Food Control 46 (2014) 539–543

Contents lists available at ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Incidence of polycyclic aromatic hydrocarbons in vegetable oil blends

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ARTICLE INFO

Article history:
Received 18 February 2014
Received in revised form
21 May 2014
Accepted 15 June 2014
Available online 21 June 2014

Keywords:
Polycyclic aromatic hydrocarbons
Vegetable oil
Oil blend
Olive oil

ABSTRACT

The consumption of extra virgin olive oil has been growing in Brazil over the years. However, as it is an expensive product, many consumers tend to substitute extra virgin olive oil with a cheaper alternative, such as vegetable oil blends (mainly a mixture of olive and soybean oils). In the preset study, different brands of oil blends were evaluated for the occurrence of 13 polycyclic aromatic hydrocarbons. The method involved liquid–liquid extraction with hexane and N,N-dimethylformamide, followed by clean-up by SPE with C18 cartridges. Analyses were carried out by HPLC with fluorescence detection. PAHs were detected in all samples analyzed and the mean sum ranged from 2.59 μg/kg to 85.30 μg/kg. A high variability in PAHs levels between brands and between different batches of the same brand was observed. Approximately 50% of the samples were in disaccording with the maximum permitted levels for BaP and PAH4 established in the Brazilian and/or European regulations.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) constitute a large class of organic compounds that are formed during incomplete combustion or pyrolysis of organic matter (EFSA, 2008; WHO, 2006). PAHs have attracted attention mostly due to their carcinogenic potential, with human exposure to PAHs occurring through the airways, skin and digestive tract. Metabolic activation is necessary for their toxic, mutagenic and carcinogenic processes (EFSA, 2008; IARC, 2010).

The International Agency for Research on Cancer (IARC) has classified benzo[*a*]pyrene in the group 1, as carcinogenic to humans (IARC, 2012). During its 64th meeting, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) evaluated 33 PAHs and concluded that 13 of them were clearly carcinogenic and genotoxic (WHO, 2005, p. 47). The European Food Safety Authority Panel on Contaminants in the Food Chain (CONTAM Panel) also evaluated the previous 13 and additionally benzo[ghi]perylene, cyclopenta[*cd*]pyrene and benzo[*c*]fluorene. The panel concluded that benzo[*a*]pyrene is no longer a suitable indicator for the presence of PAHs in food, suggesting that PAH4 (benzo[*a*]pyrene, chrysene, benz[*a*]anthracene and benzo[*b*]fluoranthene) or PAH8 (benzo[*a*]pyrene, chrysene, benz[*a*]anthracene and benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*ghi*]perylene, dibenz[*a,h*]anthracene and indeno[1,2,3-*cd*]pyrene) are better indicators for the presence of PAHs in food (EFSA, 2008).

The sources of PAH in food come from food processing and cooking (drying, smoking, roasting, grilling, and frying) and also from environmental pollution (WHO, 2005). PAHs occur as contaminants in different food categories such as oils and fats, vegetables, fruits, seafood, tea, coffee, sugar, toasted bread, infant foods and smoked food products (Alomirah et al., 2011; Camargo, Antoniolli, Vicente, & Tfouni, 2011; Camargo & Toledo, 2003; Rey-Salgueiro, García-Falcón, Martínez-Carballo, & Simal-Gándara, 2008; Rey-Salgueiro, Martínez-Carballo, García-Falcón, González-Barreiro, & Simal-Gándara, 2009; Tfouni & Toledo, 2007; Tfouni et al., 2013; Vieira et al., 2010).

In Brazil, the consumption of extra virgin olive oils has been growing over the years, mainly because of its nutritional value and health benefits (IOC, 2013). Nevertheless, as it is an expensive product, many consumers tend to replace extra virgin olive oil with a cheaper alternative, such as vegetable oil blends, made of a mixture of olive oil and another vegetable oil like soybean, canola and sunflower oil.

Different studies have identified oils and fats as important sources of PAHs in the diet. The drying step of the seeds, grains and

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http://dx.doi.org/10.1016/j.foodcont.2014.06.028
0956-7135/

olive pomace has been reported as the main source of contamination, while for olive oil the source of PAHs may come from environmental contamination (Camargo & Toledo, 2002; Costopoulou et al., 2010; Moret, Dudine, & Conte, 2000; Purcaro, Moret, & Conte, 2008; Rodriguez-Acuña et al., 2008).

Although studies from many countries report the presence of PAHs in different kind of vegetable oils (like soybean, sunflower, olive and pomace) (Alomirah et al., 2010; Ballesteros, García Sánchez, & Ramos Martos, 2006; Camargo, Antoniolli, Vicente, & Tfouni, 2011; Costopoulou et al., 2010; Moret et al., 2000; Teixeira, Casal, & Oliveira, 2007), there is a lack of information regarding the occurrence of these compounds in vegetable oil blends.

Therefore the objective of the present study was to evaluate the presence of 13 polycyclic aromatic hydrocarbons in vegetable oil blends, in order to evaluate the safety of products that has been presenting an increase in consumption over the last years.

2. Materials and methods

2.1. Materials

2.1.1. Samples

Different brands and batches of commercial vegetable oil blends were purchased at supermarkets in the cities of Campinas and Ribeirão Preto (SP, Brazil) in the years 2011 and 2012. A total of 36 samples, from different brands and batches, was analyzed in duplicate for the presence of 13 PAHs: 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[*a,l*]pyrene (DalP), dibenz[*a,h*] anthracene (DahA), indeno[1,2,3-*cd*]pyrene (IcdP), dibenzo[*a,e*] pyrene (DaeP), dibenzo[*a,i*]pyrene (DaiP) and dibenzo[*a,h*]pyrene (DahP).

The composition of the oil blend samples was as follows: 85% of soybean oil and 15% of olive oil (brands A, C, D, F, G, H, I, K and N), 90% of soybean oil (B, J and L), 85% of canola oil (E) and 85% of sunflower oil (M).

2.1.2. Standards and reagents

Analytical standards were purchased from Supelco Inc. (BaA, Chr, BbF, BkF, BaP, DahA and IcdP), Fluka (BjF, DalP, DaeP, DahP), Cambridge Isotope Laboratories (5MChr) and ChemService (DaiP). Hexane and N,N-dimethylformamide were purchased from Tedia Company Inc, acetonitrile was from J.T. Baker and methanol from Mallinckrodt (all HPLC grade). Water was obtained from a Millipore Milli-Q water purification system (Milford, MA, USA). Millex HV 0.45 μm filters were purchased from Millipore and Sep Pak C18 solid-phase extraction (SPE) columns (500 mg, 3 mL) were from Waters.

2.2. Method

Analytical method was based on the one described by Camargo, Antoniolli, and Vicente (2011) for PAHs analyses in soybean oil, as follows.

2.2.1. Extraction and clean up

Vegetable oil blends (0.5 g) were weighed, 5 mL of hexane were added and the mixture was transferred into a 60 mL separating funnel where PAHs were extracted twice with 5 mL of N,N-dimethylformamide-water (9:1, v/v). The combined extract was concentrated under a flow of nitrogen until it reached approximately 50% of its initial volume. Then, the resulting solution was diluted with 8 mL of water before SPE clean-up. The SPE cartridges were prepared by pre-washing with 5 mL of methanol, followed by 5 mL of water using a Vacuum Manifold from Supelco. Then the sample solution was applied and the column was washed with 10 mL of N,N-dimethylformamide–water (1:1,v/v), followed by 10 mL of water, all eluates were discarded. The cartridges were dried under vacuum for 20 min. PAHs were eluted with 12 mL of hexane at a flow rate of 2 mL/min and the eluate was dried under a nitrogen stream (TurboVap LV, Caliper Life Sciences). The residue was diluted in 0.5 mL of acetonitrile, filtered through a 0.45 μm filter and analyzed by HPLC with fluorescence detection.

2.2.2. HPLC

The analyses were carried out using a Shimadzu (Kyoto, Japan) HPLC apparatus equipped with an LC-20AT quaternary pump, DGU-20A5 on-line degasser, a SIL-20A autosampler (30 μL injection volume), a CTO-20A column oven and an RF-10A xl fluorescence detector. Data were acquired and processed with LCsolution software. A C18 column (Vydac 201 TP54, 250 × 4.6 mm, 5 μm particle size; Vydac, Hesperia, CA, USA) at 30 °C and a gradient mobile phase

Table 1
Limit of detection (LOD), quantification (LOQ), recovery and relative standard deviation (RSD) for the analysis of 13 PAHs in vegetable oil blend.

Mean recovery ± RSD (%)[a]							
PAH	LOD (μg/kg)	LOQ (μg/kg)	Level 1 (0.3 μg/kg)[b]	Level 2 (1.0 μg/kg)[c]	Level 3 (2.0 μg/kg)[d]	Level 4 (5.0 μg/kg)	Level 5 (20.0 μg/kg)
BaA	0.16	0.30	95 (18)	102 (7)	96 (9)	93 (7)	88 (4)
Chr	0.07	0.30	98 (6)	94 (9)	86 (13)	92 (7)	96 (6)
5MChr	0.07	0.30	83 (8)	98 (20)	99 (9)	90 (11)	115 (2)
BjF	0.52	3.00	83 (9)	86 (6)	89 (4)	–	–
BbF	0.16	0.30	76 (10)	89 (7)	98 (7)	90 (5)	84 (12)
BkF	0.03	0.30	115 (3)	96 (6)	95 (4)	89 (5)	82 (2)
BaP	0.07	0.30	96 (8)	93 (7)	90 (5)	87 (4)	80 (6)
DalP	0.03	0.30	62 (8)	74 (5)	82 (3)	81 (5)	74 (6)
DahA	0.04	0.30	65 (8)	81 (6)	88 (5)	88 (5)	80 (4)
IcdP	0.19	3.00	66 (2)	79 (5)	84 (6)	–	–
DaeP	0.10	0.30	101 (7)	91 (10)	98 (3)	90 (6)	80 (4)
DaiP	0.06	0.30	90 (7)	77 (5)	81 (4)	78 (10)	76 (9)
DahP	0.02	0.30	67 (4)	78 (5)	78 (8)	73 (16)	72 (15)

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.

[a] $n = 5$.

[b] 3.0 μg/kg for BjF e IcdP.

[c] 10.0 μg/kg for BjF e IcdP.

[d] 20.0 μg/kg for BjF e IcdP.

consisting of acetonitrile and water at a flow rate of 1 mL/min were used. The 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 the initial mobile phase composition for 15 min. The following excitation (ex) and emission (em) wavelength program was used to detect the PAHs: 0.01 min (274/414 nm) for BaA, Chr and 5MChr; 16.70 min (312/507 nm) for BjF; 18.20 min (290/430 nm) for BbF, BkF, BaP, DalP and DahA; 32.40 min (300/500 nm) for IcdP; 34.90 min (297/403 nm) for DaeP and 45 min (304/457 nm) for DaiP and DahP.

2.2.3. Quantification and method validation

The external standard plot method was used for quantification. Duplicate HPLC injections of six concentration levels (0.30–10.0 ng/mL) of PAHs standard solutions, in acetonitrile, were used to construct linear regressions lines (peak area ratios versus PAH concentration).

Accuracy and precision data were obtained through recovery studies carried out by spiking a blank sample with PAHs standard solutions at five concentration levels (0.3, 1.0, 2.0, 5.0 and 20.0 μg/kg) in five replicates. Results reported were not corrected for recovery. Precision of the method was evaluated through the relative standard deviation (RSD) associated to measurements of the PAHs performed during recovery analyses.

Limits of detection (LOD) and quantification (LOQ) were determined in accordance with INMETRO (2011, 19 pp.) guidelines. For this purpose, seven independent analyses of the blank sample spiked with PAHs at a level of 0.3 μg/kg were performed. The LODs were calculated from the standard deviation of these determinations. LOQs were established as the lower concentrations used in the calibration curves and the recovery tests.

2.3. Statistical analysis

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

3. Results and discussion

Mean recovery, RSD and LOD for the 13 PAHs analyzed are shown in Table 1. Recoveries obtained ranged from 62% to 115% with RSDs varying from 2% to 20%. Limits of detection and quantification were from 0.02 to 0.52 μg/kg and 0.3–3.0 μg/kg, respectively. The calibration curves obtained for the PAHs studied were linear with correlation coefficients between 0.9990 and 0.9998. These results are satisfactory for determinations at μg/kg levels and comply with the performance criteria for methods of BaP analysis proposed by the European Union, where the LOD must be lower than 0.3 μg/kg and recovery must be in the range of 50–120% (CEC, 2007). The only exception was BjF, which presented higher LOD than the one proposed for BaP. Despite the lower sensibility for this compound, the analytical method used may be considered suitable for the analysis of 13 PAHs in vegetable oil blends.

Table 2 presents mean PAHs levels detected in different brands of vegetable oil blends. PAHs were present in all samples analyzed, with BaA, Chr, BbF, BkF, BaP and DaeP being the most representative ones as they were detected in all samples. BjF and DalP were present in only four and two samples, respectively, whereas DahP was not quantified (<LOQ) in any sample. Individual levels of PAHs ranged from not detected to 25.51 μg/kg (BaP level on batch 1 of brand J), showing a wide range of PAHs concentrations among the different brands of oil blends.

Table 2
Mean and range levels of PAHs detected in different brands of oil blends analyzed.

Brand	Mean PAH level (μg/kg)[a] (range)												
	BaA	Chr	5MChr	BjF	BbF	BkF	BaP	DalP	DahA	IcdP	DaeP	DaiP	DahP
A	1.34 (0.58–2.46)	2.85 (1.96–4.53)	0.71 (0.39–1.20)	nd	0.86 (0.54–1.30)	0.36 (0.24–0.54)	0.51 (0.29–0.41)	(nd-tr)	0.10 (tr-0.31)	(nd-tr)	0.71 (0.66–0.78)	nd	nd
B	0.80 (0.60–0.99)	1.62 (1.39–1.84)	0.46 (0.41–0.58)	nd	0.64 (0.52–0.73)	0.13 (tr-0.38)	0.39 (0.29–0.59)	nd	(nd-tr)	nd	0.69 (0.60–0.76)	nd	nd
C	6.76 (5.26–8.82)	6.34 (5.68–7.09)	1.29 (1.08–1.50)	nd	4.24 (3.72–4.60)	1.63 (1.30–2.11)	3.74 (2.94–4.46)	nd	0.81 (0.44–1.12)	(nd-tr)	1.04 (0.80–1.38)	(nd-tr)	(nd-tr)
D	5.68 (2.99–7.61)	4.58 (3.98–4.90)	1.36 (0.40–2.70)	2.08 (n-3.25)	5.00 (2.24–6.65)	2.98 (1.05–4.01)	5.91 (1.31–8.41)	nd	1.58 (0.50–2.16)	4.27 (nd-6.45)	1.36 (0.73–1.69)	0.22 (nd-0.67)	(nd-tr)
E	4.38 (3.53–5.24)	4.71 (3.47–6.45)	0.70 (0.36–0.91)	1.40 (nd–4.20)	5.19 (2.63–9.43)	2.67 (1.35–5.00)	7.65 (1.68–17.44)	nd	1.48 (nd-3.36)	4.27 (nd-12.80)	1.12 (0.74–1.71)	0.16 (nd-0.49)	(nd-tr)
F	2.67 (1.60–3.98)	3.71 (2.16–5.05)	0.75 (0.38–1.04)	nd	1.91 (1.33–3.08)	0.82 (0.40–1.23)	1.54 (0.98–2.47)	nd	0.20 (nd-0.59)	(nd-tr)	0.65 (0.43–0.93)	(nd-tr)	nd
G	1.70 (0.82–3.28)	2.58 (1.22–4.23)	0.40 (nd-1.20)	nd	1.49 (0.87–2.67)	0.62 (0.31–1.00)	0.93 (0.52–1.59)	nd	0.41 (nd-0.75)	nd	0.58 (0.50–0.69)	nd	nd
H	3.85 (3.05–5.03)	3.95 (3.21–4.54)	1.18 (0.93–1.43)	nd	2.69 (2.28–3.36)	1.20 (0.98–1.48)	2.69 (2.28–3.27)	nd	0.61 (nd-1.07)	nd	0.86 (0.70–1.13)	(nd-tr)	(nd-tr)
I	2.39 (1.32–3.11)	3.05 (1.67–3.78)	0.38 (tr-0.66)	nd	1.80 (1.02–2.64)	0.80 (0.64–1.06)	1.29 (0.70–2.00)	0.11 (nd-0.32)	(nd-tr)	nd	0.79 (0.65–1.02)	nd	nd
J	4.83 (2.81–8.29)	4.80 (3.35–6.52)	1.19 (0.95–1.54)	1.95 (nd-5.85)	6.06 (3.16–11.77)	2.79 (1.31–5.53)	10.36 (2.31–25.51)	nd	1.32 (tr-2.95)	5.22 (nd-15.65)	1.11 (0.84–1.63)	0.18 (nd-0.55)	(nd-tr)
K	2.89 (0.66–4.21)	2.39 (0.60–3.83)	0.53 (tr-0.95)	nd	2.09 (0.64–3.00)	0.62 (tr-1.01)	1.84 (0.67–2.85)	nd	0.58 (tr-0.94)	nd	0.57 (tr-0.90)	nd	nd
L	1.46	2.35	1.15	nd	1.27	0.57	0.98	nd	0.58	nd	0.66	nd	nd
M	0.43	1.30	0.69	nd	0.51	0.39	0.36	nd	nd	nd	tr	nd	nd
N	4.04	3.94	1.06	nd	3.35	1.01	2.45	nd	1.00	nd	0.80	nd	nd

nd < LOD from Table 1.
tr < LOQ from Table 1.
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.
[a] Mean of 3 batches in duplicate (except L, M and N: one batch).

Fig. 1 presents the sum of 13 PAHs levels in the different brands and batches of oil blend samples. The summed levels ranged from 2.59 μg/kg (batch 1, brand K) to 85.30 μg/kg (batch 1, brand J). These results are in accordance with results observed in other studies. Alomirah et al. (2010) detected, in blended olive oils produced in Spain, levels from 31.01 μg/kg to 78.01 μg/kg for the sum of 16 PAHs (U.S. Environmental Protection Agency (EPA) priority PAHs). As there are few studies related to the presence of PAHs in blended oils, available data for edible oils can be used for comparison. Teixeira et al. (2007) analyzed sunflower, soybean and virgin olive oil commercialized in Portugal, finding summed levels of 15 PAHs ranging between 8.78 μg/kg and 26.35 μg/kg Fromberg, Hojgard, and Duedahl-Olesen (2007) evaluated different types of vegetable oils and the mean level of PAHs (sum of 18 compounds) varied from 5.5 μg/kg to 172 μg/kg Camargo et al. (2011a) determined 13 PAHs in 11 brands of soybean oils commercialized in Brazil, with summed levels varying from 10.4 μg/kg to 112.0 μg/kg, these results show the potential of contamination of vegetable oil blends deriving from soybean oil.

As Fig. 1 also shows, there was a high variability between batches of the same brand, with 82% of the brands presenting statistically significant difference between batches ($P < 0.05$). Most brands are produced with a blend of 15% olive oil and 85% soybean oil. Brazil imports, from different producing countries, the majority of the olive oil internally commercialized. On the other hand soybean oil is locally produced, with crops spread in a large area of the territory. Therefore, the high variability between batches, and likewise between brands, is probably due to some factors such as: a) different regions of origin - the air pollution, and consequently presence of PAHs, in the surrounding area of cultivation may vary according to the region of origin of the raw material used for oil blend production (olive, soybean, sunflower and canola) (Camargo & Toledo, 2002; Rodriguez-Acuña et al., 2008) and b) differences in the process - the contact with combustion gases during the drying process of the seeds may lead to their contamination by PAHs and subsequent transfer to the vegetable oil. Thus different variables involved in the drying and also in the oil production/refining process will result in oils with different PAHs levels (Camargo, Antoniolli, & Vicente, 2012; Rodriguez-Acuña et al., 2008).

In Brazilian regulation there are no maximum limits for PAHs in vegetable oil blends. The only regulations available are for BaP in olive pomace oil (limit of 2.0 μg/kg), smoke flavourings (0.03 μg/kg, in the final product) and drinkable water (0.7 μg/L) (Brasil, 2003, 2004; 2007). In contrast, European regulation sets limits of some PAHs for the category of oils and fats, i.e. 2.0 μg/kg for BaP and 10.0 μg/kg for PAH4 (CEC, 2011). Considering the values of BaP detected in the 36 samples analyzed (Table 2) and the limits set by Brazilian and European regulations, one can assume that BaP levels in the olive oil blends (0.29–25.51 μg/kg), may be considered relatively high, as 17 samples (47%) presented levels above the established ones and reached values up to twelve times the maximum limit.

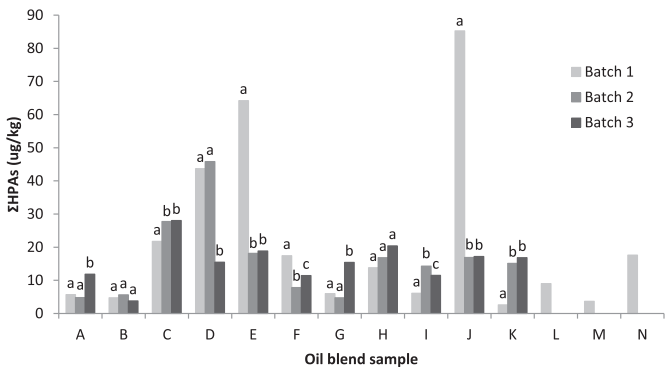

Fig. 1. Sum of 13 PAHs levels in different batches of vegetable oil blends. Different letters indicate statistic difference ($P < 0.05$) between batches within the same brand.

Table 3
Levels of PAH4 (BaA + Chr + BbF + BaP) in different brands and batches of vegetable oil blends.

PAH4 (μg/kg)[a]				
Brand	Batch 1	Batch 2	Batch 3	Mean
A	4.19	6.16	9.12	6.49
B	3.60	3.95	2.80	3.44
C	17.59	23.27	22.36	21.07
D	25.44	27.53	10.51	21.93
E	36.31	15.13	14.35	21.93
F	14.57	6.06	8.87	9.83
G	4.91	3.42	11.76	6.70
H	10.97	12.39	16.20	13.18
I	4.70	11.53	9.35	8.53
J	52.08	12.60	13.47	26.05
K	2.56	11.93	13.13	9.21
L	7.62	–	–	–
M	2.60	–	–	–
N	13.78	–	–	–

PAH4 = BaA + Chr + BbF + BaP.
[a] Mean of duplicate.

PAH4 concentrations determined in the different batches and brands of blend oils analyzed are reported in Table 3. Levels ranged from 2.80 μg/kg (brand B, batch 3) to 52.08 μg/kg (brand J, batch 1), with brand J being 5 times higher than the maximum level established by the European regulation. According to the table, 58% of the analyzed samples presented PAH4 levels above the permitted limit. Results also emphasize the high variability between brands and between different batches of the same brand.

4. Conclusion

PAHs levels present in the analyzed oil blend samples can be considered high since approximately 50% of the samples were in disaccording with the maximum permitted levels for BaP and PAH4 established in the Brazilian and/or European regulations.

Oil blends are generally consumed as a replacement for more expensive extra virgin olive oil. Nevertheless, the levels of PAHs detected in this type of products are relatively high, similar to those usually found in soybean oils. This information contrasts with the health claim of extra virgin olive oil, especially regarding the possible presence of potentially carcinogenic compounds in oil blends.

Acknowledgements

Financial support from São Paulo Research Foundation (2011/10966-4) and scholarship from PIBIC/CNPq are gratefully acknowledged.

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