020).

3. Data analysis

The results of the experiments were evaluated using means of the unidirectional analysis of variance (ANOVA). The means and standard deviation were compared by using the Tukey multiple comparison test, with significance established at $p < 0.05$. T-test, *Grubbs* test and C*ochran's* test were also performed for statistical evaluation of the validation method. The statistical evaluation was performed using the software system Statistical Package for the Social Sciences (SPSS) for Windows (version 23.0).

4. Results and discussion

4.1. Validation

The validation parameters are presented in Table 1 and Fig. 1 shows the chromatograms obtained for a milk chocolate sample and a milk chocolate spiked sample.

For selectivity test, interference was detected for B*a*A, Chr, and B*b*F, and absence of interference was found for B*a*P. For the linearity parameters, calibration curves were evaluated in two different groups: solvent (MeCN) to B*a*P, and matrix (blank chocolate sample) to B*a*A, Chr and B*b*F. The linear regression equation obtained by the least-squares method showed squared correlation coefficient (r^2) values above 0.99, as shown in Table 1, demonstrating that results are linear in the range studied. The outlier values were not detected by linearity test (*Grubbs* tests), presenting 99 % of confidence limit for the linearity parameter. *Cochran's* tests were applied to evaluate residual variances across the range, the calibration curves presented homoscedastic behavior and residues presented a random distribution, free of trends.

The LOQ for B*a*P was 0.50 μg/kg and for B*a*A, Chr and B*b*F were 1.00 μg/kg, indicating the sensitivity of the method. For B*a*P, the most carcinogenic hydrocarbon, the result obtained was lower than 0.90 μg/kg, target limit according to the European Commission (EC, 2011b).

The results obtained for recoveries, B*a*A (98.27–100.86 %), Chr (95–108.12 %), B*b*F (98.23–102.82 %), and B*a*P (97.27–100.79 %) were considered acceptable, according to the performance criteria of INMETRO (2020) and Regulation N°. 836/2011 (EC, 2011b). In the range of 40 to 120 % and 75 to 110 %, respectively. In the study of repeatability, for precision, the RSD values obtained, 0.14 to 7.25 %, were adequate

Table 1
Parameters of method validation for the PAHs analysis in chocolate bars.

PAHs	Linear range (ng/mL)[a]	r^2	Spiked level (μg/kg)[c]	Recovery (%)	RSD (%)	LOD (μg/kg)	LOQ (μg/kg)
B*a*A	1.00 – 5.00	0.9984	1.00	100.86	2.37	0.54	1.00
			2.00	98.27	2.81		
			3.00	100.66	0.63		
			4.00	100.75	0.79		
			5.00	99.53	2.43		
Chr	1.00 – 5.00	0.9904	1.00	99.88	0.51	0.57	1.00
			2.00	101.13	1.70		
			3.00	108.12	0.77		
			4.00	95.25	1.15		
			5.00	100.30	0.84		
B*b*F	1.00 – 5.00	0.9953	1.00	102.82	5.83	0.51	1.00
			2.00	98.23	3.18		
			3.00	100.72	7.25		
			4.00	98.76	0.14		
			5.00	100.70	1.53		
PAHs	**Linear range (μg/kg)[b]**	**r^2**	**Spiked level (μg/kg)**	**Recovery (%)**	**RSD (%)**	**LOD (μg/kg)**	**LOQ (μg/kg)**
B*a*P	0.50 – 5.00	0.9986	0.50	100.30	2.48	0.11	0.50
			1.00	97.27	1.23		
			2.00	97.65	2.21		
			3.00	100.15	0.58		
			4.00	100.79	2.51		
			5.00	99.84	0.57		

[a] Linearity determined in five points of the chocolate matrix in triplicate, [b]Linearity determined in six points of the MeCN solvent in triplicate, [c]Spiked levels for accuracy and precision performed on chocolate blank sample (concentrations of PAHs below the LOD) and results expressed as mean of triplicate determinations. B*a*A: benz[*a*]anthracene, Chr: chrysene, B*b*F: benzo[*b*]fluoranthene, B*a*P: benzo[*a*]pyrene. % RSD: relative standard deviation. LOD: limit of detection. LOQ: limit of quantification.

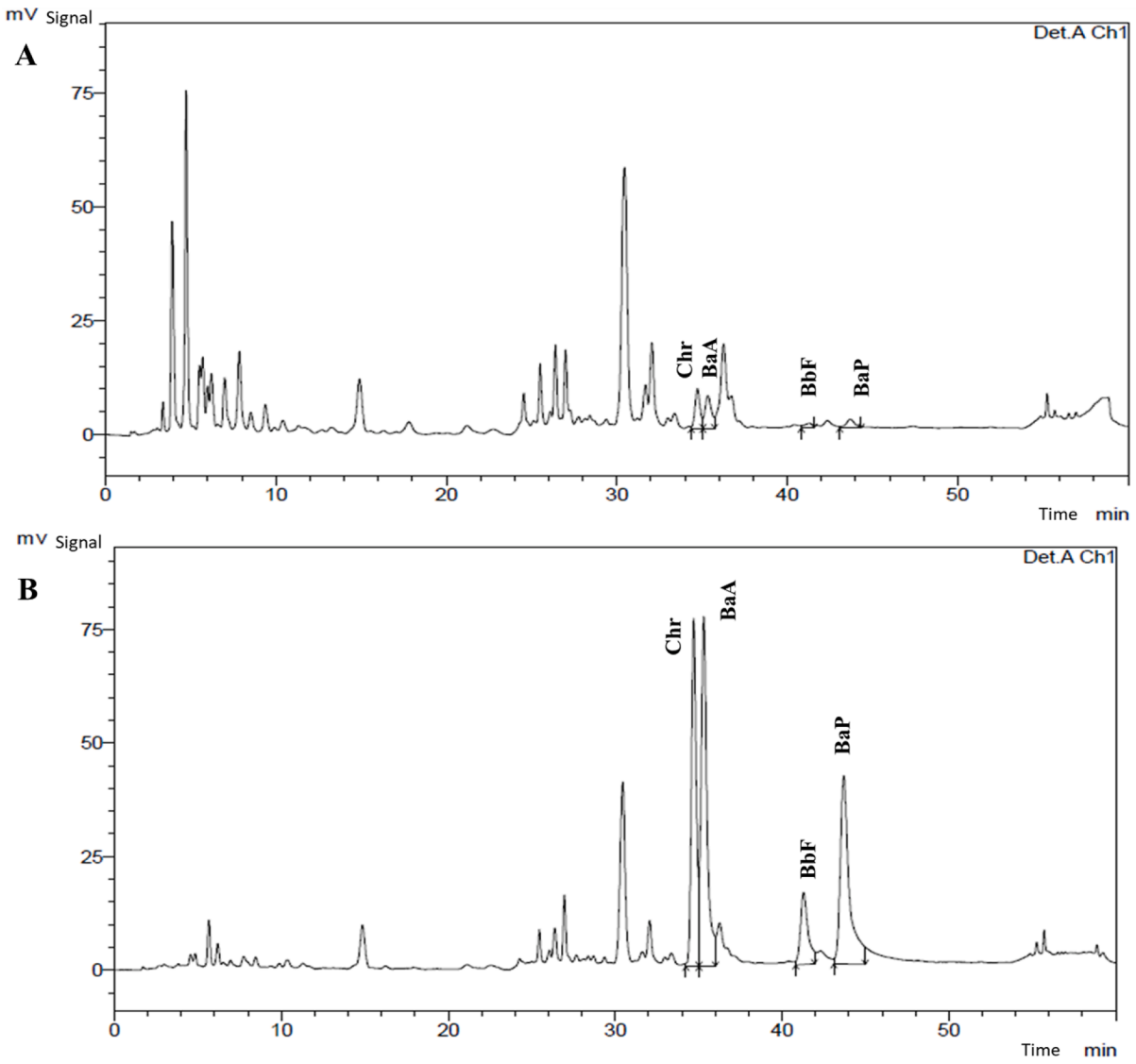

Fig. 1. Chromatograms by HPLC-FL of PAHs in milk chocolate sample (A) and milk chocolate sample spiked (B) with a solution of all four PAHs (50 μg/kg).

Table 2
Lipid content (g/100 g of chocolate bar) and PAHs levels (μg/kg of fat) in different types of chocolate bar.

Products		Lipid	B*a*A	Chr	B*b*F	B*a*P	∑4 PAHs
Milk chocolate	Mean	32.10^{a}	3.12^{a}	7.70^{ab}	2.63^{a}	1.00^{a}	14.46^{a}
(n = 14)	Min	21.71	1.09	5.56	1.59	< LOQ	8.20
	Max	62.06	7.08	13.56	4.40	2.61	26.22
White chocolate	Mean	32.26^{a}	4.76^{ab}	9.73^{ab}	3.72^{ab}	2.31^{a}	20.53^{ab}
(n = 10)	Min	33.05	3.34	8.64	3.20	1.56	16.75
	Max	26.47	2.57	6.14	2.01	0.87	11.47
40 % cocoa chocolate	Mean	26.90^{a}	2.60^{a}	9.29^{ab}	3.04^{ab}	0.79^{a}	15.73^{a}
(n = 4)	Min	10.15	1.79	5.56	1.56	< LOQ	8.57
	Max	39.38	4.51	16.02	3.56	1.87	26.38
53 % to 60 % cocoa chocolate	Mean	35.01^{a}	3.96^{a}	7.97^{ab}	2.94^{ab}	1.64^{a}	16.52^{a}
(n = 3)	Min	30.34	2.81	6.48	2.74	1.74	11.12
	Max	36.64	6.19	10.14	4.70	3.13	24.16
70 % cocoa chocolate	Mean	36.56^{a}	6.76^{b}	11.01^{b}	4.89^{b}	4.56^{b}	27.24^{b}
(n = 4)	Min	26.68	3.10	6.62	2.38	1.41	13.61
	Max	40.09	10.25	13.76	6.93	7.94	37.48
Dark chocolate undeclared cocoa (n = 3)	Mean	47.28^{ab}	3.91^{ab}	5.84^{a}	1.79^{a}	1.56^{a}	13.12^{a}
	Min	36.02	2.65	4.69	< LOQ	< LOQ	9.14
	Max	64.03	5.41	7.17	2.12	3.32	15.45

Values are means (n = 2).*Lipid content g/100 g of milk chocolate. Different letters (a,b,c) in the same column indicate statistical differences between samples ($p < 0.05$). B*a*A: benz[*a*]anthracene, Chr: chrysene, B*b*F: benzo[*b*]fluoranthene, B*a*P: benzo[*a*]pyrene. < LOQ: concentration below the limit of quantification.

for the 4 PAHs criteria (EC, 2011b; AOAC, 2016; INMETRO, 2020).

4.2. Determination of total fat content

The results of total fat composition of the chocolate samples ranged from 26.90 to 47.28 g/100 g. The 40 % cocoa chocolate showed the lowest fat values, and the undeclared cocoa chocolate, the highest values (Table 2).

Chocolate has several types of fats in its composition. The main one is cocoa butter (characteristic of chocolate), but other fats like palm oil, shea butter, and mango kernel oil can be used to reduce the cost of raw materials or modify the sensory characteristics of the final product. It should be noted that the type of fat used could interfere in the textural or rheological properties of the chocolate (Torres-Moreno et al., 2015; Chukwujindu et al., 2019).

4.3. Content of PAHs in chocolate bars

Among the 38 samples of chocolate bars analyzed individually, three of them presented values considered above safe levels according to Regulation N°. 835/2011 (Table S1 - supplementary material). Samples B3 and B10, both from the white chocolate group, presented values of 5.81 and 6.01 μg/kg of fat for B*a*P; and 41.58 and 39.35 μg/kg of fat for the ∑4 PAHs, respectively. The third sample that presented high PAHs values was the 70 % cocoa chocolate sample (G40), with 7.72 μg/kg of fat for B*a*P and 36.32 μg/kg of fat for the ∑4 PAHs.

The groups milk chocolate, 40 % cocoa chocolate, 53 % to 60 % cocoa chocolate and dark chocolate with undeclared cocoa percentage presented results below the maximum limits established for B*a*P (5.00 μg/kg of fat) and for the ∑4 PAHs (30.00 μg/kg of fat), being considered safe for consumption (Table 2). The individual concentrations of the 4 PAHs in the 38 chocolate samples are shown in Table S1 (Supplementary Material).

The mean values of B*a*P identified in the samples varied from 1.00 to 4.56 μg/kg of fat (Table 2), considered below the maximum limit (5.00 μg/kg) established by Regulation (EC) N°. 835/2011 for cocoa beans and derived products (EC, 2011a) whereas the group of chocolates with 70 % cocoa showed a higher concentration of B*a*P (4.56 μg/kg of fat). The 40 % chocolate group had the lowest concentration of B*a*P (0.79 μg/kg of fat).

These results for B*a*P were similar to others reported in the literature. Raters and Matissek (2014) presented values between 1.56 and 1.96 μg/kg of fat (respectively for dark and milk chocolate). Kumari et al. (2012) also found the highest concentration of B*a*P in 70 % cocoa chocolate samples (4.56 μg/kg of fat) between the chocolate groups analyzed in their study.

Chrysene was the most prevalent hydrocarbon, accounting for almost 50 % of the PAHs present in the chocolate samples, with a range concentration of 5.84 μg/kg of fat (undeclared cocoa in dark chocolate) to 11.01 μg/kg of fat (70 % cocoa in chocolate). This result proves, as indicated by the EFSA, that B*a*P is not the best marker for the presence of PAHs in foods and more hydrocarbons should be evaluated (da Silva et al., 2017). Chr levels were higher than the maximum value obtained by Kumari et al. (2012), corroborating the results obtained in our study.

For the ∑4 PAHs, the mean values of 14.46 μg/kg (milk chocolate) to 27.24 μg/kg of fat (70 % cocoa chocolate), were also below the maximum limit (30.0 μg/kg of fat) by the European Commission (EC, 2011a). The mean values of milk chocolate (14.46 μg/kg) presented similar values of milk chocolate (10.11 μg/kg) by Raters and Matissek (2014). The sample with 70 % cocoa in our study (27.24 μg/kg) demonstrated the highest level of contamination, about five times the concentration presented by Raters and Matissek (2014) in dark chocolate (5.25 μg/kg).

Through our results, it is noticeable that there is a relationship between higher concentrations of PAHs and the percentage of cocoa, as the 70 % cocoa chocolates present the maximum values. According to Misnawi (2012) and Raters and Matissek (2014), cocoa products can present significant concentration of B*a*P and the ∑4 PAHs, like cocoa mass (0.32 μg/kg and 3.80 μg/kg of fat, respectively to B*a*P and 4 PAHs), cocoa butter (0.96 μg/kg and 12.44 μg/kg of fat) and cocoa powder (1.07 μg/kg and 8.86 μg/kg of fat). The presence of higher concentration of liquor (derived from the processing of cocoa beans) and cocoa butter in 70 % cocoa chocolate bars analyzed (4.56 μg/kg of fat) can contribute to increase the concentrations of PAH in the final product, corroborating to Abballe et al. (2020) analysis of cocoa-derived products (cocoa beans, cocoa shell, nibs and cocoa butter).

Temperature and the presence of smoke during the processing of cocoa beans may also have a significant effect on the formation of PAHs. Żyżelewicz et al. (2016) after analyzing 12 PAHs in cocoa beans verified that samples roasted at 150 °C showed higher concentrations than those roasted at 135 °C. The presence of smoke, according to Abballe et al. (2021) in addition to temperature during the drying and roasting process contributes to the increase of PAHs such as B*a*A, Chr, B*b*F, B*k*F, B*a*P and D*ae*P. It is important to highlight that cocoa butter seems to contribute a lot to the process of contamination by PAHs, since this cocoa product is subjected to high temperatures during its extraction process and is the most common fat added to chocolates.

White chocolate, a type of chocolate not previously analyzed in the literature, reached a maximum value of 6.62 μg/kg of fat for B*a*P and 46.24 μg/kg of fat for the ∑4 PAHs (Table S1). The results can be explained by the presence of high amounts of cocoa butter in this product. White chocolate has the highest fat composition compared to other chocolates, especially cocoa butter, but not only cocoa fat. Other sources of fat can contribute to the formation of PAHs in these chocolates, such as hydrogenated fat. Abballe et al. (2020) confirmed that cocoa butter was more susceptible to the transfer of PAHs from smoke in the production process of cocoa-based products when compared to other products such as cocoa powder. This retention was associated with the high lipid content of cocoa butter, which may be associated with the high concentrations found in white chocolates.

In contrast, 40 % cocoa chocolate (0.79 μg/kg of fat) and milk chocolate (1.00 μg/kg of fat), had low B*a*P amounts compared to the other samples. The production processes of these chocolates of the analyzed brands may present variations that justify a lower presence of these contaminants (Ziegenhals, Speer and Jira, 2009; Raters and Matissek, 2014). Although milk chocolate has the lowest concentrations of liquor in its composition, these chocolates can be added to their composition of fats other than cocoa butter, suggesting that PAHs contamination in these chocolates may come from other food sources.

4.4. Health risk assessment

Table 3 presents values obtained for different risk indices, including mean ID, MOE and ILCR values.

The ID of B*a*P and ∑4 PAHs ranged, respectively, from 3.6 to 8.0 ng/day and from 50.0 to 70.8 ng/day, with a large representation of white and dark chocolate (higher percentage of cocoa).

For the ILCR results, for the different groups (adolescents, adults and the elderly) and different products (milk, white and dark chocolate), the values indicated low risk to the health from the consumption of the evaluated chocolate products, since scores below $1*10^{-6}$ indicate an acceptable level of risk (Wang et al., 2021). This conclusion was compatible with the results of the MOE, indicating minimal risk, since the values of B*a*P and ∑4 PAHs were >100,000 (Wang et al., 2021, JECFA, 2005; EFSA, 2008).

Although more studies are required to assess exposure to chocolate B*a*P consumption, especially for countries where per capita consumption of chocolate is proportionately higher than consumption in Brazil, our study demonstrates the need for policies to control the production of PAHs in food, such as chocolate, ensuring less formation of this contaminant in food raw materials. Thus, information on PAHs concentrations in foods and their dietary intake is crucial to assess their risk

Table 3
Dietary daily intake (ID), ILCR, and MOE values of PAHs in commercial chocolates from São Paulo city, Brazil.

			Milk chocolate (n = 14)	White chocolate (n = 10)	Dark chocolate (n = 14)
ID (ng/day)	B*a*P	Mean	3.6	8.0	7.7
		Maximum	8.4	20.7	26.6
	∑4 PAHs	Mean	50.0	70.8	61.7
		Maximum	81.0	143.4	119.3
ILCR (B*a*P)	Adolescents	Mean	$1.1*10^{-7}$	$1.2*10^{-7}$	$1.9*10^{-7}$
		Maximum	$6.1*10^{-7}$	$2.9*10^{-7}$	$3.6*10^{-7}$
	Adults	Mean	$3.3*10^{-7}$	$3.6*10^{-7}$	$5.4*10^{-7}$
		Maximum	$1.8*10^{-6}$	$8.6*10^{-7}$	$1.1*10^{-6}$
	Elderly	Mean	$3.3*10^{-8}$	$3.6*10^{-8}$	$5.7*10^{-8}$
		Maximum	$1.8*10^{-7}$	$8.5*10^{-8}$	$1.1*10^{-7}$
ILCR (∑4 PAHs)	Adolescents	Mean	$1.2*10^{-7}$	$2.0*10^{-7}$	$2.6*10^{-7}$
		Maximum	$6.7*10^{-7}$	$4.20*10^{-7}$	$4.5*10^{-7}$
	Adults	Mean	$3.6*10^{-7}$	$5.9*10^{-7}$	$7.9*10^{-7}$
		Maximum	$2.0*10^{-6}$	$1.2*10^{-6}$	$1.4*10^{-6}$
	Elderly	Mean	$3.6*10^{-8}$	$5.9*10^{-8}$	$7.8*10^{-8}$
		Maximum	$2.0*10^{-7}$	$1.2*10^{-7}$	$1.4*10^{-7}$
MOE	B*a*P	Mean	$1.5*10^{+6}$	$6.8*10^{+5}$	$7.1*10^{+5}$
		Maximum	$6.5*10^{+5}$	$2.6*10^{+5}$	$2.0*10^{+5}$
	∑4 PAHs	Mean	$5.3*10^{+5}$	$3.7*10^{+5}$	$4.3*10^{+5}$
		Maximum	$3.3*10^{+5}$	$1.8*10^{+5}$	$2.2*10^{+5}$

B*a*P: benzo[*a*]pyrene; ∑4 PAHs: B*a*A: benz[*a*]anthracene, Chr: chrysene, B*b*F: benzo[*b*]fluoranthene, B*a*P; ILCR: incremental life cancer risk; MOE: margin of exposure; Daily dietary intake of chocolate (g/day): for women: 4.2, for men: 2.7, for adolescents (10–19 years): 6.2, for adults (20–59 years): 3.2, for elderly (60–76 years): 1.4 (IBGE, 2010, 2020); Concentration of B*a*P and ∑4 PAHs in chocolate (μg/kg) presented in Table S1 -supplementary material.

to human health.

Considering the types of chocolate, 70 % cocoa, undeclared cocoa and milk bars had the highest levels of dietary exposure to Benzo[*a*] pyrene, as they had the highest levels of B*a*P (Table 3).

In addition, B*a*P intake was estimated for men and women. The chocolate with the highest contribution was chocolate with 70 % cocoa (0.160 μg/kg/day for men and 0.294 μg/kg/day for women) and chocolate with 40 % cocoa had the lowest contribution of dietary exposure to B*a*P for both sexes (0.058 μg/kg/day for men and 0.170 μg/kg/day for women).

Regarding the different age groups, 70 % cocoa chocolate presented the greatest contribution to the group of adolescents, with a higher estimate of dietary exposure to B*a*P in relation to the other groups, especially for women, while the elderly group had the lowest exposure. The elderly group had a lower daily intake of B*a*P compared to the other groups, especially cocoa undeclared chocolate (0.045 μg/kg/day for men and 0.50 μg/kg/day for women) and milk chocolate (0.040 μg/kg/day men and 0.045 μg/kg/day women).

Despite the need to improve the origin of the raw materials used in the production of chocolates and the improvement of production techniques due to the formation of PAHs, it is important to emphasize the positive effects of chocolates with a higher percentage of cocoa in the control of oxidative stress (Mehrabani et al., 2020), cardioprotective and hypoglycemic effects (Tan et al., 2021) and the role of their polyphenols in cognitive disease prevention (Martín, Goya and Pascual-Teresa, 2020).

5. Conclusion

The validation parameters confirm the reliability of the method to analyze 4 PAHs in chocolate bars. The method showed good accuracy (recoveries 95.25–108.12 %), precision (RSD 0.14–5.83 %), and sensibility (LOD 0.11–0.57 μg/kg and LOQ 0.50–1.00 μg/kg). Two white chocolate samples and one 70 % cocoa chocolate sample exceeded the maximum limits for B*a*P and ∑4 PAHs, according to the European Commission. For all samples, the major hydrocarbon was chrysene.

For the assessment of dietary exposure to B*a*P, women and adolescents were more exposed to PAHs due to the higher consumption in grams per day of chocolate by these groups. Considering the samples evaluated in the present study, it was found that the consumption of chocolate presents low potential health risk, but the continuous evaluation of this type of product must be carried out in order to verify the contamination by PAHs by the general population.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2022.134853.

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