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# Cocoa beans and derived products: Effect of processing on polycyclic aromatic hydrocarbons levels

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### ABSTRACT

Contamination of cocoa and its derived products by polycyclic aromatic hydrocarbons (PAHs) may occur due to high roasting temperatures and/or the presence of smoke during drying. Some PAHs are considered carcinogenic and genotoxic. Thirteen PAHs were investigated during cocoa processing. Two cocoa beans samples were used, with and without presence of smoke during drying. In both, an initial contamination was detected, 1.78  $\mu$ g/kg for non-smoked beans and 66  $\mu$ g/kg for smoked cocoa beans. After roasting, a decrease in contamination was observed. Levels detected in derived products were from not detected (shell) to 2.08  $\mu$ g/kg (cocoa butter) for non-smoked beans and 5.2  $\mu$ g/kg (cocoa powder) to 136  $\mu$ g/kg (shell) for smoked beans. Mass balance showed that, for non-smoked cocoa beans, PAHs were transferred after pressing in equal proportions to cocoa butter (34%) and powder (37%). Smoked samples presented a large portion of PAHs in the shells (31%) and, after pressing, compounds remained mainly in the cocoa butter (43%). A tendency for PAHs to migrate to cocoa butter was observed. Drying cocoa beans without contact with smoke could reduce contamination and, therefore, human exposition to potentially carcinogenic and genotoxic compounds.

### 1. Introduction

Cocoa (*Theobroma cacao* L.) is the main raw material used in the production of chocolate, a popular and worldwide appreciated product. Besides their characteristic flavour, food rich in cocoa stand out due to the presence of compounds with health benefits, such as antiinflammatory, antioxidant, antimicrobial, analgesic and vasodilator properties (Efraim et al., 2010). They may also be a source of essential elements as Ca, Fe, Mg, Mn, K and Zn (Peixoto, Oliveira, & Cadore, 2012). The characteristics presented by the cocoa beans are related to the genetic attributes of cocoa tree, form of cultivation and processing (Schmidt, Efraim, Biasi, & Ferreira; 2015, pp. 1–168).

Cocoa world production is estimated to be 4800 thousand tons in 2018/2019. Ivory Coast is presently the larger producer of cocoa beans with 1900 thousand tons per year, followed by Ghana (850 thousand tons per year). With 255 thousand tons, Brazil is the fifth larger producer (IBGE, 2019; ICCO, 2019).

Cocoa beans pre-processing occurs usually in the country of origin and has an important function on quality and flavour profile. The farms are responsible for harvesting, splitting cocoa pods, fermenting and drying, which will reduce acidity, promote biochemical processes and reduce moisture to 80 g/kg (Beckett, Fowler & Ziegler; 2017, pp. 1–760). The most common drying process, where beans are spread in the sun or in greenhouses, takes 7–14 days. Another drying option is the use of hot air dryers, which takes 2–3 days (Araujo et al., 2014); however, it results in lesser elimination of acetic acid and higher astringency and bitterness (Oetterer, Regitano-d'arce, & Spoto, 2006, pp. 1–632). The usual source of heat for air dryers is wood combustion. A critical point in this process is the generation of smoke, that, in case of bad maintenance of the dryer, may come in direct contact with cocoa beans, and can lead to unpleasant flavour and contamination by polycyclic aromatic hydrocarbons (PAHs) (Raters & Matissek, 2014).

In the processing industries, the steps of cleaning and separation of the shells and nibs are carried out before or after the roasting process. When performed before, the fragmented cotyledon will be roasted using time and temperatures varying from 100 °C to 200 °C for a period of 15 min to 2 h, which allows the occurrence of Maillard reaction, moisture loss and reduction of volatile acids, mainly acetic acid (Beckett et al.,

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2017, pp. 1–760). Roasting is an important step in the formation of desirable flavour (Djikeng et al., 2018). However, as in the drying stage, uncontrolled parameters can lead to the formation of carcinogenic compounds such as PAHs (Zyzelewicz, Oracz, Krysiak, Budryn, & Nebesny, 2017). After roasting, cocoa nibs undergo a grinding stage, after which a cocoa mass or liquor with 20–24 µm granulometry is obtained. Cocoa butter and cocoa powder (100–120 g/kg fat) are obtained by hydraulic pressing of the liquor. These products are used as ingredients in chocolate manufacture (Beckett et al., 2017, pp. 1–760).

PAHs are a class of compounds considered as contaminants and some of them present genotoxic and carcinogenic potential. Among them, benzo[a]pyrene has been classified by IARC (International Agency for Research on Cancer) in group 1, as carcinogenic to humans. Other PAHs have been classified in groups 2A and 2B (IARC, 2010). The Joint FAO/WHO Expert Committee on Food Additives (JECFA) evaluated 33 PAHs and concluded that 13 were clearly carcinogenic and genotoxic (FAO/WHO, 2005). These compounds are formed by incomplete combustion of organic matter. Therefore, they can be formed in some foods during high temperature processes, such as smoking, roasting, drying and cooking (FAO/WHO, 2005; EFSA, 2008). Food contamination by PAHs is due mainly to air and water pollution, to their presence in soils and their formation during food processing at high temperatures. In this manner, the presence of these compounds has been reported in different food categories, such as smoked products, teas, coffee, vegetable oils and fats, fruit, vegetable among others (Tfouni, Padovani, Reis, Furlani, & Camargo, 2014; Molle, Abballe, Gomes, Furlani, & Tfouni; 2017).

Data regarding the presence of PAHs in cocoa products are scarce. There is little information available concerning the contribution of the processing steps in PAHs formation and their presence in derived products. Most studies cover PAHs in raw beans, the drying stage of the process, or in the final products (cocoa butter and chocolate). The few studies involving roasting process analysed the 16 PAHs monitored by EPA (United States Environmental Protection Agency), which include nine light PAHs, not considered to be potentially carcinogenic and genotoxic, and seven that are. To our knowledge, this is the first study to evaluate the presence, fate and distribution of 13 potentially carcinogenic and genotoxic PAHs during cocoa beans processing.

Therefore, the objective of the present study was to investigate the effects of time and temperature during roasting of cocoa beans in the behaviour of 13 PAHs, as well as their behaviour throughout the process until obtaining derived cocoa products (liquor, cocoa butter and cocoa powder).

## 2. Materials and methods

## 2.1. Samples

## 2.1.1. Plant material

Cocoa beans were acquired from a cocoa processor located in the South of Bahia, Brazil. Samples were collected after fermentation and drying steps. Two samples were used in the study: 25 kg of cocoa beans fermented and dried without the presence of smoke and 25 kg of cocoa beans fermented and dried in the presence of smoke.

## 2.1.2. Roasting

Cocoa beans dried with and without smoke were roasted in 2 kg batches in an electric rotator oven (JAF Inox, Tambaú, SP, Brasil) with air circulation in temperatures of 120 °C, 135 °C and 150 °C (for nonsmoked beans) and 120 °C e 150 °C (for smoked beans). Samples (100 g) were taken at 15 min periods until reaching a total roasting time of 120 min. All roasting experiments were done in triplicate, totalling 77 samples that were used for studying PAHs kinetics. All samples were stored in metallized PET packaging in order to avoid moisture loss and contact with oxygen.

## 2.1.3. Beans fractionation

The quality of roasted cocoa beans was evaluated by total acidity and moisture content. Processing conditions selected were the ones that would guarantee the elimination of acetic acid and other undesirable compounds formed during fermentation. Beans were roasted in triplicate at 120 °C for 60 min, resulting in moisture (16.6 g/kg) and acidity (0.1229 mol/kg) values proper for processing and obtaining the derived products: liquor, cocoa powder and cocoa butter.

Beans were grounded with a stainless steel knife mill with a 0.6 mm sieve (ICMA, Campinas, SP, Brasil). Shells were separated from the nibs by sieving (material retained between sieves 5.66 mm and 2.83 mm was used) and winnowing (deshelling machine Capco Test Equipment, Ipswich, UK). Nibs were milled in a Thermomix® TM6 (Vorwerk & Co KG, Wuppertal, Germany) at 40 °C at speed 6 for 60 s and refined in a Melanger mill (Spectra 10, Coimbatore, India) to obtain the liquor. Process time was nearly 2 h at a temperature of approximately 60 °C to obtain a maximum particle size of 25  $\mu$ m, monitored with a digital micrometer model 293-561-30 (Mitutoyo, Suzano, Brasil). Afterwards, liquor was pressed in 500 g batches in a hydraulic press (Ercitec, Bauru, SP, Brasil) for the separation of cocoa butter. The remained solids, also known as cocoa cake, were milled in a Thermomix® TM6 (Vorwerk & Co KG, Wuppertal, Germany) at room temperature at speed 6 for 15 s to obtain cocoa powder.

## 2.2. Solvents and reagents

All solvents used were of HPLC grade; methanol and acetonitrile (JT Baker, Xalostoc, Mexico) and hexane and *N*,*N*-dimethylformamide (Scharlab S.L., Sentmenat, Spain). Sodium hydroxide was from Synth (Labsynth, Diadema, SP, Brasil). PAHs standards were acquired from Sigma-Aldrich (dibenzo[ai]pyrene (DaiP), benzo[k]fluoranthene (BkF), chrysene (Chy), benzo[b]fluoranthene (BbF), benzo[a]pyrene (BaP), indeno[1,2,3-*cd*]pyrene (IcdP)) (Saint Louis MO, USA), Supelco (benz [*a*]anthracene (BaA), dibenzo[ah]pyrene (DahP), dibenz[ah]anthracene (DahA), dibenzo[al]pyrene (DalP), dibenzo[ae]pyrene (DaeP), benzo[*j*] fluoranthene (BjF)) (Bellefonte, PA, USA) and IRMM BCR-08IR (5methylchrysene (5MChr)) (Geel, Belgium). Water was obtained from a Milli-Q purifying system (Millipore, Bedford, MA, USA). Solid phase extraction cartridges were used for clean-up (Waters Sep-Pak C18 Vac, 500 mg, 3 mL, Dublin, Ireland). Extracts were filtered using Millex filters (HV PVDF 0.45 µm, Millipore, Cork, Ireland).

### 2.3. Moisture content, total acidity and pH

Moisture content, total acidity and pH were determined by the following official AOAC methods: AOAC 931.04, AOAC 970.21, AOAC 942.15.

## 2.4. PAHs analysis

Method was based in Camargo, Antoniolli, and Vicente (2011).

## 2.4.1. Extraction and cleanup

To a 1 g sample (for cocoa bean, powder and liquor) 10 mL of hexane was added and mixed in ultrasound for 15 min and filtered. For cocoa butter, 5 mL of hexane was added to 0.5 g sample. Two 5 mL portions of *N*,*N*-dimethylformamide-water (9:1, mL/mL) were used for liquid-liquid extraction. The combined extract was concentrated in a stream of nitrogen (TurboVap LV, Caliper Life Science, Hopkinton, MA, USA) until reaching 50% of its original volume, and 5 mL of water was added. Before SPE clean-up, cartridges were prepared by pre-washing with 5 mL of methanol and 5 mL of water using a Vacuum Manifold (Supelco, Saint Louis, MO, USA). Sample extract was loaded and cartridges were washed with 10 mL *N*,*N*-dimethylformamide-water (9:1, mL/mL), 10 mL of water and dried under vacuum for 20 min. PAHs were eluted with 12 mL of hexane and eluate was dried under a nitrogen flow. Final

Table 1	Analvtical
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HYH	r	$r^2$	LOD (µg/	LOQ (µg/	Cocoa beans	s		Cocoa liquo	r		Cocoa powd	ler		Cocoa butter		
			kg)	kg)	R range (%)	RSDr range (%)	RSDR (%)	R range (%)	RSDr range (%)	RSDR (%)	R range (%)	RSDr range (%)	RSDR (%)	R range (%)	RSDr range (%)	RSDR (%)
BaA	8666.0	96660	0.08	0.30	97-108	1-4	6	89-101	3-7	5	90-117	2–8	9	88-99	2–9	8
Chr	0.9996	0.9992	0.19	0.30	94-102	3-9	6	86 - 100	3-6	5	93–98	3-8	9	88-108	5-10	7
5MChr	0.9996	0.9992	0.21	0.30	81 - 94	1-12	6	86-90	2–9	8	85-92	69	11	79–94	4-9	8
BjF	0.9998	0.9996	1.56	3.00	92 - 101	1-3	ŝ	98-104	2–8	7	93-107	4-5	л С	103 - 114	$1^{-7}$	7
BbF	0.9998	0.9996	0.11	0.30	86–96	3-8	ы С	86-95	4-5	5	89–93	5-10	12	92 - 100	1-11	10
BkF	0.9999	0.9998	0.08	0.30	93-106	4-9	7	80-94	5-6	9	89–94	4-10	12	86-91	$2^{-6}$	8
$\operatorname{BaP}$	0.9999	0.9998	0.11	0.30	93-102	3-8	9	84 - 102	4-7	5	83-104	2-11	11	88–96	4-5	7
DalP	0.9997	0.9994	0.13	0.30	87–99	1-8	6	75-96	5-10	6	82–99	2-10	10	81 - 100	5-9	7
DahA	0.9999	0.9998	0.20	0.30	88-102	$1^{-8}$	7	87 - 101	3-6	8	97 - 107	5-11	6	95–102	3-5	7
IcdP	0.9999	0.9998	0.33	3.00	95–98	1-5	ŋ	93-104	4-7	5	93-103	1-5	7	91 - 102	2–6	9
DaeP	0.9998	0.9996	0.07	0.30	95-101	1 - 14	7	93–98	1 - 10	8	87-105	4–14	10	96-105	2-7	c,
DaiP	0.9991	0.9982	0.13	0.30	95–107	1–8	9	92–112	$2^{-7}$	9	88-107	2-7	12	90-107	3-10	5
DahP	9666.0	0.9992	0.17	0.30	85-102	1-4	4	84 - 103	$1^{-7}$	5	82 - 101	2-11	14	88-97	3–6	9
BaA: ben	z[a]anthra	icene. Chr	: chrvsene. 5	MChr: 5-methy	vlchrvsene. B	iF. benzo[ <i>i</i> ]fluo	vranthene. E	3bF: benzo[b]1	fluoranthene. B	kF: benzoľk	lfluoranthene	e. BaP: benzola	lpvrene. Dal	A: dibenz[a.]	lanthracene. D	alP: dibenzo

ż [a,l]pyrene, IcdP: indeno[1,2,3-cd]pyrene, DaeP: dibenzo[a,e]pyrene, DaiP: dibenzo[a,i]pyrene, DahP: dibenzo[a,h]pyrene

r<sup>2</sup>: coefficient of determination. r: correlation coefficient.

LOQ: limit of quantification. LOD: limit of detection.

R: recovery (accuracy) (n = 15)

RSDr: Relative Standard Deviation under repeatability conditions (same day) (n = 15)

(different days) (n Standard Deviation under within-laboratory reproducibility conditions Relative RSDR:

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residue was reconstituted with acetonitrile (0.5 mL for cocoa butter and 1 mL for bean, liquor and powder), filtered and analysed by HPLC-FLD. Samples were analysed in duplicate.

## 2.4.2. HPLC-FLD

A Shimadzu (Kyoto, Japan) high performance liquid chromatography system with fluorescence detection (HPLC-FLD) was used for the analyses. System consisted of an LC-20AT guaternary pump, DGU-20A5 on-line degasser, SIL-20A autosampler (injection volume 30 µL), CTO-20A column oven (30  $^\circ\text{C})$  and RF-10A xl fluorescence detector. Data were acquired and processed with LCsolution software. A C18 column (Vydac 201 TP54,  $250 \times 4.6$  mm, 5 mm particle size, Vydac, Hesperia, CA, USA) and a gradient mobile phase of acetonitrile:water at a flow rate of 1 mL/min were used for peak separation. Gradient elution program was as follows: linear gradient from 70% to 75% acetonitrile in 20 min. 15 min linear gradient from 75% to 100% acetonitrile, 100% acetonitrile isocratic until 55 min, return to the initial conditions and reequilibration of the column for 15 min. PAHs detection used an excitation and emission wavelength program: 274/414 nm (for BaA, Chr and 5MChr), 312/507 nm (BjF), 290/430 nm (BbF, BkF, BaP, DalP and DahA), 300/500 nm (IcdP), 297/403 nm (DaeP) and 304/457 nm.

## 2.4.3. Quantification and quality control

Method validation was based on INMETRO guidelines (INMETRO, 2018). Compounds were quantified using external standards. Linear regression lines with six concentration levels were obtained by triplicate injections of PAHs standard solutions in acetonitrile (3.0-200.0 ng/mL for BjF and IcdP, and 0.30-20.0 ng/mL for the other PAHs). Recovery tests were used for determination of accuracy and precision. A blank sample was spiked with PAHs standard solutions at three concentration levels in five replicates. Precision (repeatability) was evaluated through the relative standard deviation (RSD) obtained during recovery tests. Reproducibility was evaluated through RSD from recovery tests performed in different days. Limits of detection (LOD) and quantification (LOQ) were obtained from regression lines. Results are displayed in Table 1. Calibration curves were linear for all compounds. The analytical method showed adequate sensibility, except for BjF and IcdP, which presented higher LODs and LOQs. Accuracy, repeatability and reproducibility were within the acceptance criteria settled by INMETRO guidelines (INMETRO, 2018). According to INMETRO (2018), for results in the order of  $\mu$ g/kg, accuracy should be in the range of 40–120% and repeatability and reproducibility should be lower than 30% (INMETRO, 2018). Results obtained were also considered satisfactory and complied with the performance criteria proposed by the European Union for BaP analysis, which states an LOD lower than 0.3 µg/kg and recovery between 50 and 120% (CEC, 2007).

## 3. Results and discussion

## 3.1. Effect of time-temperature binomial

Fig. 1 presents PAHs levels during roasting process of the cocoa beans samples. An initial contamination was detected in cocoa beans dried with and without the presence of smoke, 1.78  $\pm$  0.02  $\mu g/kg$  and  $66\pm2~\mu\text{g/kg},$  respectively, for the sum of the 13 PAHs analysed. The presence of PAHs in time zero is, for the beans dried without smoke, possibly related with environmental contamination, with particulate matter depositing in the surface (Pozo et al., 2015; Liu et al., 2015). Aikpokpodion, Oduwole, and Ademola (2013) showed that, although some plantations are far away from urban areas, smoke exhaustion from automobiles is an important source of contamination of cocoa beans by PAHs.

The beans dried in presence of smoke have another possible source of contamination, the smoke generated in the drying process. The initial contamination in this case is probably due to the artificial drving systems which uses rotating dryers with burning wood as heat source. The

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**Fig. 1.** Polycyclic aromatic hydrocarbons (PAH) levels detected in fermented and dried cocoa beans, with and without the presence of smoke, during process of roasting at different times and temperatures. Mean of triplicate of roasting process. Analyses in duplicate. (n = 6). Results expressed in dry basis.  $\blacktriangle$ : benz[*a*] anthracene, x: chrysene,  $\Diamond$ : 5-methylchrysene,  $\Box$ : benzo[*b*]fluoranthene,  $\blacklozenge$ : benzo[*k*]fluoranthene,  $\circ$ : benzo[*a*]pyrene,  $\textcircled{\bullet}$ : dibenzo[ae]pyrene.





wood combustion may lead to PAHs formation and the consequent deposit in the cocoa beans (EFSA, 2008). Studies by Wandan, Elleingand, and Ndouba (2011) and Lowor et al. (2012) showed that cocoa beans processed with dryers present higher BaP levels then the ones dried in the sun. The presence of PAHs in cocoa beans dried both with and without smoke was also verified in other studies (Aikpokpodion et al., 2013; Raters & Matissek, 2014; Wandan et al., 2011).

In non-smoked cocoa beans, 4 out of 13 PAHs (BaA, Chy, BaP and DaeP) were detected throughout different roasting temperatures and times, with single levels between  $0.32\pm0.01~\mu\text{g/kg}$  and  $0.50\pm0.02~\mu\text{g/kg}$ . Smoked beans, however, presented 6 compounds (BaA, Chy, BbF, BkF, BaP and DaeP) in higher levels, from 0.6  $\pm$  0.2  $\mu\text{g/kg}$  to 23.35  $\pm$  0.07  $\mu\text{g/kg}$ .

Results obtained during roasting of non-smoked beans showed small variation between different processing times at a same temperature (maximum of 0.07  $\mu$ g/kg for BaP at 150 °C). When comparing with the initial contamination (time zero) there was no increase in PAHs levels when increasing process time and temperature. This contrasts with data reported by Zyzelewicz et al. (2017) for 12 PAHs, where whole and crushed cocoa beans roasted at 150 °C contains more PAHs than those roasted at 135 °C. However, Żyżelewicz et al. (2017) analysed another set of PAHs in their study, which may explain this discrepancy.

Smoked cocoa beans samples were roasted at 120  $^{\circ}$ C and 150  $^{\circ}$ C. Comparing with initial PAHs concentration at time zero, lower PAHs levels were detected at the end of the roasting process. There was a small variation in PAHs levels between 30 and 105 min. However, levels declined at 30 and 120 min, possibly due to compounds degradation. When cocoa beans were roasted at the highest temperature and time, 150 °C for 120 min, PAHs with 3 and 4 aromatic rings (BaA, Chy, BbF, BkF and BaP) presented a reduction in concentration of the order of 64%–78%, while 6-ring compounds, 5 MChy and DaeP, suffered a smaller reduction of 36%–43%. These results are in accordance with other studies where PAHs stability at high temperatures for long times was evaluated. Chen and Chen (2001) and Houessou et al. (2007) observed that PAHs with low molecular weight can degrade while the ones of high molecular weight are more difficultly degraded.

## 3.2. Effect of processing in cocoa derived products

Cocoa beans moisture content, acidity and pH were determined in order to monitor roasting process. Results are presented on Fig. 2.

The selection of time and temperature appropriate to carry on the process for obtaining liquor, cocoa powder and cocoa butter was based on moisture and acidity values that would guarantee the elimination of acetic acid and other undesirable compounds that are formed during fermentation, so a more qualitative product could be obtained. Acidity is an important parameter in roasted cocoa beans, since it indicates the concentration of total free acids that can interfere in the final products flavour (Efraim et al., 2010), while pH influences on colour, flavour and dispersibility of cocoa (Efraim, Alves, & Jardim, 2011).



Fig. 2. Moisture content, total acidity and pH in cocoa beans fermented and dried, with and without the presence of smoke, during roasting process at different times and temperatures. Mean of triplicate of roasting process. Analyses in duplicate. (n = 6). Results expressed in dry basis.  $\blacktriangle$ : 120 °C Non-smoked,  $\bigstar$ : 135 °C Non-smoked, x: 150 °C Non-smoked,  $\circlearrowright$ : 120 °C Smoked,  $\Box$ : 150 °C Smoked.

#### Table 2

Polycyclic aromatic hydrocarbons (PAH) mean levels detected in smoked and non-smoked cocoa beans, roasted at 120 °C for 60 min, and in the derived products of cocoa process: *liquor*, shell, cocoa powder and cocoa butter.

		PAH (µg/kg) (	Mean $\pm$ CI) <sup>a</sup>						
		BaA	Chr	5MChr	BbF	BkF	BaP	DaeP	ΣΗΡΑ
Non-smoked beans	Rostaed cocoa beans	$0.39\pm0.01$	$0.34\pm0.01$	<loq< th=""><th><loq< th=""><th><loq< th=""><th><math display="block">0.32\pm0.01</math></th><th><math display="block">0.44\pm0.02</math></th><th><math display="block">1.44\pm0.02</math></th></loq<></th></loq<></th></loq<>	<loq< th=""><th><loq< th=""><th><math display="block">0.32\pm0.01</math></th><th><math display="block">0.44\pm0.02</math></th><th><math display="block">1.44\pm0.02</math></th></loq<></th></loq<>	<loq< th=""><th><math display="block">0.32\pm0.01</math></th><th><math display="block">0.44\pm0.02</math></th><th><math display="block">1.44\pm0.02</math></th></loq<>	$0.32\pm0.01$	$0.44\pm0.02$	$1.44\pm0.02$
	Liquor	$\textbf{0.57} \pm \textbf{0.04}$	$\textbf{0.67} \pm \textbf{0.02}$	<loq< th=""><th><loq< th=""><th><loq< th=""><th><math display="block">\textbf{0.41} \pm \textbf{0.03}</math></th><th><math display="block">0.37 \pm 0.03</math></th><th><math display="block">1.99 \pm 0.05</math></th></loq<></th></loq<></th></loq<>	<loq< th=""><th><loq< th=""><th><math display="block">\textbf{0.41} \pm \textbf{0.03}</math></th><th><math display="block">0.37 \pm 0.03</math></th><th><math display="block">1.99 \pm 0.05</math></th></loq<></th></loq<>	<loq< th=""><th><math display="block">\textbf{0.41} \pm \textbf{0.03}</math></th><th><math display="block">0.37 \pm 0.03</math></th><th><math display="block">1.99 \pm 0.05</math></th></loq<>	$\textbf{0.41} \pm \textbf{0.03}$	$0.37 \pm 0.03$	$1.99 \pm 0.05$
	Shell	<loq< th=""><th><loq< th=""><th><loq< th=""><th><loq< th=""><th><loq< th=""><th><loq< th=""><th><loq< th=""><th><loq< th=""></loq<></th></loq<></th></loq<></th></loq<></th></loq<></th></loq<></th></loq<></th></loq<>	<loq< th=""><th><loq< th=""><th><loq< th=""><th><loq< th=""><th><loq< th=""><th><loq< th=""><th><loq< th=""></loq<></th></loq<></th></loq<></th></loq<></th></loq<></th></loq<></th></loq<>	<loq< th=""><th><loq< th=""><th><loq< th=""><th><loq< th=""><th><loq< th=""><th><loq< th=""></loq<></th></loq<></th></loq<></th></loq<></th></loq<></th></loq<>	<loq< th=""><th><loq< th=""><th><loq< th=""><th><loq< th=""><th><loq< th=""></loq<></th></loq<></th></loq<></th></loq<></th></loq<>	<loq< th=""><th><loq< th=""><th><loq< th=""><th><loq< th=""></loq<></th></loq<></th></loq<></th></loq<>	<loq< th=""><th><loq< th=""><th><loq< th=""></loq<></th></loq<></th></loq<>	<loq< th=""><th><loq< th=""></loq<></th></loq<>	<loq< th=""></loq<>
	Cocoa powder	$0.37 \pm 0.04$	$0.34\pm0.01$	<loq< th=""><th><loq< th=""><th><loq< th=""><th><math display="block">0.32\pm0.01</math></th><th><math display="block">0.42\pm0.01</math></th><th><math display="block">1.44\pm0.03</math></th></loq<></th></loq<></th></loq<>	<loq< th=""><th><loq< th=""><th><math display="block">0.32\pm0.01</math></th><th><math display="block">0.42\pm0.01</math></th><th><math display="block">1.44\pm0.03</math></th></loq<></th></loq<>	<loq< th=""><th><math display="block">0.32\pm0.01</math></th><th><math display="block">0.42\pm0.01</math></th><th><math display="block">1.44\pm0.03</math></th></loq<>	$0.32\pm0.01$	$0.42\pm0.01$	$1.44\pm0.03$
	Cocoa butter	$0.60\pm0.06$	$0.75\pm0.05$	<loq< th=""><th><loq< th=""><th><loq< th=""><th><math display="block">0.32\pm0.02</math></th><th><math display="block">0.42\pm0.05</math></th><th><math display="block">2.08\pm0.07</math></th></loq<></th></loq<></th></loq<>	<loq< th=""><th><loq< th=""><th><math display="block">0.32\pm0.02</math></th><th><math display="block">0.42\pm0.05</math></th><th><math display="block">2.08\pm0.07</math></th></loq<></th></loq<>	<loq< th=""><th><math display="block">0.32\pm0.02</math></th><th><math display="block">0.42\pm0.05</math></th><th><math display="block">2.08\pm0.07</math></th></loq<>	$0.32\pm0.02$	$0.42\pm0.05$	$2.08\pm0.07$
Smoked beans	Rostaed cocoa beans	$\textbf{3.8} \pm \textbf{0.2}$	$5.01 \pm 0.08$	$0.38\pm0.02$	$\textbf{2.3} \pm \textbf{0.1}$	$1.43\pm0.08$	$\textbf{2.8} \pm \textbf{0.1}$	$\textbf{0.48} \pm \textbf{0.02}$	$15.9 \pm 0.04$
	Liquor	$2.63\pm0.3$	$\textbf{4.71} \pm \textbf{0.09}$	$1.1\pm0.1$	$1.35\pm0.06$	$1.20\pm0.07$	$\textbf{2.2} \pm \textbf{0.2}$	$0.33\pm0.02$	$13.3\pm0.2$
	Shell	$30.6\pm0.7$	$47 \pm 1$	$\textbf{3.3}\pm\textbf{0.3}$	$17.7\pm0.9$	$12.1\pm0.9~\text{d}$	$\textbf{22.2} \pm \textbf{0.9}$	$\textbf{4.3} \pm \textbf{0.3}$	$136\pm5$
	Cocoa powder	$1.2\pm0.2~\text{a}$	$2.1\pm0.2$	$0.35\pm0.02$	$0.46\pm0.05$	$0.40\pm0.09$	$\textbf{0.48} \pm \textbf{0.07}$	$0.35\pm0.02$	$\textbf{5.2} \pm \textbf{0.4}$
	Cocoa butter	$\textbf{8.3}\pm\textbf{0.2}$	$11.1\pm0.5$	$0.56\pm0.02$	$3.5\pm0.1$	$\textbf{3.2} \pm \textbf{0.2}$	$\textbf{4.1}\pm\textbf{0.2}$	$0.93 \pm 0.06$	$31.6 \pm 0.2$

BaA: benz[*a*]anthracene, Chr: chrysene, 5MChr: 5-methylchrysene, BbF: benzo[*b*]fluoranthene, BkF: benzo[k]fluoranthene, BaP: benzo[*a*]pyrene, DaeP: dibenzo[ae] pyrene.

<sup>a</sup> Mean of triplicate of roasting process. Analyses in duplicate. (n = 6). Results expressed in dry basis < LOQ: Lower than limit of quantification (<0.3  $\mu$ g/kg). CI: Confidence interval.



Fig. 3. Polycyclic aromatic hydrocarbons mass balance (%) for smoked (A) and non-smoked (B) roasted cocoa beans samples during processes of grinding, for obtaining liquor, and pressing, for obtaining, cocoa powder and cocoa butter.

Non-smoked cocoa beans acidity varied between 0.1134  $\pm$  0.0006 and  $0.1563 \pm 0.0001$  mol/kg. A reduction in acidity was observed while time and temperature of the roasting process raised, meanwhile there was an increase in pH. The same pattern was observed by Jinap and Dimick (1991) and García-Alamilla, Lagunes-Gálvez, Barajas-Fernández, and García-Alamilla (2017), in their studies acidity varies between 18.79 meq NaoH/100 g at 150 °C and 11.10 meq NaoH/100 g at 110 °C. Acetic acid is the main acid present in cocoa beans, being formed during cocoa fermentation by the oxidation of ethanol. As it is a volatile acid, a decrease in concentration is expected during drying and roasting steps (Fowler, 2008). So, the longer the drying or roasting time, lower the acetic acid concentration and consequently lower the total acidity and higher the pH.

Smoked samples presented higher acidity values (Fig. 2); probably due to a less careful drying. Mechanical dryers employ higher temperatures, around 65 °C, to accelerate the process, as consequence beans are dried faster and the elimination of acetic acid from the cotyledon to the surface becomes more difficult (Oetterer et al., 2006, pp. 1–632).

Moisture contents on time zero were 74.7  $\pm$  0.4 g/kg (non-smoked

cocoa beans) and 76.8  $\pm$  0.1 g/kg (smoked cocoa beans), values below the established by Instrução Normativa 38/2008, which states a maximum of 80 g/kg in order to assure preservation and avoid fungi growth (BRASIL, 2008). As expected, throughout roasting, moisture content decreased until final lowest values of 4.4  $\pm$  0.2 g/kg and 5.2  $\pm$  0.3 g/kg, for non-smoked and smoked cocoa beans, respectively.

To evaluate PAHs behaviour and their levels on cocoa derived products, it was given sequence to the process using roasting condition of 120 °C for 60 min. As stated before, for non-smoked cocoa beans these conditions resulted in moisture and acidity proper for processing (16.6 g/kg and 0.1229 mol/kg, respectively). In order to allow an adequate comparison, smoked beans were processed in the same conditions. The use of a longer roasting time can cause an intense dehydration of the surface of cotyledons, resulting in sensorial loss and lower acceptance due to bitter and burnt flavour; while shorter periods will not lead to the formation of flavour compounds and elimination of acetic acid (Afoakwa, 2015, pp. 1–374; Beckett et al., 2017).

Table 2 displays PAHs levels in the products obtained from beans roasted at 120  $^\circ\text{C}$  for 60 min. In the products obtained from non-smoked

beans sample, the same four PAHs present in the roasted cocoa beans were detected, with single levels from not detected to  $0.75 \pm 0.05 \,\mu$ g/kg. Liquor and cocoa butter presented the highest concentrations for the sum of the compounds ( $\Sigma$ PAH),  $1.99 \pm 0.05$  and  $2.08 \pm 0.07 \,\mu$ g/kg, respectively. No PAH was detected in the cocoa beans shells. The seven PAHs present in smoked cocoa beans were detected in their derived products and individual levels were from  $0.33 \pm 0.02$  to  $30.6 \pm 0.7 \,\mu$ g/kg. Shells and cocoa butter were the products with highest  $\Sigma$ PAH levels,  $136 \pm 5$  and  $31.6 \pm 0.2 \,\mu$ g/kg, respectively. Results are similar to the ones reported by Misnawi (2012), where cocoa butter derived from non-smoked cocoa beans present lower PAHs levels than the one obtained from beans dried with wood burn.

In order to analyse PAHs behaviour during processing, a mass balance was done using the mass of the products obtained and the concentrations of  $\Sigma$ PAH presented in Table 2. During processing it was observed that there was a liquor residue left in the mill and in the press, probably due to lack of efficiency of both equipment's, as process was done in a pilot scale.

Mass balance is presented in Fig. 3. For non-smoked sample the largest portion of PAHs was retained in the liquor (91%) and subsequently was transferred in almost equal portions to the cocoa butter (34%) and cocoa powder (37%). The remaining portion was retained in the residue in the press (20%). No PAH was detected in the cocoa beans shells. For the smoked sample, on the other hand, only 55% of the PAHs were present in the liquor, and later transferred in higher proportion to the cocoa butter (43%). After milling and pressing, a large portion of the PAHs was retained in the shells and a considerable portion was present in the residue left in the mill.

According to data presented in Table 2 and Fig. 3, there is a tendency for PAHs, which are nonpolar compounds, to migrate to the lipophilic fraction of the cocoa beans, in this case the cocoa butter. When comparing cocoa beans shells from smoked and non-smoked samples, a significant higher  $\Sigma$ PAHs level was detected in the previous one, and also a higher PAHs transference to this product. Therefore, the contamination probably occurred on the hot air drying step, when cocoa beans came in direct contact with the smoke generated by wood burn. After roasting, the shells are eliminated from the process, however the high temperatures employed for roasting may facilitate PAHs migration from the shells to the cotyledons, and consequently to the derived products (Aikpokpodion et al., 2013; Ziegenhals, Speer, & Jira, 2009).

## 4. Conclusions

Drying cocoa beans in the presence of smoke resulted in higher PAHs contamination, possibly because of inadequate use of hot air dryers. There was no increase in PAHs concentration with the temperatures and processing times used during roasting of both non-smoked and smoked cocoa beans, meaning that no PAHs formation occurred during roasting step. Higher temperatures and longer roasting periods result in roasted cocoa beans with inadequate flavour and aroma, and, therefore, the possible PAHs formation under these conditions was not investigated. During processing, PAHs mainly remain in the shells or transfer to the most lipidic fraction, cocoa butter. When contaminated, cocoa butter, used as an ingredient in many products, from food to cosmetics, can lead to the contamination of final products that are highly and regularly consumed, such as chocolate.

The data obtained, encompassing 13 potentially carcinogenic and genotoxic PAHs, are important to support recommendations to minimize the formation and presence of PAHs in cocoa beans and their derived products. An adequate maintenance of the dryers, avoiding contact of cocoa beans with smoke can reduce the contamination by PAHs. Removing the shells before roasting, as already done in some industries, could also eliminate and/or reduce PAHs levels in both cocoa beans and derived products. The use of cocoa butter blends, i.e., mixture of cocoa butters from different batches or producers, with different levels of contamination, could be a viable alternative for reducing polycyclic

aromatic hydrocarbons in final products, as chocolate. These actions could help reduce human exposure to these potentially carcinogenic and genotoxic compounds. Data obtained can be used in future studies to estimate human exposure to these contaminants.

## CRediT authorship contribution statement

Caroline Abballe: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing, Visualization. Fernanda Moralez Leme Gomes: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing - original draft. Beatriz Dezembro Lopes: Conceptualization, Validation, Formal analysis, Investigation, Data curation, Writing - original draft. Ana Paula Ferreira de Oliveira: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & edit-Visualization. Maria Isabel ing. Berto: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing - original draft, Writing - review & editing, Visualization, Supervision. Priscilla Efraim: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Writing original draft, Supervision. Sílvia Amélia Verdiani Tfouni: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing - original draft, Writing - review & editing, Visualization, Supervision, Project administration, Funding acquisition.

## Declaration of competing interest

The authors declare that there is no potential competing interest with respect to the research, authorship, and/or publication of this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2020.110019.

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