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Evaluation of polycyclic aromatic hydrocarbons content in different stages of soybean oils processing

Mônica C. Rojo Camargo *, Paula Ramos Antoniolli, Eduardo Vicente

Food Science and Quality Center, Institute of Food Technology – ITAL, Av. Brasil, 2880, 13070-178 Campinas – SP, Brazil

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ABSTRACT

A study was conducted in order to determine the levels of 13 polycyclic aromatic hydrocarbons (PAHs) in crude soybean oils produced in Brazil and to evaluate the influence of the refining process in their reduction. Analysis of intermediary products (neutralized, bleached and deodorized oils) showed that all compounds were reduced through refining (up to 88%). Neutralization and deodorization steps contributed effectively to the PAHs decrease. The mean total PAHs content in crude and deodorized oil samples ranged, respectively, from 10 to 316 and 3 to 69 μg/kg. Since vegetable oils have been shown to be the major sources of PAHs in the diet, a monitoring program should be developed by the refining industries and the use of activated carbon during oil processing is highly recommended.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) represent an important group of chemical carcinogens formed during incomplete combustion of organic material (World Health Organization (WHO), 2005). These compounds occur as contaminants in different food categories including vegetable oils that, owing to their lipophilic nature, are easily contaminated (Larsson, Erikson, & Cervenka, 1987; Pupin & Toledo, 1996). Two main routes of PAHs contamination have been suggested: environmental pollution and direct drying of the raw material with combustion smoke before oil extraction (Moret & Conte, 2000; Pupin & Toledo, 1996). Nevertheless, the amount of PAHs in crude vegetable oils can be reduced during refining, particularly using activated carbon in the bleaching step (Camargo & Toledo, 1998; Cejpek, Hajslová, Kocourek, Tomaniová, & Cmolík, 1998; Larsson et al., 1987; Teixeira, Casal, & Oliveira, 2007). The deodorization step reduces PAHs with up to four aromatic rings, while bleaching with activated charcoal is effective to reduce the compounds with 5 and 6 rings (Larsson et al., 1987; Moret & Conte, 2000; Teixeira et al., 2007).

The refining consists in a set of operations used to obtain an edible product including degumming, neutralization, bleaching and deodorization. The first step or degumming is carried out to remove phospholipids and mucilaginous gums (Jung, Yoon, & Min, 1989). Neutralization or alkali refining and bleaching are used to eliminate free fatty acids and pigments that can promote fat oxidation and lead to undesirable colours in the final product. The neutralized oil is treated with bleaching agents such as bleaching earth and activated carbon. Finally, the deodorization removes volatile compounds and decomposes peroxides to improve the oil flavour quality and stability (Jung et al., 1989). The resulting product is referred as refined oil and is ready to be consumed or for the manufacture of other products.

Among vegetable oils, soybean has played a leading role in production and in use worldwide for years. Although world supplies of other vegetable oils, especially palm and rapeseed, have been growing in some countries, soybean remains the primary oilseed produced in Brazil. In 2011, the production was 6.85 million tons and a 1.9% year-to-year increase was projected to 2020/2021. The production is mainly to supply the domestic market, once the edible oil is one of the most consumed in the country and its consumption for 2011/2012 is estimated at 5.22 million tons (MAPA. Ministério da Agricultura, 2011). Additionally, in the coming years soybean oil production for biodiesel is expected to rise around 3% and the export forecasts stands at 0.5% per year between the period of 2010/2011 and 2020/2021 (MAPA, 2011).

Moreover, the latest European official food regulation regarding maximum levels of PAHs in oils and fats intended for direct human consumption or use as an ingredient in food established 2.0 μg/kg for benzo[*a*]pyrene and 10.0 μg/kg for the sum of benz[*a*]anthracene, chrysene, benzo[*a*]pyrene and benzo[*b*]fluoranthene (EU, 2011).

A study conducted by Camargo, Antoniolli, Vicente, and Tfouni (2011b) showed relatively high and variable levels of PAHs in soybean oils commercially available in the Brazilian market. Thus, considering the importance of soybean oil in national diet, it is

* Corresponding author. Tel.: +55 19 3743 1778; fax: +55 19 3242 4585.
E-mail address: monicarojo@ital.sp.gov.br (M.C. Rojo Camargo).

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important to identify the main source of crude oils contamination by PAHs, be acquainted with the extension of this contamination and evaluate the possible influence of each step of the refining process on PAHs concentration decrease. Additionally, in Brazil oil refineries do not use charcoal treatment during the oil processing and the regulation for oils and fats does not set maximum levels for any PAH.

The aim of this study was to determine the levels of PAHs in crude, neutralized, bleached and deodorized soybean oils from four different Brazilian regions. The PAHs selected for the study were the 13 compounds identified by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) as clearly carcinogenic and genotoxic, namely benz[*a*]anthracene, chrysene, 5-methylchrysene, benzo[*j*]fluoranthene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, dibenzo[*al*]pyrene, dibenz[*ah*]anthracene, dibenzo[*ae*]pyrene, dibenzo[*ah*]pyrene, dibenzo[*ai*]pyrene, and indeno[*1,2,3-cd*]pyrene (World Health Organization (WHO), 2005).

2. Material and methods

2.1. Oil samples

A total of 112 samples of crude soybean oil and their corresponding neutralized, bleached and deodorized ones were provided by a Brazilian soybean oil producer and refining company. The samples were acquired directly from the producing sites located in four different states: Goiás, Paraná, Minas Gerais and Bahia, corresponding to the Central West, South, Southeast and Northeast regions of the country, respectively (Fig. 1). Sampling was performed in the years of 2007 and 2008, representing two different harvests. Samples were collected sequentially on the production line, during the purification step sequence. Then, the samples were taken to the laboratory, packed in plastic bags and were stored in darkness until the analyses were carried out (within a month).

2.2. Chemicals and materials

PAHs standards were purchased from Supelco Inc. (St. Louis, MO, USA) (benzo[*a*]anthracene (B[*a*]A), chrysene (Chy), benzo[*b*]fluoranthene (B[*b*]F), benzo[*k*]fluoranthene (B[*k*]F), benzo[*a*]pyrene (B[*a*]P), dibenzo[*ah*]anthracene (D[*ah*]A) and indeno[1,2,3-*cd*]pyrene (Indeno)), Fluka (Munich, Germany) (benzo[*j*]fluoranthene (B[*j*]F), dibenzo[*al*]pyrene (D[*al*]P), dibenzo[*ae*]pyrene (D[*ae*]P) and dibenzo[*ah*]pyrene (D[*ah*]P)), Cambridge Isotope Laboratories Inc. (Andover, MA, USA) (5-methylchrysene (5MeChy)) and Chem-Service Inc. (PA, USA) (dibenzo[*ai*]pyrene (D[*ai*]P)). Hexane, methanol and *N,N*-dimethylformamide (HPLC grade) were acquired from Tedia Brazil Ltda (Rio de Janeiro, RJ, Brazil). Acetonitrile (HPLC grade) was supplied by J.T. Baker (Mexico City, Mexico). Water was purified on a Milli-Q system, Millipore Corp. (Bedford, MA, USA). For clean-up procedures, C_{18} AccuBond^{II} (500 mg, 3 ml) cartridges from Agillent Technologies Inc. (Allentown, PA, USA) were used. The polyvinylidene fluoride membranes (PVDF, Millex-HV) were also purchased from Millipore Corp. (Bedford, MA, USA).

2.3. Sample preparation and SPE extraction

Based on the method described by Camargo, Antoniolli, and Vicente (2011a) modified from Grimmer and Bohnke (1975) and Barranco et al. (2003), the soybean oil samples were prepared in duplicate by mixing 0.5 g of oil in 5.0 ml of hexane, which were placed into a 60 ml separating funnel. The PAHs were extracted twice with *N,N*-dimethylformamide–water (DMF–H_2O) (9:1, v/v) (5 ml) and the combined extracts were diluted with 8 ml of water. The resulting solution was cleaned up using the AccuBond^{II} SPE cartridges (500 mg, 3 ml), preconditioned with methanol (5 ml) and water (5 ml). Then, the sample extract was quantitatively transferred to the cartridge that was washed with 10 ml of DMF–H_2O (1:1, v/v) and 10 ml of water. Subsequently, the cartridges were dried for 20 min using vacuum. Finally, the analytes were eluted with 10 ml of hexane at a flow rate of 2 ml min^{-1} and the eluate collected was dried under a flow of nitrogen. The residue was dissolved in 0.5 ml of acetonitrile and analyzed by liquid chromatography with fluorescence detection. The SPE clean-up was performed in a 24-port Visiprep solid phase extraction Vacuum Manifold from Supelco® (USA).

2.4. HPLC analysis and quantification

A Shimadzu LC-20A Prominence HPLC (Kyoto, Japan) coupled to a RF-10AXL fluorescence detector was used for the analysis. The system was also equipped with a LC-10AT pump, an in-line degasser and a SIL-20A auto injector with 30 µl injection volume. The chromatographic separation of the compounds was achieved with a C18 Vydac 201 TP54 column (5 µm, 250 × 4.6 mm) operating at 30 °C. A linear binary gradient composed of acetonitrile (A) and water (B) was used according to the following scheme: t_0 min 70% A, t_{20} min 75% A, t_{35} min 100% A, maintained isocratic conditions (100% A) for 20 min, when the initial conditions were restored and the column was re-equilibrated for 15 min. The flow rate of the eluent was 1 ml min^{-1}. The excitation and emission wavelengths were set at 0.01 min (268/398 nm) for B[*a*]A, Chy, 5MeChy; 16.70 min (312/507 nm) for B[*j*]F; 18.20 min (290/430 nm) for B[*b*]F, B[*k*]F, B[*a*]P, D[*al*]P, D[*ah*]A; 32.40 min (300/500 nm) for

State/Region	Nº of samples
(1) Goiás/Central West	24
(2) Paraná/South	48
(3) Minas Gerais/Southeast	20
(4) Bahia/Northeast	20

Fig. 1. Soybean oil samples collection areas in Brazil.

Indeno; 34.90 min (297/403 nm) for D[*ae*]P and 45 min (304/457 nm) for D[*ai*]P, D[*ah*]P.

The compounds were quantified using external calibration curves for each PAH with seven concentration levels, ranging from 0.5 to 250 ng ml^{-1}. Mixed standard stock solutions with PAHs in different concentrations were prepared in acetonitrile and duplicate injections of 30 μl were used to construct linear regression lines (peak area ratios versus PAH concentration).

2.5. Method validation

A single-laboratory validation was conducted based on the following parameters: recovery, linearity, repeatability, intermediate precision, limits of detection (LOD) and quantification (LOQ), according to the Institute of Metrology, Standardization and Industrial Quality (Inmetro) guidelines, under ISO 17025 criteria (Inmetro – Instituto Nacional de Metrologia, 2010). Linearity was observed through correlation coefficients (r^2) of the analytical curves constructed with seven points of standard solutions (0.5–250 μg/kg depending on the PAH). The recovery experiments were carried out by spiking a blank sample of oil with PAHs at 0.5, 1.2 and 5.0 μg/kg and analyzed in triplicate. Recoveries were calculated from the differences in total amounts of each PAH between the spiked and unspiked samples. Repeatability and intermediate precision were evaluated using the relative standard deviation (% RSD) associated to measurements of each PAH performed during recovery tests at the same day and within three different days by two different analysts, respectively. The LODs were determined considering the analysis of seven independent replicates of blank samples, fortified with the lowest levels of the calibration curves, and calculated as three times the standard deviation of the mean fortified blank sample determinations. The same procedure was applied for the LOQs and the values were assessed as five times the standard deviation of the mean fortified blank sample determinations.

2.6. Statistical analysis

The software Statistica for Windows 5.5 (StatSoft Inc., Tulsa, OK, USA) was used to perform the analysis of variance (ANOVA). The PAHs levels in different steps of the refining process were compared by Tukey test (95% confidence).

3. Results and discussion

3.1. Optimization of the analytical procedure and validation study

The analytical procedure was based on previous one related to PAHs analysis in oils (Camargo et al., 2011a), however some modifications were introduced and the method was re-validated.

The calibration curves obtained for each PAH showed a linear response with correlation coefficients between 0.9967 and 0.9999. The LODs and LOQs ranged from 0.11 to 1.01 μg/kg and from 0.19 to 1.69 μg/kg, respectively, expressing adequate sensitivity of the method for the target compounds. Taking into account each fortified level, the average recovery values ranged from 70% to 120%, considered satisfactory for determinations at μg/kg levels. The repeatability study revealed a precise method for most PAHs in the same day, and likewise in different days with RSDs less than 10%. The validation parameters are summarized in Table 1.

3.2. PAHs in oils

Tables 2 and 3 present the PAHs content determined in each step of the refining oil process, from different Brazilian regions, considering 2007 and 2008 harvests.

Soybean oils from 2007 are much less contaminated than those from 2008. In the first year crude oils contained 10–208 μg/kg of summed PAHs, while in 2008 the levels raised to 26–316 μg/kg. This might be attributed to different soybean seed drying processes, which is the main responsible for oils contamination. In Brazil, the use of direct drying of the plant material with combustion smoke is a common practice that permits the direct contact between the PAHs present in the smoke and the soybean seeds. These compounds remain concentrated in the surface of the beans and during processing for oil production they are transferred to the crude product.

Evaluating the regions individually, the contamination profile presented in crude oils from both seasons varied considerably and many factors may contribute to this variation. The samples provenance is an important parameter to be considered, since Brazil presents a large territory with different regions and different weather conditions, where artificial drying is always necessary. According to the producers, the soybean from South region, where a humid subtropical and cold climate predominates, is used to be dried twice. Differently, the soybean produced in the other regions,

Table 1
Method performance: recoveries, repeatability, intermediate precision, linearity, LOD and LOQ.

PAH[a]	Recovery ± RSD[b] (%)			Intermediate precision (RSD%)[c]	Linearity (μg/kg)	LOD (μg/kg)	LOQ (μg/kg)
	Level 1	Level 2	Level 3				
B[a]A	81 ± 3	94 ± 5	83 ± 4	2–3	0.9994	0.32	0.53
Chy	115 ± 6	92 ± 4	75 ± 3	2–8	0.9994	0.17	0.29
MeChy	105 ± 1	79 ± 2	70 ± 0	1–3	0.9998	0.17	0.29
B[j]F	77 ± 8	85 ± 3	78 ± 3	2–3	0.9999	1.01	1.69
B[b]F	75 ± 4	82 ± 1	74 ± 3	1–5	0.9993	0.21	0.35
B[k]F	81 ± 13	74 ± 2	71 ± 2	1–6	0.9990	0.16	0.27
B[a]P	120 ± 4	115 ± 2	88 ± 7	2–3	0.9998	0.15	0.26
D[al]P	81 ± 8	88 ± 5	70 ± 7	1–6	0.9993	0.11	0.19
D[ah]A	73 ± 4	84 ± 3	75 ± 4	3–9	0.9984	0.15	0.24
Indeno	76 ± 3	77 ± 6	71 ± 3	6–10	0.9997	0.74	1.23
D[ae]P	77 ± 3	75 ± 2	75 ± 2	1–7	0.9999	0.12	0.20
D[ai]P	74 ± 4	76 ± 5	76 ± 7	1–4	0.9967	0.15	0.25
D[ah]P	85 ± 13	94 ± 14	88 ± 15	1–4	0.9993	0.45	0.75

[a] B[*a*]A: benz[*a*]anthracene; Chy: chrysene; 5MeCri: 5-methylchrysene; B[*j*]F: benzo[*j*]fluoranthene; B[*b*]F: benzo[*b*]fluoranthene; B[*k*]F: benzo[*k*]fluoranthene; B[*a*]P: benzo[*a*]pyrene; D[*al*]P: dibenzo[*al*]pyrene; D[*ah*]A: dibenz[*ah*]anthracene; Indeno: indeno[1,2,3-*cd*]pyrene; D[*ae*]P: dibenzo[*ae*]pyrene; D[*ai*]P: dibenzo[*ai*]pyrene; D[*ah*]P: dibenzo[*ah*]pyrene.

[b] Level 1: 0.5 μg/kg; Level 2: 1.2 μg/kg; Level 3: 5.0 μg/kg; Recovery (mean ± RSD, $n = 3$).

[c] $n = 3$ Determinations in three different days (RSD range).

Table 2
PAHs levels in soybean oils during refining process in different regions (soybean harvest time: 2007).

PAH[2]	PAHs levels (mean ± SD) (μg/kg)[1]															
	Central West Oils				South Oils				Northeast Oils				Southeast Oils			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
B[a]A	38 ± 1	13.4 ± 0.05	9 ± 1	9.1 ± 0.1	8.4 ± 0.5	3.1 ± 0.1	1.9 ± 0.3	0.98 ± 0.08	2.12 ± 0,04	1.6 ± 0.5	1.4 ± 0.4	1.54 ± 0.03	1.28 ± 0.02	0.75 ± 0.07	0.39 ± 0.04	0.35 ± 0.01
Chy	58 ± 2	22 ± 1	19.1 ± 0.3	13.7 ± 0.7	15.5 ± 0.9	7 ± 1	2.4 ± 0.2	1.99 ± 0.04	2.7 ± 0.1	1.2 ± 0.4	1.2 ± 0.4	1.67 ± 0.02	3.1 ± 0.4	2.4 ± 0.5	0.86 ± 0.07	0.46 ± 0.05
MeChy	3.0 ± 0.3	1.9 ± 0.1	1.4 ± 0.3	1.32 ± 0.07	2 ± 1	1.3 ± 0.3	1.0 ± 0.1	1.3 ± 0.01	1.10 ± 0.09	0.8 ± 0.2	0.6 ± 0.2	1.74 ± 0.04	0.8 ± 0.1	0.40 ± 0.06	0.27 ± 0.05	0.35 ± 0.02
B[j]F	16.5 ± 0.8	5.65 ± 0.02	5.1 ± 0.7	4.5 ± 0.5	<LQ	n.d.	n.d.	n.d.	n.d.	<LQ	<LQ	<LQ	n.d.	n.d.	n.d.	n.d.
B[b]F	22.8 ± 0.7	9.2 ± 0.3	7.3 ± 0.3	6.5 ± 0.1	1.6 ± 0.2	0.5 ± 0.1	0.35 ± 0.03	0.37 ± 0.04	1.0 ± 0.2	1.7 ± 0.5	1.6 ± 0.5	1.76 ± 0.06	0.36 ± 0.04	<LQ	0.61 ± 0.05	0.57 ± 0.02
B[k]F	9.5 ± 0.5	3.4 ± 0.3	2.28 ± 0.04	5.1 ± 0.1	0.8 ± 0.1	0.6 ± 0.1	<LQ	0.25 ± 0.02	1.60 ± 0.03	1.0 ± 0.3	0.8 ± 0.3	0.80 ± 0.03	0.35 ± 0.09	0.26 ± 0.06	<LQ	<LQ
B[a]P	21.8 ± 0.3	8.5 ± 0.2	4.9 ± 0.1	6.5 ± 0.2	16 ± 1	<LQ	<LQ	<LQ	2.4 ± 0.2	2.5 ± 0.8	1.7 ± 0.8	1.9 ± 0.1	0.25 ± 0.03	<LQ	<LQ	<LQ
D[al]P	0.87 ± 0.01	0.29 ± 0.07	0.29 ± 0.03	0.29 ± 0.02	0.22 ± 0.01	0.21 ± 0.03	n.d.	<LQ	0.7 ± 0.03	0.3 ± 0.1	0.2 ± 0.1	0.37 ± 0.03	n.d.	n.d.	n.d.	n.d.
D[ah]A	30 ± 1	13.8 ± 0.3	8.2 ± 0.6	11.6 ± 0.6	1.9 ± 0.1	1.3 ± 0.3	0.9 ± 0.2	0.88 ± 0.08	3.0 ± 0.1	5 ± 1	4 ± 1	3.47 ± 0.06	0.91 ± 0.03	0.9 ± 0.1	0.86 ± 0.07	0.84 ± 0.07
Indeno	2.0 ± 0.1	<LQ	2.4 ± 0.1	2.15 ± 0.06	2.0 ± 0.3	<LQ	<LQ	<LQ	2.0 ± 0.2	2.9 ± 0.8	2.4 ± 0.8	2.84 ± 0.08	2.0 ± 0.1	n.d.	n.d.	n.d.
D[ae]P	1.85 ± 0.05	1.3 ± 0.2	0.53 ± 0.02	0.96 ± 0.06	0.9 ± 0.1	0.69 ± 0.02	0.7 ± 0.1	0.30 ± 0.02	0.83 ± 0.08	0.5 ± 0.2	0.4 ± 0.2	0.45 ± 0.03	0.41 ± 0.04	0.43 ± 0.03	0.29 ± 0.01	0.40 ± 0.02
D[ai]P	2.3 ± 0.3	0.57 ± 0.02	1.14 ± 0.03	1.02 ± 0.1	<LQ	<LQ	0.30 ± 0.05	0.21 ± 0.02	<LQ	0.25 ± 0.07	0.29 ± 0.08	0.23 ± 0.02	0.27 ± 0.07	0.31 ± 0.02	<LQ	<LQ
D[ah]P	1.53 ± 0.07	<LQ	n.d.	<LQ	<LQ	n.d.	n.d.	n.d.	<LQ	n.d.	n.d.	<LQ	n.d.	n.d.	n.d.	n.d.
ΣHPAs	208	80	61	63	49	15	8	6	17	18	16	17	10	5	3	3

[1] SD: standard deviation; oil 1: crude; oil 2: neutralized; oil 3: bleached; oil 4: deodorized (mean of $n = 8$ determinations); n.d.: not detected; <LOQ: lower than quantification limit (Table 1).
[2] B[*a*]A: benz[*a*]anthracene; Chy: chrysene; 5MeCri: 5-methylchrysene; B[*j*]F: benzo[*j*]fluoranthene; B[*b*]F: benzo[*b*]fluoranthene; B[*k*]F: benzo[*k*]fluoranthene; B[*a*]P: benzo[*a*]pyrene; D[*al*]P: dibenzo[*al*]pyrene; D[*ah*]A: dibenz[*ah*]anthracene; Indeno: indeno[1,2,3-*cd*]pyrene; D[*ae*]P: dibenzo[*ae*]pyrene; D[*ai*]P: dibenzo[*ai*]pyrene; D[*ah*]P: dibenzo[*ah*]pyrene.

Table 3
PAHs levels in soybean oils during refining process in different regions (soybean harvest time: 2008/2009).

PAH[2]	PAHs levels (mean ± SD) (μg/kg)[1]															
	Central West Oils				South Oils				Northeast Oils				Southeast Oils			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
B[a]A	35 ± 4	9.6 ± 0.9	9.8 ± 0.5	5.6 ± 0.4	42 ± 2	7.5 ± 0.8	9.4 ± 0.6	5.7 ± 0.1	21.1 ± 0.3	11.6 ± 0.3	10 ± 1	7.2 ± 0.1	3.6 ± 0.2	1.43 ± 0.01	1.69 ± 0.03	1.30 ± 0.02
Chy	58 ± 2	20.9 ± 0.8	14.9 ± 0.8	9.7 ± 0.4	23.3 ± 0.6	6.5 ± 0.2	5.2 ± 0.2	3.6 ± 0.4	30.9 ± 0.5	6.6 ± 0.5	5.3 ± 0.1	3.68 ± 0.09	3.35 ± 0.02	1.70 ± 0.04	1.56 ± 0.05	1.26 ± 0.03
MeChy	15.1 ± 0.3	12 ± 2	12.1 ± 0.2	5.6 ± 0.4	21 ± 2	7.8 ± 0.8	6.9 ± 0.7	7.2 ± 0.4	9.34 ± 0.03	4.0 ± 0.2	4.4 ± 0.4	3.6 ± 0.3	4.2 ± 0.2	1.45 ± 0.06	1.1 ± 0.1	0.89 ± 0.05
B[j]F	7 ± 0.5	2.0 ± 0.5	1.8 ± 0.2	1.2 ± 0.2	4 ± 1	<LQ	n.d.	n.d.	7.0 ± 0.2	<LQ	<LQ	<LQ	<LQ	n.d.	n.d.	n.d.
B[b]F	33 ± 1.5	20.2 ± 0.7	14.8 ± 0.2	15.5 ± 0.4	17 ± 3	6.4 ± 0.6	3.9 ± 0.4	4.1 ± 0.1	20.79 ± 0.07	10.1 ± 0.3	8.1 ± 0.2	6.7 ± 0.3	4.7 ± 0.2	2.45 ± 0.07	0.61 ± 0.01	0.42 ± 0.01
B[k]F	11.35 ± 0.04	5.6 ± 0.4	2.5 ± 0.3	4.4 ± 0.3	3.4 ± 0.5	0.9 ± 0.3	0.37 ± 0.06	0.25 ± 0.06	11.4 ± 0.1	8.5 ± 0.2	7.7 ± 0.2	1.5 ± 0.1	0.50 ± 0.07	0.46 ± 0.02	<LQ	0.40 ± 0.01
B[a]P	58 ± 2	11 ± 1	8.2 ± 0.1	6.0 ± 0.6	29 ± 1	12 ± 1	6.4 ± 0.04	4.0 ± 0.6	30 ± 2	6.33 ± 0.02	7.0 ± 0.2	4.7 ± 0.2	0.32 ± 0.01	0.31 ± 0.01	<LQ	<LQ
D[al]P	9.1 ± 0.4	6.65 ± 0.06	3.9 ± 0.3	4.0 ± 0.1	11 ± 2	1.00 ± 0.06	0.41 ± 0.02	<LQ	2.48 ± 0.05	2.18 ± 0.05	1.87 ± 0.04	1.4 ± 0.3	0.72 ± 0.07	n.d.	n.d.	n.d.
D[ah]A	53.6 ± 0.2	16.8 ± 0.2	9.5 ± 0.5	3.7 ± 0.2	21 ± 3	11 ± 1	9.5 ± 0.5	4.4 ± 0.5	12 ± 1	8.31 ± 0.05	7.5 ± 0.2	1.4 ± 0.3	1.1 ± 0.2	0.92 ± 0.07	0.86 ± 0.02	0.28 ± 0.03
Indeno	15.2 ± 0.6	7.0 ± 0.3	6.3 ± 0.5	6.1 ± 0.2	2.8 ± 0.4	2.3 ± 0.4	2.3 ± 0.3	<LQ	15.2 ± 0.3	3.5 ± 0.3	4.1 ± 0.2	3.0 ± 0.3	<LQ	n.d.	n.d.	n.d.
D[ae]P	9.1 ± 0.8	3.34 ± 0.01	2.3 ± 0.2	1.50 ± 0.06	5.5 ± 0.8	4.6 ± 0.9	2.3 ± 0.1	0.6 ± 0.1	9.6 ± 0.3	5.6 ± 0.1	4.66 ± 0.05	3.8 ± 0.3	4.3 ± 0.1	1.56 ± 0.04	1.87 ± 0.02	1.93 ± 0.04
D[ai]P	7.5 ± 0.1	2.8 ± 0.8	2.1 ± 0.2	2.7 ± 0.3	2.2 ± 0.4	1.4 ± 0.3	1.24 ± 0.1	1.3 ± 0.2	7.8 ± 0.7	3.9 ± 0.4	1.7 ± 0.2	2.0 ± 0.1	1.5 ± 0.3	0.99 ± 0.06	0.52 ± 0.02	0.26 ± 0.04
D[ah]P	3.8 ± 0.6	3.29 ± 0.09	3.2 ± 0.2	3.2 ± 0.4	3.2 ± 0.6	2.4 ± 0.3	2.43 ± 0.2	1.4 ± 0.1	3.8 ± 0.1	1.6 ± 0.1	1.58 ± 0.08	1.21 ± 0.07	1.73 ± 0.09	0.87 ± 0.01	n.d.	0.35 ± 0.04
ΣHPAs	316	121	91	69	185	64	50	33	181	72	64	40	26	12	8	7

[1] SD: standard deviation; oil 1: crude; oil 2: neutralized; oil 3: bleached; oil 4: deodorized (mean of $n = 8$ determinations); n.d.: not detected; <LOQ: lower than quantification limit (Table 1).
[2] B[*a*]A: benz[*a*]anthracene; Chy: chrysene; 5MeCri: 5-methylchrysene; B[*j*]F: benzo[*j*]fluoranthene; B[*b*]F: benzo[*b*]fluoranthene; B[*k*]F: benzo[*k*]fluoranthene; B[*a*]P: benzo[*a*]pyrene; D[*al*]P: dibenzo[*al*]pyrene; D[*ah*]A: dibenz[*ah*]anthracene; Indeno: indeno[1,2,3-*cd*]pyrene; D[*ae*]P: dibenzo[*ae*]pyrene; D[*ai*]P: dibenzo[*ai*]pyrene; D[*ah*]P: dibenzo[*ah*]pyrene.

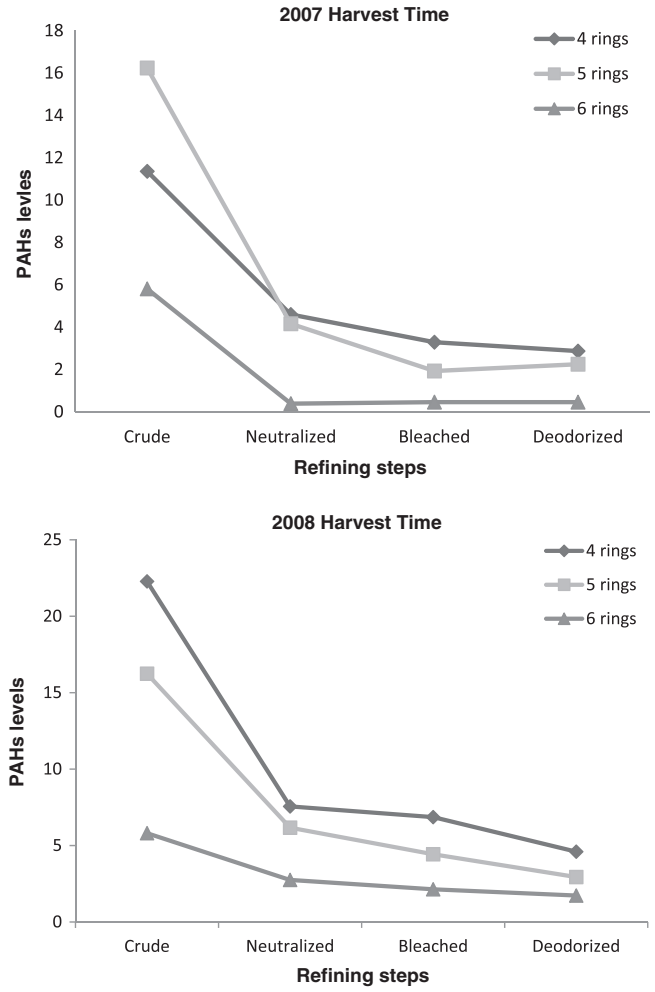

Fig. 2. PAHs levels through oil refining according to the molecular weight (mean of four regions).

due to the higher temperatures, requires a moderate drying process. However, the obtained results are not exactly in accordance with this information. Crude oils from Central West region, for example, presented in both years higher PAHs levels (208 μg/kg/2007 and 316 μg/kg/2008) in comparison with those collected in South region (50 μg/kg/2007 and 186 μg/kg/2008). In the year of 2008, the Northeast also provided crude oils with relatively higher contamination levels. This can be explained, in part, by the fact that in the latest years pluviometric indexes in these regions were higher than expected and more drying steps were required.

Analysis of other intermediary products (neutralized, bleached and deodorized oils) showed that, with exception to the Northeast region in 2007, all compounds showed reduction in their levels. Since in Brazil soybean oils are not treated with activated charcoal, only tonsil activated earth is used during the bleaching step, the decrease observed is due exclusively to the refining process. In other words, neutralization and deodorization steps contributed effectively to the PAHs decrease (Tukey, $p < 0.05$). Taking into account the crude oils from Central West region that in the study presented the highest PAHs concentrations, the levels of the corresponding deodorized oils were 63 μg/kg in 2007 and 69 μg/kg in 2008, representing 88% and 83% dropping off, respectively (Tables 2 and 3).

In order to evaluate the influence of the molecular weight of the compounds in the contamination reduction during refining, PAHs were separated in three groups according to the number of aromatic rings: four (group 1: B[*a*]A, Chy, 5MeChy) five (group 2: B[*j*]F, B[*b*]F, B[*k*]F, B[*a*]P, D[*ah*]A) and six (group 3: D[*al*]P, D[*ae*]P, D[*ah*]P, D[*ai*]P, Indeno). As shown in Fig. 2, it is possible to observe a decrease of PAHs levels from all the three groups, in higher or lower percentage, independent of the region evaluated; although, there is no pattern for this diminution. The neutralization contributed to a sharply reduction among group 2 in 2007 and group 1 in 2008, corresponding to 64% and 66%, respectively. The refining was responsible for a maximum reduction of 77% (group 1), 82% (group 2) and 72% (group 3) PAHs content from crude soybean oil produced in 2008.

The results are not aligned to those obtained by other authors. Teixeira et al. (2007) determined a decrease of 87% in total PAHs content. When 5–6 rings compounds are the focus of comparison, a lower reduction (49%) was observed for this PAHs fraction. In this study, the authors stated that activated charcoal was used during the bleaching step, which is considered very efficient in removing PAHs. As mentioned by Teixeira et al. (2007), two situations may contribute to PAHs decrease during oil refining: the very high initial contamination level and the application of activated charcoal in the bleaching process. In this manner, the relatively high initial contamination of Brazilian samples can be the main contributor to the huge PAHs decrease observed in the present study. In addition, for some compounds the levels slightly raised after bleaching (Tables 2 and 3), which was also described earlier by Cejpek et al. (1998) and Teixeira et al. (2007).

Once the crude oils are contaminated, the refined edible ones will also be contaminated. The summed PAHs found for soybean oil in this study was very similar to those determined in commercial samples (10.4–112.0 μg/kg) by Camargo et al. (2011b). PAHs profile in both studies is practically the same.

In Brazil, there is no legislation regarding levels of PAHs in edible oils. There are only maximum benzo[*a*]pyrene levels established for smoke flavourings (0.03 μg/kg), olive pomace oil (2.0 μg/kg) and drinkable water (0.7 μg/L) (Brasil., 2003; Brasil., 2004; Brasil., 2007). When using the maximum limit established by the European Union for B[*a*]P or the sum for B[*a*]A, Chy, B[*b*]F and B[*a*]P it is possible to observe two situations: in 2007 only one region provided deodorized oils with values higher than 2.0 or 10 μg/kg, but in 2008 three regions reached this mark, with concentrations varying between twice and three times these limits.

4. Conclusion

Throughout the monitoring performed it was possible to observe that due to the different variables involved in oil production and the difficult of controlling the drying by the industry, it is hardly possible to predict the PAHs levels present. The content of PAHs in the crude soybean oils plays an important role in the contamination of the corresponding refined oils. It was noted that although the refining process reduces the amount of PAH originally present in the crude oil, this effect can be marginal, enhancing the necessity of a better control of the crude oil contamination. Since vegetable oils have been shown to be the major source of PAHs in the diet, a monitoring program should be developed by the oil refining industries and the use of activated carbon during processing is highly recommended.

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