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Polycyclic aromatic hydrocarbons in canola, sunflower and corn oils and estimated daily intake

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ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) are compounds formed during incomplete combustion of organic matter; some are considered to be potentially carcinogenic. The drying step of the seeds or grains is considered the main source of contamination of vegetable oils. Presence of 13 PAHs was evaluated in canola, sunflower and corn oils from the Brazilian market by HPLC-FLD. PAHs were present in 69 of the 70 samples. Levels of summed 13 PAHs varied from not detected to 31.70 μg/kg for canola oil, 0.65 to 17.88 μg/kg for sunflower and 2.61 to 38.23 for corn. There were statistical differences between different types of oil, brands batches. Levels of benzo[a]pyrene and PAH4 were not in accordance with maximum limit established by European regulation in 36 and 33 samples, respectively. Estimated daily intakes were from 7 ng/kg bw/day for to 15.1 ng/kg bw/day. Any action to reduce and/or control their presence in this type of products should be encouraged.

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1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a large class of compounds formed during incomplete combustion of organic matter. They are considered to be both environmental and food contaminants. PAHs presence in food is due to food processing and cooking, or environmental contamination (WHO, 2005; EFSA, 2008). PAHs can be present as contaminants in a wide range of food, such as smoked food products, seafood, tea, coffee, toasted bread, infant foods, fruits, vegetables, alcoholic drinks, and oils and fats (Camargo & Toledo, 2003; Camargo, Antoniolli, Vicente, & Tfouni, 2011a; Fasano, Yebra-Pimentel, Martínez-Carballo, & Simal-Gándara, 2016; García-Falcón & Simal-Gándara, 2005; Rey-Salgueiro, Martínez-Carballo, García-Falcón, González-Barreiro, & Simal-Gándara, 2009; Tfouni, Padovani, Reis, Furlani, & Camargo, 2014; Tfouni et al., 2013; Vieira et al., 2010).

Some PAHs are considered to be potentially carcinogenic. The International Agency for Research on Cancer (IARC) has classified benzo[a]pyrene as carcinogenic to humans (group 1) (IARC, 2010). The Joint FAO/WHO Expert Committee on Food Additives (JECFA) evaluated 33 PAHs and came to the conclusion that 13 of them were clearly carcinogenic and genotoxic (WHO, 2005). The European Food Safety Authority Panel on Contaminants in the Food Chain evaluated 16 PAHs and concluded that benzo[*a*]pyrene is no longer a suitable indicator for the presence of PAHs in food, suggesting that a sum of four or eight specific PAHs (PAH4 and PAH8) are better indicators for PAHs presence in food (EFSA, 2008).

Previous studies have reported the category of oils and fats as one of the most important sources of PAHs in the diet. The main source of vegetable oils contamination is the drying step of the process of seeds or grains before oil extraction (Camargo & Toledo, 2002; Moret, Dudine & Conte, 2000; Purcaro, Moret, & Conte, 2008; Rodríguez-Acuña, Pérez-Camino, Cert, & Moreda, 2008). Although there are some studies regarding the presence of PAHs in Brazilian soybean oil, there is a lack of information about the occurrence of these compounds in those vegetable oils that are considered to be healthier, like sunflower, canola and corn.

Therefore the objective of the present study was to evaluate the presence of 13 polycyclic aromatic hydrocarbons in canola, sunflower and corn oils and estimate their daily intake from the consumption of these products.

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2. Materials and methods

2.1. Materials

2.1.1. Samples

A total of 70 vegetable oil samples of different brands and batches were collected at supermarkets of the State of São Paulo, as follows: 23 samples of canola oil, 26 samples of sunflower oil and 21 samples of corn oil. Samples were analysed in duplicate for the presence of 13 HPAs: benz[a]anthracene (BaA), chrysene (Chr), 5-methylchrysene (5MChr), benzo[*j*]fluoranthene (BjF), benzo[*b*]fluoranthene (BbF), benzo[*k*]fluoranthene (BkF), benzo[*a*]pyrene (BaP), dibenzo[*al*]pyrene (DalP), dibenz[*ah*]anthracene (DahA), indeno[1,2,3-*cd*]pyrene (IcdP), dibenzo[*ae*]pyrene (DaeP), dibenzo [*ai*]pyrene (DaiP) and dibenzo[*ah*]pyrene (DahP).

2.1.2. Standards and reagents

Standards were from Supelco (BaA, DahP, DahA, DalP, DaeP, BjF), Sigma-Aldrich (DaiP, BkF, Chy, BbF, BaP, IcdP) and IRMM (BCR-08IR, 5-MeChy). The HPLC grade solvents used were hexane, N,N-dimethylformamide, methanol and acetonitrile (JT Baker). Water was from a Milli-Q purifying system (Millipore). Solid phase extraction cartridges were used for clean-up (Waters Sep-Pak C18 Vac, 500 mg, 3 mL). Extracts were filtered with Millex HV PVDF 0.45 μm (Millipore).

2.2. Method

Method was based on the one which Camargo, Antoniolli, and Vicente (2011b) used for PAHs analyses in soybean oil.

2.2.1. Extraction and clean up

Hexane (5 mL) was added to a 0.5 g sample and transferred to a separation funnel. Afterwards, extraction was done with two 5 mL portions of N,N-dimethylformamide-water (9:1, v/v). Using a flow of nitrogen (TurboVap LV, Caliper Life Science) the combined extract was concentrated until reaching 50% of its original volume. The remaining extract was then dissolved by adding 5 mL of water. Before SPE clean-up, cartridges were prepared by pre-washing with 5 mL of methanol, followed by 5 mL of water using a Vacuum Manifold from Supelco. Sample extract was loaded and cartridges were washed with 10 mL N,N-dimethylformamide-water (9:1, v/v) followed by 10 mL of water. Cartridges were dried under vacuum for 20 min. PAHs were eluted with 12 mL of hexane and eluate was dried under a nitrogen stream. Final residue was reconstituted with 0.5 mL of acetonitrile, filtered and analysed by HPLC-FLD.

2.2.2. HPLC-FLD

PAHs were analysed by HPLC-FLD using a Shimadzu system composed of the following modules: LC-20AT quaternary pump, DGU-20A5 on-line degasser, SIL-20A autosampler (30 μL injection volume), CTO-20A column oven (30 °C) and RF-10A xl fluorescence detector. Data were acquired and processed with LCsolution software. For peak separation a C18 column (Vydac 201 TP54, 250 × 4.6 mm, 5 μm particle size) and a gradient mobile phase of acetonitrile and water at a flow rate of 1 mL/min were used. Gradient elution program started with a linear gradient from 70% to 75% acetonitrile in 20 min, followed by a 15 min linear gradient from 75% to 100% acetonitrile and maintained 100% acetonitrile isocratic until 55 min, when finally returned to the initial conditions and the column was re-equilibrated with initial mobile phase composition for 15 min. For PAHs detection an excitation and emission wavelength program was used: 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 (DaiP and DahP).

2.2.3. Quantification and method validation

Method was validated based on INMETRO (2011) guidelines. Compounds were quantified using the external standard plot method. Linear regression lines were obtained by triplicate injections of six concentration levels of PAHs standard solutions in acetonitrile (3.0–200.0 ng/mL for BjF and IcdP, and 0.30 to 20.0 ng/mL for the others PAHs).

Accuracy and precision were obtained through recovery tests carried out by spiking a blank sample with PAHs standard solutions at three concentration levels (1.0, 2.0 and 5.0 μg/kg) in five replicates. Precision of the method was evaluated through the relative standard deviation (RSD) obtained during recovery analyses. Limits of detection (LOD) were calculated from the standard deviation of seven independent analyses of the blank sample spiked with PAHs at a level of 1.0 μg/kg. Limits of quantification (LOQ) were established as the lower concentrations used in the calibration curves. Reproducibility was evaluated under within-laboratory reproducibility conditions through RSDs obtained from recovery tests performed in different days (two days, five replicates each day).

2.2.4. Statistical analysis

Data were processed by analysis of variance one-way ANOVA with means comparison (Tukey test) with 95% confidence using software Statistica (Statistica 5.5, Stat Soft Inc.).

2.2.5. Estimated daily intake

In order to estimate the PAHs daily intake from the consumption of vegetable oils some considerations were made. Vegetable oil consumption data was obtained from the National Household Survey conducted by IBGE (Instituto Braileiro de Geografia e Estatística) (IBGE, 2010). The worst case scenario was considered, which means it was used: the highest PAHs summed level determined for each type of oil, the highest consumption of oil (8.622 kg per person per year in the Middle-West region) and the assumption that all oil consumed was of a same type. An average body weight of 60 kg was considered in the calculations.

3. Results and discussion

Table 1 presents results obtained for different parameters studied for method validation: recovery, RSD, LOD and LOQ. Calibration curves obtained were linear with correlation coefficients between 0.9933 and 0.9989. Recovery ranged from 71% to 110% with RSD varying from 4% to 20%. LODs determined were between 0.07 μg/kg (BbF) and 0.30 μg/kg (Chr), while LOQ was established as 0.3 μg/kg. The analytical method however showed to be less sensitive for BjF and IcdP, with LODs of 1.95 μg/kg and 1.32 μg/kg, respectively, and LOQ of 3.0 μg/kg for both PAH. Results obtained can be considered satisfactory for determinations at μg/kg levels and comply with the performance criteria proposed by the European Union for BaP analysis, where LOD should be lower than 0.3 μg/kg and recovery should be in the range of 50–120% (CEC, 2007).

Table 2 presents PAHs mean levels and range detected in different vegetable oil samples analysed. PAHs were present in 69 of the 70 samples with individual levels ranging from not detected to 13.11 μg/kg. Levels of the summed 13 PAHs varied from not detected to 31.70 μg/kg for canola oil, 0.65 to 17.88 μg/kg for sunflower oil and 2.61 to 38.23 for corn oil. Among PAHs analysed, the ones that were more frequently present in the three types of oil were BaP, Chr, BbF and BaA, present in 99%, 97%, 97% and 96% of the samples studied, respectively. DaiP and DahP however, were not detected in any sample. Results obtained for PAH4 were up to

Table 1
Recovery, relative standard deviation (RSD), limit of detection (LOD) and quantification (LOQ) for the analysis of 13 PAHs.

PAH	Mean recovery (%) (RSDr) (n = 5)			RSDR (n = 10)	LOD (μg/kg)	LOQ (μg/kg)
	(1.0 μg/kg)	(2.0 μg/kg)	(5.0 μg/kg)			
BaA	75 (8)	85 (10)	92 (8)	4	0.23	0.3
Chr	84 (14)	104 (6)	104 (9)	3	0.30	0.3
5MChr	90 (9)	89 (15)	92 (9)	8	0.26	0.3
BjF	98 (4)	87 (8)	90 (6)	7	1.95	3.0
BbF	71 (4)	88(6)	98 (11)	3	0.07	0.3
BkF	89 (5)	85 (4)	94 (10)	6	0.15	0.3
BaP	94(4)	98 (9)	95 (20)	8	0.10	0.3
DalP	77 (20)	84 (16)	83 (12)	12	0.29	0.3
DahA	103 (5)	96 (10)	88 (7)	8	0.18	0.3
IcdP	102 (4)	93 (6)	96 (6)	5	1.32	3.0
DaeP	91 (6)	90 (7)	103 (16)	8	0.17	0.3
DaiP	110 (16)	106 (4)	101 (6)	6	0.23	0.3
DahP	105 (8)	106 (7)	106 (4)	6	0.24	0.3

BaA: benz[*a*]anthracene, Chr: chrysene, 5MChr: 5-methylchrysene, BjF, benzo[*j*]fluoranthene, BbF: benzo[*b*]fluoranthene, BkF: benzo[*k*]fluoranthene, BaP: benzo[*a*]pyrene, DahA: dibenz[*a,h*]anthracene, DalP: dibenzo[*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.
RSDr: Relative Standard Deviation under repeatability conditions (same day).
RSDR: Relative Standard Deviation under within-laboratory reproducibility conditions (different days).

Table 2
Polycyclic aromatic hydrocarbons mean levels in different types of vegetable oil.

Vegetable oil	Mean PAH level (μg/kg) (range)												
	BaA	Chr	5MChr	BjF	BbF	BkF	BaP	DalP	DahA	IcdP	DaeP	Σ13 PAH	PAH4
Canola	1.99	3.84	1.15	0.27	2.03	0.44	2.82	nd	nd	0.20	nd	13.56 a	11.18 a
(n = 23)	nd-4.69	nd-7.94	nd-2.77	nd-4.78	nd-4.78	nd-1.05	nd-6.25			nd-3.20		nd-31.70	nd-22.15
Sunflower	1.31	2.68	0.74	nd	1.12	0.26	1.39	nd	nd	nd	0.01	7.56 b	6.52 b
(n = 26)	nd-3.39	nd-6.36	nd-2.29		0.29–2.48	nd-0.54	0.31–3.27				nd-0.49	0.65–17.88	0.65–15.61
Corn	3.06	6.04	1.63	0.43	2.70	0.57	3.03	0.02	0.02	0.65	0.10	17.20 a	14.19 c
(n = 22)	0.41–6.67	1.14–13.11	nd-3.51	nd-3.05	0.57–5.40	nd-1.12	0.27–7.46	nd-0.48	nd-0.43	nd-3.53	nd-1.15	2.61–38.23	2.61–30.98

BaA: benz[*a*]anthracene, Chr: chrysene, 5MChr: 5-methylchrysene, BjF, benzo[*j*]fluoranthene, BbF: benzo[*b*]fluoranthene, BkF: benzo[*k*]fluoranthene, BaP: benzo[*a*]pyrene, DalP: dibenzo[*a,l*]pyrene, DahA: dibenz[*a,h*]anthracene, IcdP: indeno[1,2,3-*cd*]pyrene, DaeP: dibenzo[*a,e*]pyrene.
Values in the same column with the same letter are not statistically different ($p < 0.05$).
nd: not detected.

22.15 μg/kg (canola), 15.61 μg/kg (sunflower) and 30.98 μg/kg (corn), which indicate that the summed levels of BaA, Chr, BbF and BaP represent a major contribution to the sum of the 13 PAHs. As can be seen in Table 1, mean summed levels of the 13 PAHs showed to be statistically lower ($p < 0.05$) in sunflower oil than in canola and corn oils, while levels of PAH4 were statistically different for the three types of oil.

Levels detected in the present study are relatively lower than the ones previously reported by other authors. Jiang et al. (2015) reported the sum of 16 PAHs ranging from 3.71 μg/kg to 185.72 μg/kg in Chinese corn oil. For sunflower oil purchased in Portugal, Teixeira, Casal, and Oliveira (2007) detected levels of the same 16 PAHs varying from 8.78 μg/kg to 9.72 μg/kg. In 2010 Alomirah et al. reported, for sunflower oil acquired in Kuwait, the sum of 16 PAHs ranging from 0.42 μg/kg to 41.30 μg/kg. However, for corn and canola oils Alomirah et al. (2010) detected similar levels to the ones in the present study, 0.30 to 34.49 and 10.29 to 12.23, respectively. Camargo et al. (2011a) detected much higher levels of PAHs in soybean oil commercialized in Brazil, mean of 10.4 μg/kg to 112 μg/kg for the sum of 13 PAHs and 0.5 μg/kg to 15.8 μg/kg for BaP.

In Brazilian regulation there are no maximum limits for PAHs in vegetable oil. Therefore, European regulation which sets limits for some PAHs in the category of oils and fats was used for comparison (2.0 μg/kg for BaP and 10.0 μg/kg for PAH4) (CEC, 2011). Taking into account BaP and PAH4 levels, both were shown to be up to three times higher than the ones permitted. According to results showed in Fig. 1, levels of PAH4 detected were not in accordance with the regulation in 33 samples – i.e., 15 samples of corn oil, 11 samples of canola and 7 of sunflower. As for BaP, 36 samples surpassed the maximum limit established by European regulation: 15 corn oil samples, 13 canola oils and 8 sunflower oils.

Fig. 2 shows the mean levels of the sum of 13 PAHs in different brands of the three types of oil evaluated. As can be observed, there was statistical difference ($p < 0.05$) between different brands of the same type of vegetable oil. There was also variability between different batches of the same brand. These results are in accordance to studies by Tfouni et al. (2014) and Camargo et al. (2011a) where were also found differences among different brands and batches of oil blends and soybean oils.

As stated before, there were statistical differences between different types of oil, different brands of the same oil and also between different batches. These differences may be related to environmental pollution, where levels of PAHs may vary according to region of the crops, and/or different variables involved in the drying process and also during the oil production/refining (Camargo & Toledo, 2002; Camargo, Antoniolli, & Vicente, 2012; Rodríguez-Acuña et al., 2008).

Estimated daily intakes of PAHs from the consumption of vegetable oil in Brazil were calculated considering the worst case scenario. Thus the highest summed levels of the 13 PAHs analysed were used: 31.70 μg/kg for canola oil, 17.88 μg/kg for sunflower and 38.23 μg/kg for corn. A vegetable oil consumption of 23.62 g per person per year and a body weight of 60 kg were assumed. As certain consumers are known to be faithful to a specific type or brand of product, it was also considered that all vegetable oil consumed was of a same type. The daily intakes obtained for this

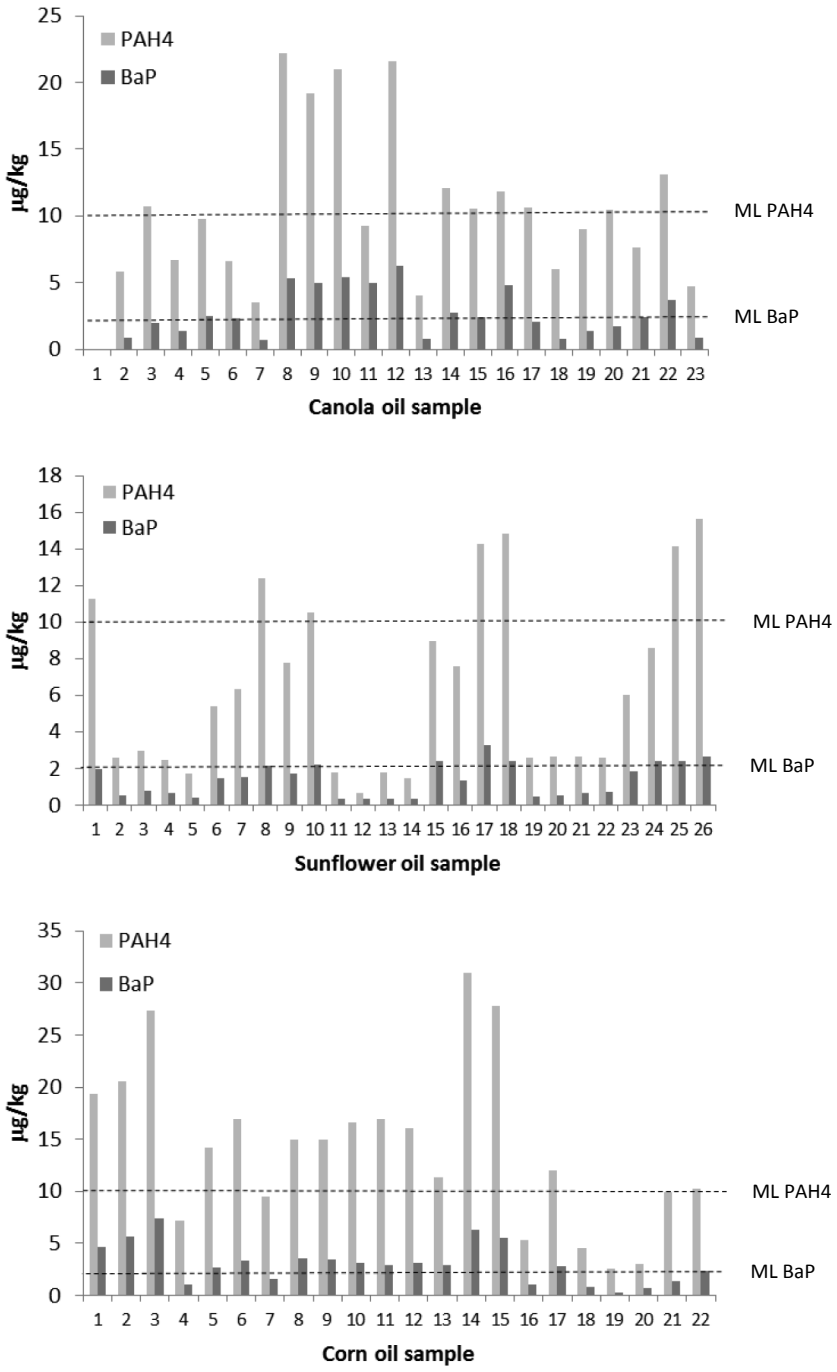

Fig. 1. Levels of PAH4 (sum of benz[*a*]anthracene, chrysene, benzo[*b*]fluoranthene and benzo[*a*]pyrene) and BaP (benzo[*a*]pyrene) in canola, sunflower and corn oil samples. ML: Maximum level according to EU regulation (2 µg/kg - BaP, 10 µg/kg - PAH4).

scenario were 7 ng/kg bw/day for sunflower oil, 12.5 ng/kg bw/day for canola oil and 15.1 ng/kg bw/day for corn oil. These daily intakes are relatively lower than the one of 19.1 ng/kg bw/day reported by Camargo et al. (2011a) from soybean oil. A study from Catalonia, Spain, estimated intakes of summed 16 PAHs from oils and fats ranging from 0.416 µg/day to 0.504 µg/day (Martorell et al., 2010). In the present study, daily intakes from vegetable oils, using the same unity (µg/day), were somewhat higher and ranged from 0.42 to 0.91 µg/day.

4. Conclusion

Analytical method was shown to be suitable for PAHs analysis in vegetable oils.

There was a high variability in PAHs levels in canola, sunflower and corn oils, with approximately half the samples exceeding European regulation limits. Therefore, it is recommended that oil refineries use active carbon in the refining process. Active carbon is known to be highly efficient to reduce PAHs presence in oils; however this practice has not yet been adopted in Brazil.

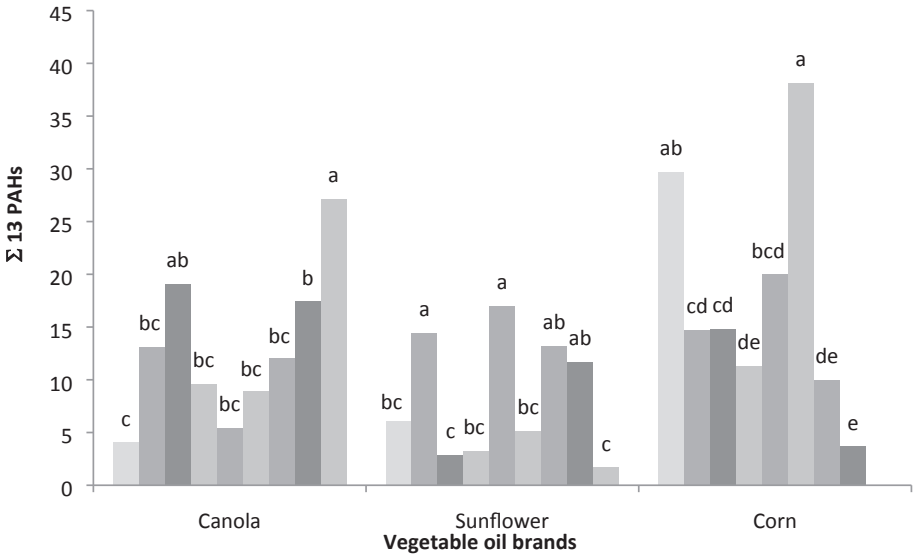

Fig. 2. Mean Σ 13 PAHs in different brands of canola, sunflower and corn oils. Different letters indicate statistic difference ($p < 0.05$) between brands within the same type of oil.

Estimated intakes were in accordance with results obtained from previous studies. The present study showed that vegetable oil is still a product subject to contamination by these potentially carcinogenic compounds, so any action to reduce and control their presence in this type of products should be encouraged. It is strongly recommended that maximum limits for PAHs presence in fats and oils are set by Brazilian regulation in order to improve the safety of these products.

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