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Polycyclic aromatic hydrocarbons content and fatty acids profile in coconut, safflower, evening primrose and linseed oils

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ABSTRACT

This study aimed at evaluating the polycyclic aromatic hydrocarbons (PAHs) contamination of commercial vegetable oils and examined the identity through the fatty acids profiles. Coconut, safflower, evening primrose, and linseed oils marketed in São Paulo (Brazil) were investigated totaling 69 samples. Four PAHs, benzo[*a*]anthracene (BaA), chrysene (Chr), benzo[b]fluoranthene (BbF), and benzo[a]pyrene (BaP), were detected in 96% of the samples at individual levels ranging from not detected to 14.99 μg kg^{-1}. Chrysene was the abundant hydrocarbon found among all types of oils, with the highest median values. The results of the fatty acid profiles revealed that 43% showed different profiles according to the ones on their labels, with a higher incidence of adulteration of evening primrose oils. The maximum tolerable limits by European Regulation No. 835/2011 were exceeded for BaP in 12%, and for total 4 PAHs in 28%, with a greater contribution of adulterated samples.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a group of over 200 different organic compounds with two or more fused aromatic rings (Domingo & Nadal, 2015). Humans are exposed to PAHs from dietary and non-dietary sources, but the first form is considered the most relevant (Bansal & Kim, 2015).

The International Agency for Research on Cancer (IARC) of World Health Organization has determined that benzo[a]anthracene (BaA), chrysene (Chr), benzo[b]fluoranthene (BbF) are in the Group 2B, possibly carcinogenic to humans and benzo[a]pyrene (BaP) was included in the Group 1A, carcinogenic to animals and humans (IARC, 2010, 2012). The PAHs biotransformation on the body includes a series of reactions performed by enzymes distributed across all tissues, with the purpose that the metabolite is transformed into a compound more hydrophilic than its precursor, and thereby facilitate the excretion per biological fluids. However, some PAHs are transformed into substances with high power of covalently binding to DNA, forming adducts, which can cause genetic mutation, with activity to generate tumors, as well as the risks of bad formations for embryos (Purcaro, Moret, & Conte, 2013; Liu et al., 2016). Because of its carcinogenic action, BaP is the most common hydrocarbon studied (Su et al., 2014).

Food can be contaminated by PAHs because of the contamination of air, water or soil, and during industrial process, as heating, drying and smoking process. PAHs were found in different foodstuffs, including vegetables, fruit, cereals, oils and fats, smoked fish and meat, coffee and tea (Camargo, Antoniolli, & Vicente, 2011; Bansal & Kim, 2015).

For vegetable oils, the contamination with PAHs could be generated by environmental pollution of the vegetable raw material, the contamination from seed drying (specially with combustion gases before oil extraction), the extraction with solvent, burning of soil, the material of packing, residues of mineral oils, and migration from contaminated water or soils (Pandey, Mishra, Khanna, & Das, 2004; Camargo et al., 2011; Ciecierska & Obiedziński, 2013; Bansal & Kim, 2015). The presence of PAHs in vegetable oils have investigated in some studies including linseed, mustard, olive, palm, soybean, sesame, safflower, borage, evening primrose, sunflower oils and others samples (Pandey et al., 2004; Camargo et al., 2011; Roszko, Szterk, Szymczyk, & Waszkiewicz-Robak, 2012; Ciecierska & Obiedzinski, 2013). The oils and fats are one of the classes of foods with the highest levels of PAHs (Domingo & Nadal, 2015).

Considering the risk characterization of PAHs, the EFSA recommended the analysis of 4 PAHs as possible indicators for carcinogenic potency in food: BaA, Chr, BbF, and BaP (EFSA, 2008). The levels of fat and oils have

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been regulated by European Commission (EC) Regulation No 835/2011 and the maximum permitted are 2.0 μg kg^{-1} and 10.0 μg kg^{-1} for BaP and total 4 PAHs, respectively. For coconut oils, the sum is 20.0 μg kg^{-1}, higher than other oils, because the proportionally higher presence of BaA and Chr. The separate maximum level for BaP was maintained in Regulation EC to ensure comparability of previous and future data (Commission of the European Communities, 2011a). In Brazil, the only regulation for oils is an old one that establishes the BaP maximum level of 2.0 μg kg^{-1} in pomace olive oil (Brazil., 2003).

Studies suggest that cold-pressed vegetable oils, like safflower, coconut, evening primrose and linseed oils are considered functional, since many of them have biologically active lipids, such as polyunsaturated fatty acids omega-3 (n-3), and omega-6 (n-6). Moreover, when obtained by cold pressing, they exceed nutritional advantages over refined oils, with higher compounds such as tocopherols, phenolic, and sterols, which provide health benefits, and diseases prevention (Teh & Birch, 2013).

However, these oils may be showed adulteration. Studies about adulterated vegetable oils are common and the main causes are related to high demand and/or added value and/or a possibility of potential gain. For example, cold-pressed vegetable oils have a lower efficiency process than oils extracted with solvent; therefore, have higher prices and thus may be auspicious to adulteration. In addition, oils may be added or substituted by other oils of lower value (Rohman & Man, 2012; Hirashima, Silva, Caruso, & Aued-Pimentel, 2013; Stankova, Kremmyda, Tvrzicka, & Žák, 2013; Aued-Pimentel, Castro, de Sousa, Mello, & Abe-Matsumoto, 2015; Azadmard-Damirchi & Torbati, 2015).

Among the cold-pressed vegetable oils, the four types are the most available in the Brazilian market, sold in pharmacies and natural food stores, and presented in glass bottles, plastic bottles or encapsulated. These oils are regulated by the National Health Surveillance Agency (ANVISA), by Health Ministry in Brazil, and registered in the "new foods" category according to RDC Resolution 16/1999 (Brazil., 1999). Quality and identity parameters are established according to Resolution by ANVISA (Brazil., 2005), which uses Codex Stan 210 as reference (Codex Alimentarius., 2015); however, it does not present parameters for evening primrose and linseed oils.

To our knowledge, there are no published works that relate the identity of the oils and the contamination by 4 PAHs. Furthermore, there is a lack of research about the identity and occurrence of PAHs in vegetable oils, especially those from unconventional sources.

Therefore, this study had two objectives: (1) investigate the level of 4 PAHs including benzo[a]anthracene, chrysene, benzo[b]fluoranthene and benzo[a]pyrene of cold-pressed vegetable oils marketed in São Paulo city, Brazil; (2) evaluate the identity in these oils through the fatty acid profile.

2. Material and methods

2.1. Material

2.1.1. Sampling

Sixty-nine cold-pressed vegetable oils were randomly purchased in natural food stores and pharmacies of São Paulo city (Brazil) between 2014 and 2016, including 16 of coconut oils (*Cocos nucifera* L.), 19 of safflower oils (*Carthamus tinctorius* L.), 17 of evening primrose oils (*Oenothera biennis* L.), and 17 of linseed oils (*Linum usitatissimum* L.), with different brands and batches. The samples were presented in gelatinous capsules or in amber glass bottles and were chosen because they are the encapsulated oils most consumed by local people, especially by consumers that search for health promotion. All products declared in their labels the national manufacturing (encapsulation or packaging), mostly from São Paulo State, Brazil, and did not indicate any information about the origin of the oils. The oils were kept in their original flasks protected from light and were stored at 4 °C in the dark until the analysis.

2.1.2. Chemicals, solutions and materials

PAHs standards were obtained from Supelco (Bellefonte, PA, USA) and included BaA (code 40,070), Chr (code 40,074), BbF (code 40,072), and BaP (code 40,071). Individual stock solutions of PAHs were prepared in acetonitrile with a concentration of 40 μg mL^{-1} (for BbF, Chr, and BaP) and 36 μg mL^{-1} (for BaA), and stored at −18 °C in an amber flask. Mixed working solution (with 40 ng mL^{-1} for BaA and BaP, 60 ng mL^{-1} for Chr, and 160 ng mL^{-1} for BbF) were prepared monthly in acetonitrile.

The fatty acids methyl esters (FAME) standards were: a mixture of 37 FAME from 4 to 24 carbon atoms with certificated quantities of each compound (CRM47885, Supelco, Bellefonte, PA, USA); mixture of cis/trans isomers of FAME linoleic (code CRM47791) and FAME linolenic (code CRM47792) of Supelco (Bellefonte, PA, USA). Fame individual solutions of FAME were prepared in *n*-hexane with a concentration of 1.0 mg mL^{-1}, and stored at −18 °C in an amber flask. In the verification of the analytical methodology, a FAME mixture indicated for identification and quantification of oils was used (FAME Mix RM-1, 100 mg, AOCS Reference Mixtures, code O7006, Supelco).

Solvents were HPLC grade: methanol, acetonitrile, N,*N*-dimethylformamide (Tedia, Fairfield, OH, USA), ethanol, and acetone (Carlo Erba, Rodano, Italy). The *n*-hexane used was of nanograde® quality, with a certificate of residue analysis (Mallinckrodt, Paris, KY, USA). All other reagents were purchased from Synth (LabSynth, Sao Paulo, SP, Brazil) and were analytical grade: sulfuric acid, ammonium chloride, sodium chloride, and sodium hydroxide. Deionized water was obtained with the Milli-Q purification system (Millipore, Bedford, MA, USA).

Solid-phase extraction (SPE) cartridge Bond Elut C18 (500 mg, 3 mL) were used from Agilent Technology (Palo Alto, CA, USA) and the polytetrafluoroethylene (PTFE) filters (0.20 μm, 15 mm) were purchased from Macherey-Nagel (Duren, Germany).

The glassware's of PAHs were washed with detergent and water, dried and rinsed with ethanol, acetone, and *n*-hexane before use.

2.2. Analysis of fatty acid methyl esters (FAME)

Preparation of FAME was done by the method described by Hartman and Lago (Hartman & Lago, 1973; Instituto Adolfo Lutz, 2005) with some modifications: sample (30 mg) was diluted in 1.0 mL of *n*-hexane and saponified under boiling with 1.3 mL of 0.5 M sodium hydroxide (in methanol); after cooling, 1.7 mL of the esterification reagent (ammonium chloride solution in methanol and sulphuric acid) was added and heated to boiling; to the cold solution, 2.0 mL of saturated NaCl solution were added and the top layer was taken for FAME analysis. FAME were separated and quantified using a Shimadzu gas chromatograph model GC-2010 (Kyoto, Japan) equipped with a split capillary injector, flame ionization detector and a fused silica capillary column (15 m × 0.1 mm, 0.1 μm, DB-FFAP, J & W Scientific, Agilent, Palo Alto, CA, USA). GC oven temperature program was as follows: 100 °C for 1 min, increase to 155 °C (45 °C min^{-1}), 155 °C for 21 min, increase to 240 °C (15 °C min^{-1}), 240 °C for 1 min, increase to 243 °C (1.5 °C min^{-1}) and 243 °C for 1 min. The others chromatographic conditions were: hydrogen as carrier gas, flow 0.27 mL min^{-1}, split 1:350, injection volume 1 μL, injector temperature 250 °C, and detector temperature 260 °C. Data were processed by GCSolution Software (Shimadzu, Kyoto, Japan). Fatty acids were quantified in triplicate using normalization (Instituto Adolfo Lutz, 2005). To identify the FAME, retention times and elution order were compared with individual standards, when available, and with analyses carried out in the literature with the same column (Masood, Stark, & Salem, 2005), as well as the analysis of authentic oils: coconut, safflower, evening primrose, linseed, soybean, olive and fish, which have characteristic FAME profiles.

2.3. Analysis of 4 PAHs

Sample extraction and clean-up procedures were performed based on the method described by Silva, Sampaio, and Torres (2017). The oil (500 mg) was dissolved with *n*-hexane and PAHs were extracted twice with 5 mL of N,*N*-dimethylformamide (DMF): water (9:1, v/v) in a separatory funnel. The combined extracts were diluted with water until they reached a 1:2 (v/v) proportion of DMF:water and carried out through SPE. Clean-up was performed on a Gilson GX-274 ASPEC system (Gilson Inc., Middleton, WI, USA) using SPE cartridge with the C18 sorbent. After activation and conditioning, the sample solution was loaded, followed by washing. The cartridge was dried and PAHs were eluted with *n*-hexane and, after complete evaporation, the residue was dissolved in 500 μL of acetonitrile, filtered through PTFE membrane and collected in vials. Samples were analyzed in duplicate, and extracts were injected twice in the ultra-high performance liquid chromatography (UHPLC) with fluorescence detector. On each day of analysis, a blank was included to verify contamination of the solvents used consisted of all reagents excluding the sample and the analyte.

The UHPLC analysis was performed using a Shimadzu Nexera® System (Kyoto, Japan) equipped with a LC pump (LC-30AD), an on-line degasser (DGU-20A), a column oven (CTO-20), an automatic injector (SIL-30AC) and a fluorescence detector (RF-20A). Data were acquired and processed by LabSolution Software (Shimadzu, Kyoto, Japan). The column used was Zorbax Rapid Resolution High Definition Eclipse PAH (100 × 2.1 mm, 1.8 μm, Agilent, Palo Alto, CA, USA), protected by a guard column (5 × 2.1 mm, 1.8 μm, Eclipse Plus, Agilent, Palo Alto, CA, USA) maintained at 30 °C. A mobile phase that constituted of A (acetonitrile) and B (water) with flow rate 0.4 mL min^{-1} was used with gradient method: 50% A for 0–0.9 min, 50% to 75% A for 0.9–7.0 min, 75% A for 7.0–17.0 min, 75% to 100% A for 17.0–20.0 min, and 100% A for 20.0–24.0 min, returning to the initial conditions. The excitation and emission wavelengths were 270/390 nm (for BaA and Chr) and 290/430 nm (for BbF and BaP).

2.4. Method validation

The Guidelines of the Brazilian Institute of Metrology, Quality and Technology (INMETRO, 2016) and study of Camargo et al. (2011) were used to evaluate the parameters: linearity, accuracy (recovery), precision (repeatability and intermediate precision), limits of detection (LOD) and quantification (LOQ). Linearity was tested through square correlation coefficients (r2). Accuracy was evaluated using spiked oil samples at four levels of concentration (ranging from 0.25 to 20.0 μg kg^{-1}) and recoveries were calculated. For precision, the sample was spiked with two levels (ranging from 0.25 to 8.0 μg kg^{-1}) on two different days, by a single analyst using the same equipment, and the relative standard deviation (RSD) was determined. The precision for BaP was also evaluated by participation to inter-laboratory comparison test: a sample of olive oil sent by International Olive Council (IOC) in 2016 (Code M4, COI CHEM/2016, Proficiency Testing, Madrid, Spain) was evaluated. The LOD and LOQ were calculated for each PAH as three times and ten times the standard deviation (SD) obtained from the concentrations of six replicates at the lowest fortification level (ranging from 0.25 to 1.0 μg kg^{-1}).

To verify the accuracy of FAME analysis, a mixture was evaluated (RM-1, AOCS Reference Mixtures) with similar composition to the fatty acids found in the following oils: corn, cotton, poppy, rice, safflower, sesame, soybean, sunflower, and nuts. Considering that it is indicated for the identification and the quantification of oils, and has a certificate with the FAME% w/w that constitutes it, solutions were prepared in quadruplicate (4 ng mL^{-1}). Using the same solution, the LOD and LOQ were estimated considering the signal-to-noise ratio of 3:1 and 10:1, respectively.

Table 1
Parameters of validation of 4 PAHs in vegetable oils.

PAHs	Linearity range (ng mL^{-1})	r2	LOD (μg kg^{-1})	LOQ (μg kg^{-1})	Recovery[a] (%)	RSD[b] (%)
BaA	0.25–5.00	0.9992	0.08	0.25	90.46	4.37
Chr	0.30–7.50	0.9987	0.09	0.30	92.53	3.42
BbF	1.00–20.00	0.9999	0.30	1.00	96.78	1.90
BaP	0.25–5.00	0.9995	0.08	0.25	91.32	4.55

[a] Mean recoveries of four different spiking levels in triplicate in the same day.
[b] Mean relative standard deviations (RSD) of two different spiking levels in triplicates in two different days.

2.5. Statistical analysis

All the results were presented as mean and standard deviation. When appropriate, Action 2.5 software was used for Student's and ANOVA test.

3. Results and discussion

3.1. Validation

The results of the validation of PAHs are summarized in Table 1. Linearity was determined using external standard plot method. The high r2 values indicated good linearity over the concentration range. Recovery experiments were considered satisfactory according to performance criteria of Commission Regulation No. 836/2011 (Commission of the European Communities, 2011b). The precision was adequate, with RSD < 10%, and the mean value found to BaP in inter-laboratory comparison test (IOC-2016) was 1.52 μg kg^{-1}, within the acceptable range for this component (1.38 ± 0.30 μg kg^{-1}). The greater values for LOD and LOQ were found for BbF, 0.30 and 1.00 μg kg^{-1}, respectively. More information about 4 PAHs validation was presented in the study of Silva et al. (2017).

For fatty acids, the accuracy of RM-1 (AOCS) was performed for the following FAME: palmitic (16:0), stearic (18:0), oleic (18:1, 9c), linoleic (18:2, 9c, 12c), α-linolenic (18:3, 9c, 12c, 15c or 18:3, n-3), and arachidic (20:0). Mean recovery values ranged from 99.6 to 100.6% and were considered adequate. The calculated values for the LOD and LOQ were 0.03% and 0.10%, respectively.

3.2. Fatty acids profile in the commercial samples

The fatty acids compositions of vegetable oils analyzed are presented in Table 2. The results for coconut oils showed that 94% (15/16) of the samples presented the profiles according to legislation by Codex, characterized by the high content of lauric (12:0) and myristic acids (14:0) (Codex Alimentarius, 2015). One sample presented a higher percentage of α-linolenic (0.7%), higher than allowed (0.2%), indicating adulteration (Table 2).

For the safflower oils, the results indicated that 47% (9/19) of the samples were authentic, with FAME contents allowed by Codex Alimentarius (2015) and high linoleic values, a characteristic of this oil. In some samples, α-linolenic levels were higher (maximum of 0.2%) than the legislation (< 0.1%), however very close to it and similar to those found in the literature (Oz, 2016). Ten samples (53%) were considered adulterated, suggesting a possible mixture of vegetable oils (Table 2), with high levels to 18:1c (27.3–69.0%) and 18: 3, n-3 (1.0–7.0%), and lowest to 18:2c (20.2–55.1%), in disagreement with the range allowed by Codex Alimentarius (2015).

For the evening primrose samples, only 18% (3/17) were authentic, with a high content of α-linolenic and γ-linolenic (18:3, n-6). The others fourteen (82%) were considered adulterated: eleven samples presented a profile compatible with soybean oil and one was considered a

Table 2
Fatty acids profile (%) in different vegetables oils marketed in São Paulo City, Brazil.

Fatty acids	Coconut oils (n = 16)				Safflower oils (n = 19)					Evening Primrose oils (n = 17)					Linseed oils (n = 17)				
	Authentic (n = 15)		Adulterated (n = 1)	Ref.[1]	Authentic (n = 9)		Adulterated (n = 10)		Ref.[1]	Authentic (n = 3)		Adulterated (n = 14)		Ref.[2]	Authentic (n = 12)		Adulterated (n = 5)		Ref.[2]
	Min	Max			Min	Max	Min	Max		Min	Max	Min	Max		Min	Max	Min	Max	
6:0	0.3	0.5	0.3	< 0.05–0.7	ND	ND	ND	ND	< 0.05	ND	ND	ND	ND	–	ND	ND	ND	ND	–
8:0	5.6	7.3	6.3	4.6–10.0	ND	ND	ND	ND	< 0.05	ND	ND	ND	ND	–	ND	ND	ND	ND	–
10:0	4.8	6.3	5.1	5.0–8.0	ND	ND	ND	ND	< 0.05	ND	ND	ND	ND	–	ND	ND	ND	ND	–
12:0	47.3	52.2	47.5	45.1–53.2	ND	ND	ND	ND	< 0.05	ND	ND	ND	ND	0.03	ND	ND	ND	ND	–
14:0	17.5	20.3	19.3	16.8–21.0	0.1	0.1	0.1	0.1	< 0.05–0.2	ND	0.1	ND	0.3	0.07	ND	0.1	0.1	0.2	–
16:0	7.6	9.4	8.6	7.5–10.2	6.2	6.9	5.3	9.0	5.3–8.0	6.4	6.9	8.5	11.9	6–10	5.0	6.6	6.4	7.7	5.7–7
16:1	ND	ND	ND	< 0.05	0.1	0.1	0.1	0.2	< 0.05–0.2	ND	0.1	0.1	0.2	0.04	0.1	0.7	0.1	1.1	–
17:0	ND	ND	ND	< 0.05	ND	< LOQ	< LOQ	0.1	< 0.05–0.1	ND	ND	ND	ND	–	ND	0.1	< LOQ	0.1	–
17:1	ND	ND	ND	< 0.05	ND	ND	ND	0.1	< 0.05–0.1	ND	ND	ND	ND	–	ND	ND	ND	ND	–
18:0	2.6	4.0	2.8	2.0–4.0	2.4	2.7	2.5	3.8	1.9–2.9	1.9	2.2	2.1	4.5	1.5–3.5	2.8	5.1	4.2	5.8	3–4
18:1c	4.6	7.7	7.2	5.0–10.0	11.3	21.3	27.3	69.0	8.4–21.3	7.1	8.0	18.1	28.2	5–12	14.8	23.7	20.7	26.3	20–20.3
18:2c	0.7	2.0	2.1	1.0–2.5	67.2	78.2	20.2	55.1	67.8–83.2	70.9	72.7	48.3	61.4	65–80	12.4	17.0	14.0	30.1	17–17.3
18:3n-6	ND	ND	ND	–	ND	ND	ND	ND	–	9.8	9.9	ND	4.3	8–14	ND	ND	ND	ND	–
18:3n-3	ND	0.1	0.7	< 0.05–0.2	0.1	0.2	1.0	7.0	< 0.05–0.1	0.5	0.5	2.7	7.8	0.2	49.9	59.4	31.1	51.8	52–54
20:0	ND	0.1	0.1	< 0.05–0.2	0.3	0.5	0.3	0.5	0.2–0.4	0.3	0.3	0.4	0.5	0.3	0.1	0.3	0.2	0.3	0–0.1
20:1	ND	0.1	< LOQ	< 0.05–0.2	0.1	0.4	0.2	0.3	0.1–0.3	0.3	0.4	0.2	0.2	0.2	0.1	0.2	0.1	0.3	–
22:0	ND	ND	ND	< 0.05	0.2	0.4	0.4	0.7	< 0.05–1.0	0.1	0.3	0.2	0.5	0.1	0.1	0.2	0.2	0.3	–
24:0	ND	< LOQ	< LOQ	< 0.05	0.1	0.2	0.2	0.3	< 0.05–0.2	< LOQ	0.1	< LOQ	0.2	0.1	0.1	0.3	0.2	0.3	–
24:1	ND	ND	ND	< 0.05	0.1	0.2	ND	0.2	< 0.05–0.2	ND	ND	ND	ND	–	0.1	0.2	ND	0.1	–
Others	ND	< LOQ	ND	–	ND	0.2	ND	0.3	–	0.5	0.5	ND	0.6	–	ND	0.7	ND	ND	–

Mean values (n = 3), ND: not detect (< LOD), LOD: limit of detection (0.03%), LOQ: limit of quantification (0.10%).

[1] Reference: Codex Alimentarius (2015).

[2] Reference: Firestone (2005).

Table 3
Individual PAHs and total 4 PAHs in different vegetables oils marketed in São Paulo City, Brazil.

			BaA	Chr	BbF	BaP	Total 4 PAHs
Coconut oils (n = 16)	Authentic (n = 15)	Mean (μg kg^{-1})	1.75	2.34	1.29	1.22	6.60
		Range (μg kg^{-1})	ND-13.71	ND-14.81	ND-8.06	ND-10.69	ND-47.27
		< LOQ	3 (20%)	4 (27%)	11 (73%)	9 (60%)	7 (47%)
	Adulterated (n = 1)	Mean (μg kg^{-1})	0.61	1.38	ND	< LOQ	2.12
Safflower oils (n = 19)	Authentic (n = 9)	Mean (μg kg^{-1})	0.68	1.23	< LOQ	0.40	2.94
		Range (μg kg^{-1})	0.26–1.14	0.40–2.29	ND-1.49	ND-1.23	< LOQ-5.37
		< LOQ	0 (0%)	0 (0%)	7 (78%)	6 (67%)	1 (11%)
	Adulterated (n = 10)	Mean (μg kg^{-1})	2.07	3.76	1.23	0.78	7.84
		Range (μg kg^{-1})	0.28–4.81	0.49–8.80	ND-2.52	ND-1.63	< LOQ-17.77
		< LOQ	0 (0%)	0 (0%)	5 (50%)	4 (40%)	3 (30%)
Evening primrose oils (n = 17)	Authentic (n = 3)	Mean (μg kg^{-1})	6.12	11.24	1.94	0.63	19.93
		Range (μg kg^{-1})	2.94–7.73	4.30–14.98	1.14–2.43	0.61–0.63	9.00–25.78
		< LOQ	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	Adulterated (n = 14)	Mean (μg kg^{-1})	3.97	6.52	2.79	1.92	15.20
		Range (μg kg^{-1})	0.30–9.50	< LOQ-14.99	< LOQ-5.95	< LOQ-4.18	< LOQ-34.56
		< LOQ	0 (0%)	1 (7%)	2 (14%)	1 (7%)	1 (7%)
Linseed oils (n = 17)	Authentic (n = 12)	Mean (μg kg^{-1})	1.11	2.01	< LOQ	0.34	4.06
		Range (μg kg^{-1})	ND-4.34	ND-7.58	ND-2.19	ND-1.45	< LOQ-15.41
		< LOQ	5 (42%)	2 (17%)	10 (91%)	9 (83%)	5 (42%)
	Adulterated (n = 5)	Mean (μg kg^{-1})	1.55	2.58	< LOQ	0.55	5.63
		Range (μg kg^{-1})	ND-3.92	ND-6.80	ND-2.26	ND-1.36	< LOQ-14.22
		< LOQ	1 (20%)	1 (20%)	3 (60%)	3 (60%)	2 (40%)

Mean values (n = 4); ND: not detect (< LOD), LOD: limit of detection, LOQ: limit of quantification, LOQ (total 4 PAHs): 1.80 μg kg^{-1}.

mixture, with higher values to 18:1c and 18:3, n-3, and lower to 18:3, n-6 (Table 2) (Firestone, 2005).

For linseed oils, 29% (5/17) were adulterated, suggesting that they are mixtures of linseed and soybean oils. Other samples (71%) were considered authentic and presented high levels of α-linolenic, characteristic of this product. For the authentic oils, some differences were found for FAME like 18:0, 18:1c, 18:2c, and 18:3, n-3, that were outside the range suggested by Firestone (2005) but compatible with the literature (Anastasiu et al., 2016). It is important to consider the factors that may influence the FAME profile of the oils produced by different genotypes, their origins and the environment in which the plant was grown (Anastasiu et al., 2016; Zhang & et al., 2016).

Thus, from the FAME profile analysis, 43% of samples evaluated were not within the characteristic profile than those declared on their labels, with a higher adulteration in evening primrose oils. Problems with adulterations in vegetable oils have been reported frequently in literature, in different regions and countries (Zhang & et al., 2014; Azadmard-Damirchi & Torbati, 2015; Sun & et al., 2015). In Brazil, frauds were found in encapsulated oils of safflower, linseed and evening primrose, being first with higher incidence due to the addition of soybean oil or the presence of conjugated linoleic acid (Hirashima et al., 2013). In the evaluation of commercial coconut oils, it was found an adulteration with a mixture of coconut and soybean oils (Aued-Pimentel et al., 2015).

Coconut and linseed oils were products that had the lowest adulteration incidence, and they are produced on a small scale in Brazil (FAOSTAT., 2015). But safflower and evening primrose oils are imported, India and Argentina are the largest producers of safflower oil (FAOSTAT, 2015). None labels indicated any information about the origin of the oils, only that the products were encapsulated or packaged in Brazil.

Some oils, like cold-pressed oils, have a high value and could be intentionally adulterated by the addition or substitution of other oils of lower nutritional value and commercial value (Azadmard-Damirchi & Torbati, 2015). It can result in economic losses and reduced health benefits.

To evaluate the results of fatty acid profiles of the evening primrose and linseed oils, information of scientific literature was used (Firestone, 2005; Anastasiu et al., 2016; Zhang et al., 2016), since the Codex Stan presents parameters only for coconut and safflower oils. Thus, it is reinforced the importance of researches with vegetable oils' identity, as a way of generating results that can be used by governmental organizations to create new legislation and to review existing ones.

3.3. PAHs in commercial samples

Table 3 shows the individual PAHs and total 4 PAHs for authentic and adulterated vegetable oils, and the occurrences (%) of BaP and total 4 PAHs in these oils were represented in Fig. 1. Among the sixty-nine samples, 96% were found to be positive for any of the 4 PAHs analyzed with their concentration levels in the range of not detected to 47.27 μg kg^{-1}. BaA and Chr were detected in 94% of the oils, while BbF and BaP were detected in 81% and 85%, respectively.

Among all types of oils evaluated, Chr was the most abundant hydrocarbon, with the highest individual value (14.99 μg kg^{-1}) found in an adulterated evening primrose oil, with soybean oil profile. For BaA, BaP, BbF, and 4 PAHs, the highest values were observed in an authentic coconut oil (respectively 13.71, 10.69, 8.06, and 47.27 μg kg^{-1}).

The individual values of PAHs and the total 4 PAHs were higher in the authentic coconut samples, while for linseed and safflower oils were higher for adulterated samples. For evening primrose oils, the highest values of BaA and Chr were observed in the authentic samples, and for BbF and BaP in adulterated samples. Evaluating the mean results, considering the category of each group, the descending sequence of the total 4 PAHs was: authentic evening primrose > adulterated evening primrose > adulterated safflower > authentic coconut > adulterated linseed > authentic linseed > authentic safflower > adulterated coconut.

In Brazil, olive pomace oil is the only vegetable oil with maximum BaP levels established in legislation (2.0 μg kg^{-1}) (Brazil, 2003). The Regulation No. 835/2011 by the European Union establishes the limits for PAHs, and the maximum value for BaP is 2.0 μg kg^{-1} (Commission of the European Communities, 2011a). Twelve percent of samples (8/69) exceeded the BaP limit: six evening primrose oils (adulterated, with FAME profile of soybean oil) and two of authentic coconut oil, and one of them presented the highest BaP content (10.69 μg kg^{-1}). Safflower and linseed oils (authentic and adulterated) did not exceed the recommended limits (Fig. 1).

The same regulation determines a limit for the total 4 PAHs of

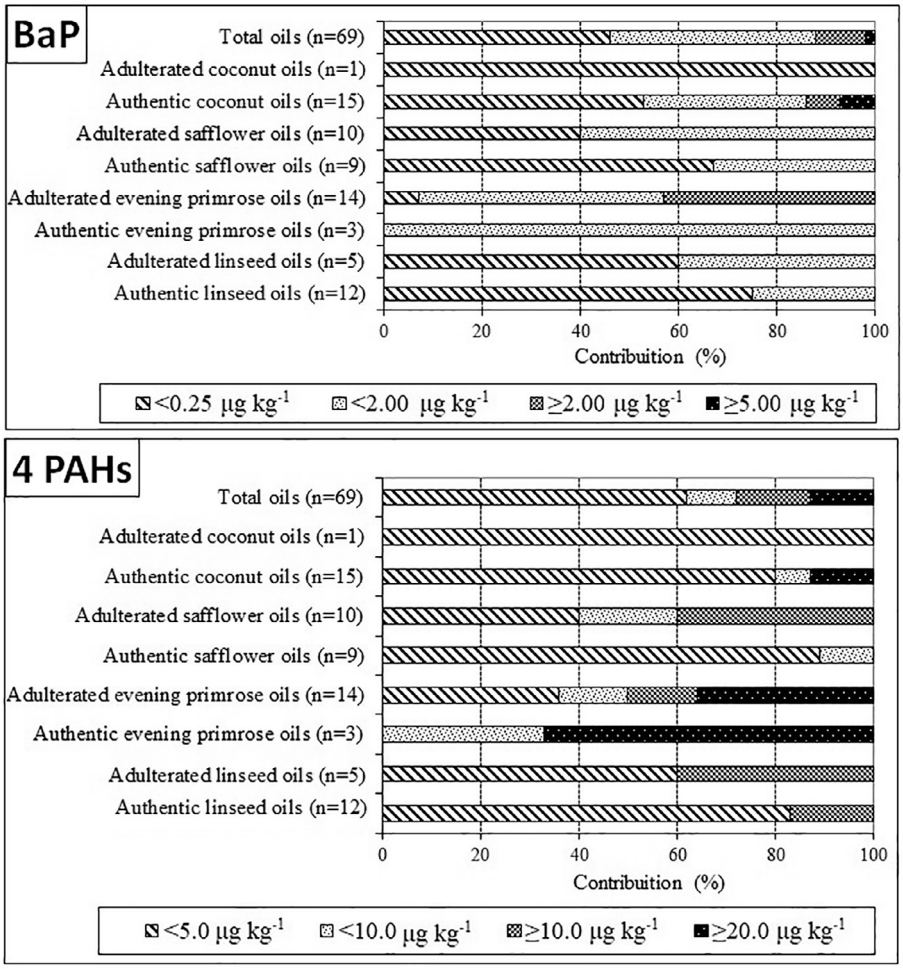

Fig. 1. BaP and total 4 PAHs: occurrences in vegetables oils marketed in São Paulo City, Brazil.

10.0 µg kg^{-1} for vegetable oils, and 20 µg kg^{-1} for coconut oil (Commission of the European Communities, 2011a). Concentrations were above the maximum value to 28% of the samples: 2 coconut oils (authentic), 4 safflower (adulterated), 2 evening primrose (authentic), 7 evening primrose (adulterated), 2 linseed (authentic), and 2 linseed (adulterated). No authentic safflower oils have exceeded the recommended limits (Fig. 1). Besides that, it is found that the highest levels for BaP and for total 4 PAHs were more frequently verified in adulterated samples (safflower, linseed and evening primrose).

Of the 19 samples that were presented results above the legislation for total 4 PAHs, seven were above for BaP. One sample was above for BaP content, but not for the total 4 PAHs. > 50% of samples of coconut (authentic and adulterated) and linseed oils (authentic and adulterated) presented BaP values lower to LOQ. The contribution relative to the BaP can be considered small for the total 4 PAHs (Fig. 1). The results indicated that the hydrocarbon that presented the highest average values was Chr, followed by BaA (Table 3). These results are in agreement with Alomirah and et al. (2010), who found other PAHs in oils in which BaP was not present. Thus, it is verified that BaP alone is not a good indicator of the concentration of other PAHs, but rather the total 4 PAHs based on data relating to occurrence and toxicity, as published by EFSA (2008).

The presence of PAHs in different types of vegetable oils has been verified by several authors (Pandey et al., 2004; Wegrzyn, Grzeskiewicz, Poplawska, & Glod, 2006; Alomirah et al., 2010; Camargo et al., 2011; Roszko et al., 2012; Ciecierska & Obiedzinski, 2013; Shi, Zhang, & Liu, 2016; Zachara, Gałkowska, & Juszczak, 2017). Researches with vegetable oils from unconventional sources, such as those in this study, are limited.

Ciecierska and Obiedzinski (2013) analyzed cold-pressed unconventional oils, including linseed, safflower, and evening primrose oils, and means values for total 4 PAHs were not detected, 2.93 and 4.03 µg kg^{-1}, respectively, and BaP concentrations were lower than 0.12 µg kg^{-1}. The results found in our study were higher for BaP and total 4 PAHs for linseed and evening primrose oils. Similar concentrations were obtained for safflower oils in total 4 PAHs.

The study with evening primrose oils by Roszko et al. (2012) indicated that results of total 4 PAHs was 3.40 µg kg^{-1} and of BaP was 0.58 µg kg^{-1}, inferior to this study. Pandey et al. (2004) evaluated Chr and BaP in linseed and safflower oils, and the most common hydrocarbon was Chr, with averages of 20.7 and 26.6 µg kg^{-1} respectively, higher than we obtained, although Chr had also been the contaminant with higher average values for all oils. In the same study of Pandey et al. (2004), the BaP was found in linseed (1.5 µg kg^{-1}) and safflower (2.6 µg kg^{-1}), with higher results than those of our study.

For coconut oils, our results were higher than those obtained by Pandey et al. (2004): 2.0 µg kg^{-1} for Chr and 0.8 µg kg^{-1} for BaP. Zachara et al. (2017) studied unrefined coconut oils and levels ranged from ND to 2.20 µg kg^{-1} for BaP, and this oil presented the highest levels of BaP contamination, as we found, comparing only authentic oils. Wegrzyn et al. (2006) obtained values of 40.6 µg kg^{-1} for BaP and 263.6 for total 4 PAHs for crude coconut oil, and for refined coconut the only hydrocarbon detected was BaP (0,10 µg kg^{-1}).

There are several causes that may justify the presence of PAHs in vegetable oils, but mainly the contact of the plant and seeds with contaminated areas, the drying seeds process and accidental

contamination. The first one relates the environmental contamination of the places in which the plants were cultivated. Air pollution contains dust and particles with a high quantity of PAHs, which can deposit on the surface of the plants and contaminate them, and during processing are transferred to the final product. In industrial areas and near to highways, this factor can be significantly higher. The contamination of the soil in which the plant was cultivated can be a favorable factor for the accumulation of PAHs in the plant (Bansal & Kim, 2015).

Another cause of contamination of the oils is the drying process of the seeds. In Brazil, the use of direct drying of the gas-fired seed is a common practice, and the PAHs present in the smoke can come in direct contact with the seeds and contaminate them. Therefore, during extraction of the oils from these seeds, PAHs can be transferred to the oils, especially considering their lipophilic characteristics (Camargo et al., 2011). Although less frequent, accidental contamination of oils is another way products can be exposed to PAHs, and this contamination can occur during processing by contact with waste with mineral oils (Bansal & Kim, 2015).

4. Conclusion

The evaluation of the fatty acid profile revealed that 43% of samples could be considered adulterated, since they did not present the characteristics of fatty acids profile of oils described in their labels, with more frauds in evening primrose oils. The PAHs results indicate that 96% of samples were found to be positive for any of the 4 PAHs analyzed. Concentrations of BaP ranged from not detected to 10.69 $\mu g\,kg^{-1}$, and of total 4 PAHs from not detected to 47.27 $\mu g\,kg^{-1}$. Considering the maximum levels allowed by the European Community, eight oils (12%) exceeded the BaP limit: six adulterated evening primrose oils, with FAME profile of soybean oil, and 2 authentic coconut oils. These same oils exceeded the limits for total 4 PAHs, and a total of 19 samples (28%) presented results above the legislation. The hydrocarbons with the highest occurrence were Chr and BaA. Good manufacturing practices should be adopted by the industries throughout the vegetable oil production, from the raw material collection until the final product, with the objective of minimizing contamination by PAHs. It is suggested to carry out monitoring programs with the objective of generating more results to send to competent governmental organizations to review legislations existing and verifying the contamination of the products being offered to the population. Thus, future studies should be conducted with the objective of evaluating the Brazilian vegetable oils contamination, especially with refined oils.

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Conflict of interest

The authors declare no conflict of interest.

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