J Am Oil Chem Soc (2017) 94:1351–1359
DOI 10.1007/s11746-017-3046-3

ORIGINAL PAPER

Adulteration and Presence of Polycyclic Aromatic Hydrocarbons in Extra Virgin Olive Oil Sold on the Brazilian Market

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Received: 13 January 2017 / Revised: 14 August 2017 / Accepted: 5 September 2017 / Published online: 15 September 2017

Abstract Seventy samples sold in the Brazilian market as extra virgin olive oil (EVOO) were evaluated for the presence of the 13 polycyclic aromatic hydrocarbons (PAH) classified as carcinogenic and genotoxic by the Joint FAO/WHO Expert Committee on Food Additives (JECFA), to verify if the products were adulterated and to evaluate if there is a correlation between PAH presence and adulteration. PAH were detected in 93% of the samples, with summed levels varying from not detected to 41.10 µg/kg. Five samples showed BaP concentration above acceptable levels set by European legislation and by Brazilian regulation (2.0 µg/kg) and 7 presented PAH4 levels above the limit set by European legislation (10.0 µg/kg). The levels of fatty acid composition, sterols content, stigmastadiene and specific extinction did not comply with both Brazilian and International Olive Council (IOC) standards in 18, 31, 30 and 21% of the samples, respectively. The tolerance levels for these analyses in the Brazilian standards are 55.0–83.0 g/100 g (oleic acid), 3.5–21.0 g/100 g (linoleic acid), ≤0.05 g/100 g (*trans*-oleic acid), ≤0.05 g/100 g (*trans*-linoleic + *trans*-linolenic acid), ≤0.15 mg/kg (stigmastadiene), ≤2.50 (K232), ≤0.22 (K270), ≤0.01 (ΔK), 1000–1600 mg/kg (Σ sterols). Results indicate that 19 samples were adulterated. According to principal component analysis, samples were distinguished as: (1) EVOO with addition of vegetable oil from another source, (2) EVOO with addition of refined oil and (3) samples possibly not adulterated. The variable ΣPAH was related mainly to samples of EVOO with addition of vegetable oil from another source.

Keywords Olive oil · Polycyclic aromatic hydrocarbon · Adulteration

Introduction

Polycyclic aromatic hydrocarbons (PAH) are organic compounds formed during incomplete combustion or pyrolysis of organic matter. Their presence in food is mainly related to food processes involving high temperatures, such as drying, smoking and grilling. Another source of PAH contamination in food is by deposition from the environment [1]. The presence of PAH have been reported in different types of food, such as soybean oil, bivalves, infant foods, vegetable and fruits [2–5].

Over the years, these compounds have been evaluated by the International Agency for Research on Cancer (IARC), the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the European Food Safety Authority (EFSA) Panel on Contaminants in the Food Chain. IARC has classified benzo[*a*]pyrene as carcinogenic to humans; the JECFA concluded that 13 PAH were carcinogenic and genotoxic; while the EFSA has suggested, as a replacement for benzo[*a*]pyrene, no longer considered a suitable indicator, the combined presence of different PAH as indicators of the occurrence of these compounds in food. Thus, the panel suggests the use of 8 PAH (PAH8) or a subgroup of 4 (PAH4) [1, 6, 7].

Several studies have reported oils and fats as important sources of PAH intake in the human diet, and the main cause of contamination of this group of food was identified to be the drying process by which the seeds, grains and olive

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pomace are subjected during oil and fat processing [5, 8]. In Brazil, the use of direct drying with combustion smoke generated from wood is a common practice, which allows direct contact of the PAH present in the smoke with the seeds or grains [5]. Brazilian soybean oil has been shown to be contaminated with PAH. In a study from 2011, 42 samples of soybean oil were analyzed for the presence of 13 PAH. In the results, individual PAH levels ranged from 0.2 to 26.1 μg/kg and summed PAH levels were up to 112.0 μg/kg [4].

Compared to other commodities, the total production of olive oils of different grades is small and, thus, that of extra virgin olive oil (EVOO) is even smaller, being a product that has even higher added value and, therefore, is a frequent object of fraud and adulteration. One of the most common adulteration processes is the addition of other vegetable oils of lower commercial value, such as refined olive oil, pomace oil and seed oils [9, 10].

Virgin olive oils (VOO) are oils obtained from the fruit of the olive tree (*Olea europaea* L.) solely by mechanical or other physical means under conditions, mainly thermal, that do not lead to alterations in the oil, and which have not undergone any treatment other than washing, decantation, centrifugation and filtration [11]. As there is no drying process involved in VOO production, the presence of PAH may be due to the fraudulent addition of other vegetable oils that were already contaminated, or also may be due to environmental pollution [8]. A previous study by Tfouni *et al*. [12] has shown a high incidence of PAH in vegetable oil blends, which are made of a mixture of olive oil and another vegetable oil like soybean, canola or sunflower. As EVOO is an expensive product, many consumers tend to replace it with this cheaper alternative commonly sold in Brazilian markets. The olive oil content in these products is between 10 and 15%. The study by Tfouni *et al*. [12] reported levels that might be considered high for the sum of 13 PAH in oil blends (2.59 to 85.30 μg/kg) as well as for PAH4, since 58% of the analyzed samples presented PAH4 above the limit permitted by the European Union (10.0 μg/kg) [21].

In order to contribute to the development of the sector and prevent adulteration or erroneous labelling, MAPA-Ministério da Agricultura, Pecuária e Abastecimento (Brazilian Ministry of Agriculture, Livestock and Food Supply) set, in 2012, a regulation establishing official standards of identity, quality and classification for olive oil and olive pomace oil [13]. This regulation was established based on International Olive Council (IOC) standards and presents the same parameters defined in COI/T.15/NC No.3/Rev. 11 [11]. The designation EVOO is given according to pre-established quality parameters set by both MAPA and IOC [11, 13]. The evaluation of authenticity of an EVOO involves the analysis of different parameters, such as the methyl esters for the determination of the trans/cis fatty acids (fatty acid composition), stigmasta-3,5-diene, sterols content and specific extinction, which may, together, provide information regarding the presence of refined oils from olive or seeds.

The aim of this study was to evaluate EVOO for the presence of the 13 PAH identified as being genotoxic and carcinogenic by the JECFA, verify if the products are adulterated with the addition of a vegetable oil with lower commercial value and evaluate if the presence of PAH is related to EVOO adulteration.

Materials and Methods

Samples

Samples acquired were sold in the Brazilian market as EVOO. Samples collected were from 37 different brands, two lots from 33 brands (66 samples) plus one lot from 4 brands (4 samples), totalling 70 samples of EVOO.

Samples were from six countries of origin: Portugal (27), Spain (24), Italy (12), Argentina (3), Greece (2) and Chile (2), 24 samples being packed in the country of origin, 11 samples packed in Brazil and 35 samples with no information in the label regarding packing.

The 70 samples collected were analyzed for the presence of 13 PAH [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)], stigmasta-3,5-diene (stigmastadiene), sterols content (campesterol, stigmasterol, β-sitosterol, brassicasterol e cholesterol), fatty acid composition and specific extinction.

Standards and Reagents

The following analytical standards were acquired from Cambridge Isotope Laboratories (5MChr), ChemService (DaiP), Fluka (BjF, DalP, DaeP, DahP), Supelco Inc. (BaA, Chr, BbF, BkF, BaP, DahA, IcdP and 37 component FAME mix) and Sigma (5α-cholestan, cholesta-3,5-diene, campesterol, stigmasterol, β-sitosterol, brassicasterol). High-performance liquid chromatography (HPLC)-grade hexane, cyclohexane, isopropanol and N,N-dimetilformamide were from Tedia, acetonitrile from J.T. Baker, methanol from Mallinckrodt and water was obtained from a Millipore Milli-Q purification system. Millex HV filters (0.45 μm) were purchased from Millipore and solid-phase extraction (SPE) columns were from Waters (Sep Pak C18, 500 mg, 3 mL).

Polycyclic Aromatic Hydrocarbons Analysis

The analytical method was the one previously described and validated by Camargo *et al.* [14] and Tfouni *et al.* [12]. PAH determination was performed in duplicate and involved extraction with N,N-dimethylformamide-water (9:1, v/v), clean-up with SPE C18 cartridges and analysis by HPLC with fluorescence detection (HPLC-FLD) using Shimadzu equipment comprised of a quaternary pump, on-line degasser, autosampler (30-µL injection volume), column oven and fluorescence detector. The following conditions were used for chromatographic separation: C18 Vydac 201 TP54 column (250 × 4.6 mm, 5-µm particle size, maintained at 30 °C) and a gradient mobile phase of acetonitrile and water with a flow rate of 1 mL/min. For PAH 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).

Specific Extinction, Fatty Acids and Stigmastadiene

Official analytical methods were used to determine specific extinction [15], fatty acid composition [16–18] and stigmastadiene content [19].

For specific extinction, 0.25 g of EVOO sample was dissolved in cyclohexane and the specific extinction was determined in a spectrophotometer using wavelengths of 232 and 270 nm. For fatty acid composition, 0.1–0.2 g of the sample was transesterified with ammonium chloride and sulfuric acid in methanol and analyzed by gas chromatography with flame ionization detection (GC-FID). For stigmastadiene determination, the EVOO unsaponified matter was obtained from 20 g sample by addition of ethanolic KOH and extraction with hexane. The solution was then washed with ethanol:water (1:1, v/v), dried and suspended in hexane followed by clean up in a silica gel column and analysis by an Agilent gas chromatograph with a flame ionization detector (GC-FID) using an internal standard (cholesta-3,5-diene).

Sterols Content

The analytical method used was the one previously described by Almeida [20] for sterols analyses in olive oil. The method involved saponification with 3% KOH and extraction with hexane. The extract was injected (1 µL) into an Agilent CG-FID system. Hydrogen was used as the carrier gas at a flow rate of 1 mL/min. The injector was operated at 250 °C in the split mode with split ratio of 1:50. Nitrogen was used as make-up at 30 mL/min. The separation was performed on a DB-5 capillary column (30 m, 0.25-mm i.d., df 0.25 µm, Agilent) and the oven temperature program was: 150 °C (held for 1 min), 10 °C/min to 300 °C (held for 10 min). Detector temperature was 300 °C. Samples were analyzed in triplicate and internal standard (5α-cholestane) was used for quantification.

Statistical Analysis

One-way analysis of variance (ANOVA) with means comparison (Tukey test) with 95% confidence was used for data processing (Statistica 5.5, Stat Soft Inc.).

Principal component analysis (PCA) was carried out with software Pirouette (InfoMetrix, Woodinville, WA, USA) version 2.01, with autoscaled data and cross-validation.

Results and Discussion

Polycyclic Aromatic Hydrocarbons in EVOO Samples

In Brazil, there is no regulation regarding levels of PAH in EVOO. Maximum BaP levels are established only for smoke flavorings, drinkable water and olive pomace oil, while European regulation sets limits for some PAH for the category of oils and fats, i.e. 2.0 µg/kg for BaP and 10.0 µg/kg for PAH4 (sum of BaA, Chr, BbF and BaP) [21].

Table 1 presents PAH levels in different brands and lots of EVOO. PAH were detected in 93% of the 70 analyzed samples. Among the 13 PAH evaluated, 11 were detected; Chr and 5MChr were the most representative, being present in 93 and 44% of the samples, respectively, while IcdP was detected in only one sample and BjF and DahP were not detected. Individual levels of PAH ranged from not detected to 22.86 µg/kg (Chr level on brand 27 lot 2).

Summed levels of the 13 PAH in the different EVOO samples evaluated ranged from not detected to 41.10 µg/kg. Results show a high variation in PAH levels among brands and also among different lots of the same brand. Considering the sum of the 13 PAH, 70% of the brands showed significant difference among lots ($p < 0.05$). This variation may be due to (1) different regions of origin (air pollution, and, consequently, the presence of PAH, in the surrounding areas of olive cultivation may vary according to the region of origin), or (2) adulteration by the addition of other vegetable oils contaminated with PAH in different concentrations [8, 22]. A previous study showed that in 42 samples of soybean oil commercialized in Brazil, the summed levels of the 13 PAH varied from 10.4 to 112.0 µg/kg, highlighting the potential for contamination from this source [4].

PAH levels found in the present study are relatively lower than others previously reported. Ergönül and Sánchez [23] analyzed the 16 US Environmental Protection Agency (EPA) priority PAH in 9 EVOO samples from Spain and Turkey and obtained summed levels between 23.25 and 62.55 µg/

Table 1 PAHs levels in different brands and lots of extra virgin olive oil

Sample	PAHs level (μg/kg)[a,b] (batch 1–batch 2)											
	BaA	Chr	5MChr	BbF	BkF	BaP	DalP	DahA	IcdP	DaeP	DaiP	ΣPAHs
01	nd–nd	1.46–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	1.46[a]–nd[a]
02	nd–nd	1.34–0.90	0.38–0.41	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–1.85	1.72[a]–3.16[b]
03	nd–0.43	1.52–1.40	nd–0.92	nd–nd	nd–nd	nd–nd	nd–nd	nd–0.48	nd–nd	nd–nd	nd–1.19	1.52[a]–4.42[b]
04	nd–nd	1.59–0.83	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	1.59[a]–0.83[a]
05	0.33–nd	2.26–1.31	0.41–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	3.00[a]–1.31[b]
06	nd–nd	nd–1.39	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd[a]–1.39[b]
07	nd–1.31	1.50–2.05	nd–1.64	nd–nd	nd–nd	nd–1.48	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	1.50[a]–6.48[b]
08	nd–2.18	0.83–2.42	0.35–0.45	nd–1.62	nd–0.81	nd–1.54	nd–nd	nd–0.39	nd–nd	nd–0.45	nd–nd	1.18[a]–9.86[b]
09	nd–nd	1.34–0.66	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	1.34[a]–0.66[a]
10	nd–0.48	1.76–1.79	0.75–0.99	nd–nd	nd–nd	nd–nd	nd–nd	nd–0.37	nd–nd	nd–nd	nd–1.39	2.51[a]–5.02[b]
11	nd–nd	1.56–nd	0.37–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	1.93[a]–nd[b]
12	nd–nd	0.57–0.64	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	0.57[a]–0.64[a]
13	nd–nd	1.62–1.71	0.42–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	2.04[a]–1.71[b]
14	nd–nd	0.59–1.04	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	0.59[a]–1.04[a]
15	nd–nd	1.11–1.32	nd–0.34	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–1.45	1.11[a]–3.11[b]
16	nd–nd	1.24–0.56	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	1.24[a]–0.56[b]
17	1.66–1.00	3.30–2.67	1.54–0.79	1.29–0.94	0.59–nd	0.62–0.34	nd–0.45	nd–nd	nd–nd	0.32–nd	nd–nd	9.32[a]–6.19[b]
18	2.91–nd	6.78–1.50	2.05–nd	6.87–nd	2.48–nd	5.95–nd	nd–nd	2.05–nd	6.55–nd	3.90–nd	nd–nd	39.54[a]–1.50[b]
19	nd–nd	1.28–1.48	nd–0.89	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	1.28[a]–2.37[a]
20	2.25–1.91	5.01–4.39	0.84–nd	1.59–0.95	0.51–0.37	0.87–0.60	nd–nd	0.33–nd	nd–nd	0.35–0.45	nd–nd	11.75[a]–8.67[b]
21	5.06–0.85	11.27–1.91	2.41–0.60	5.17–0.57	1.66–nd	4.07–nd	nd–nd	0.71–nd	nd–nd	0.74–nd	nd–nd	31.09[a]–3.93[b]
22	nd–nd	1.31–1.69	nd–0.51	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	1.31[a]–2.20[b]
23	nd–nd	1.65–1.74	nd–0.50	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–1.51	1.65[a]–3.75[b]
24	nd–nd	1.45–1.43	0.38–0.66	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	1.83[a]–2.09[a]
25	1.64–nd	3.42–0.89	2.47–nd	1.44–nd	0.34–nd	0.98–nd	nd–nd	0.41–nd	nd–nd	0.30–nd	nd–nd	11.00[a]–0.89[b]
26	1.77–2.81	3.76–5.41	1.18–2.04	1.08–3.20	nd–1.06	0.79–3.57	nd–nd	nd–0.73	nd–nd	nd–0.42	nd–nd	8.58[a]–19.24[b]
27	0.47–9.54	2.46–22.86	nd–6.29	nd–1.77	nd–nd	nd–0.64	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	2.93[a]–41.10[b]
28	nd–nd	nd–1.19	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nda–1.19[a]
29	nd–nd	1.32–0.91	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	1.32[a]–0.91[a]
30	2.92–2.75	6.57–7.93	0.93–3.47	2.86–3.45	0.86–0.68	2.51–2.39	nd–0.43	0.52–nd	nd–nd	0.35–nd	nd–nd	17.52[a]–21.10[b]
31	nd	0.80–0.83	nd–0.31	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	0.80[a]–1.14[a]
32	nd–nd	nd–0.95	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd[a]–0.95[b]
33	nd–nd	0.56–0.77	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	nd–nd	0.56[a]–0.77[b]
34[c]	nd	1.61	nd	nd	nd	nd	nd	nd	nd	nd	nd	1.61

Table 1 (continued)

Sample	PAHs level (μg/kg)[a,b] (batch 1–batch 2)												
	BaA	Chr	5MChr	BbF	BkF	BaP	DaIP	DahA	IcdP	DaeP	DaiP	ΣPAHs
35[c]	nd	0.93	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.93
36[c]	3.54	10.60	1.79	2.53	0.72	1.54	nd	0.75	nd	nd	nd	21.47
37[c]	nd	0.78	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.78

[a]Mean of duplicate

[b]BjF and DahP were not detected

[c]Only one batch

nd not detected: <LOD = 0.16 μg/kg (BaA, BbF), 0.07 μg/kg (Chr, 5MChr, BaP), 0.52 μg/kg (BjF), 0.03 μg/kg (BkF, DahP), 0.04 μg/kg (DalP), 0.19 μg/kg (IcdP), 0.10 μg/kg (DaeP), 0.06 μg/kg (DaiP), 0.02 μg/kg (DahP)

Values of ΣPAH in the same line with the same letter are not statistically different ($p < 0.05$)

kg. Krajian and Odeh [24] evaluated 7 EVOO samples from Syria for the same 16 PAH and the summed results varied from 7.55 to 159 μg/kg. Alomirah *et al*. [25] reported the summed 16 EPA PAH values ranging from 1.09 to 181.22 μg/kg for 21 EVOO samples from different origins. The highest concentrations were of naphthalene, acenaphtalene and benzo[g,h,i]perylene, PAH not present in JECFA's list of carcinogenic and genotoxic PAH, and, therefore, not analyzed in the present study.

According to Table 1, BaP was detected in 15 of the 70 samples analyzed with levels up to 5.95 μg/kg. Considering the limits set by the Brazilian regulation for olive pomace oil and the European regulation for fats and oils, five samples showed BaP concentration above acceptable levels (2.0 μg/kg). The levels of PAH4 are shown in Fig. 1 and ranged from not detected to 34.81 μg/kg. Of the 70 EVOO samples, 10% presented PAH4 levels above the limit set by European legislation (10.0 μg/kg), and among these, five samples also showed BaP concentrations above permitted levels. Samples with levels above the limit were from three different origins: Portugal, Spain and Argentina. In 2014, Krajian and Odeh [24] reported PAH4 levels below the limit (0.56 to 6.94 μg/kg) for seven Syrian EVOO samples, contrasting with the findings in the present study.

EVOO Samples Adulteration

Results obtained for fatty acid composition, specific extinction, stigmastadiene levels and sterols content are presented in Table 2.

As a result of fatty acid analysis, 15 fatty acids were detected. For all samples, C16:0, C17:0, C17:1, C18:0 and C24:0 were in accordance with the acceptable limits set by IOC and Brazilian regulation, while some samples were discordant for the following fatty acids: C14:0, C16:1, C18:1, C18:2, C18:3, C20:1, C22:0 and for *trans* isomers C18:1*T* and C18:2*T* + C18:3*T*. Out of the 70 samples evaluated, 21% presented levels of C16:1 and C18:1 below established ones, and 21% presented levels above maximum limits for C14:0, C18:2, C18:3, C20:1, C22:0 and *trans* isomers C18:1*T* and C18:2*T* + C18:3*T*. Overall, 18% of the samples presented levels that do not agree with the current regulations.

Each vegetable oil presents a very specific fatty acid composition which facilitates identifying possible adulteration. Table 2 presents the levels of the most important fatty acids used to verify EVOO adulteration by addition of vegetable oil from other sources. According to IOC and Brazilian regulation for olive oils [11, 13], 19% of the samples presented C18:1ω9 and C18:2ω6 levels above the ones tolerated, which indicates possible adulteration by addition of other vegetable oils. Among these samples, both lots of six brands presented both fatty acid levels above the limit. Additionally, the presence of *trans* fatty

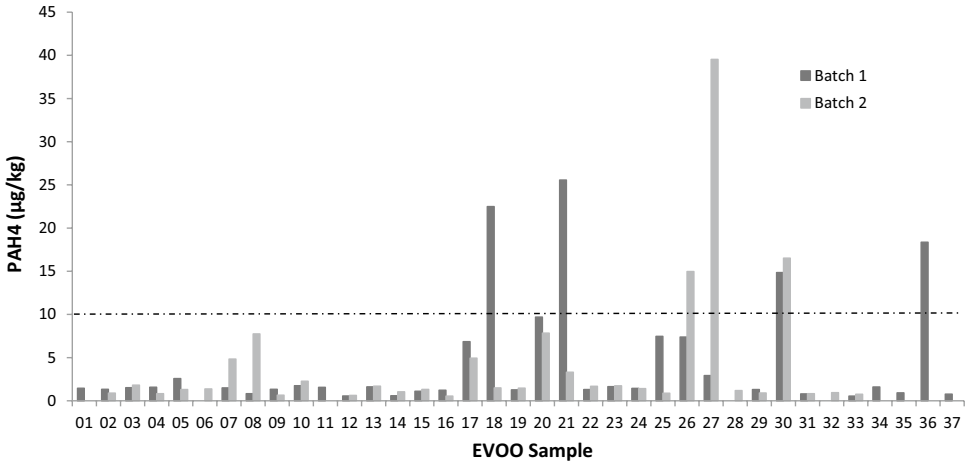

Fig. 1 Levels of PAH4 in different brands and batches of EVOO

Table 2 Parameters analyzed for EVOO adulteration ($n = 70$)

Parameter	Range detected	Tolerance[a]	% of non–compliance
Fatty acids (g/100 g)			
C18:1ω9 (oleic acid)	26.13–76.50	55.0–83.0	19
C18:2ω6 (linoleic acid)	4.3–46.81	3.5–21.0	19
C18:1ω9*t* (*trans*-oleic acid)	nd–0.1	≤0.05	1
C18:2ω6*t* + C18:3*t* (*trans*-linoleic + *trans* linolenic)	nd–1.61	≤0.05	19
Stigmastadiene (mg/kg)	nd–59.72	≤0.15	29
Specific extintion			
K232	1.76–6.89	≤2.50	32
K270	0.14–3.93	≤0.22	42
ΔK	0.00–0.61	≤0.01	29
Sterols (mg/kg)			
Campesterol	28.4–340.7	–	–
Stigmasterol	29.9–592.2	–	–
β-sitosterol	1010.1–2682.1	–	–
Σ sterols	1074.7–2836.0	1000–1600[b]	31

[a]Brasil (2012) [9]

[b]Sum of five sterols

acids indicates that the oil underwent a refining process. This occurred with 13 samples, with 5 brands exceeding the limit in both lots.

Specific extinction is an important tool to evaluate the oxidative state of olive oils. High values for K270 indicate the presence of compounds originated from oxidation or from oil refining. Of the 70 samples analyzed, 21% were above the values accepted for the three parameters (K232, K270 and ΔK). These results show possible alteration in the oxidative state of these samples, suggesting that the quality of the samples is compromised.

Stigmastadiene is not naturally present in EVOO. This compound is formed during refining of vegetable oils due to high temperatures used in the process; therefore, its occurrence in the final product is related to the addition of refined oil. The levels of stigmastadiene found in this study suggest that 20 samples had some type of refined oil added, indicating adulteration of these EVOO samples.

Sterols are naturally present in olive oils. Each vegetable or olive oil has a specific sterols composition; consequently, this analysis has been used to identify possible adulterations in olive oils. According to IOC and Brazilian regulation, olive oil must present a total sterols levels between 1000 and 1600 mg/kg. Of the 70 samples analyzed, 21 presented levels of sterols (sum of campesterol, stigmasterol and β-sitosterol) over 1600 mg/kg, and one presented a level below 1000 mg/kg. Therefore, 31% of the samples are not considered to be olive oil according to this item of the regulation, indicating that they may be adulterated with vegetable oils from other origins. Sterols brassicasterol and cholesterol were not detected in any sample, which is in accordance with Brazilian regulation for VOO and EVOO where it is stated that maximum limits are 0.1 and 0.5%, respectively.

The results shown in Table 2 suggest that 19 of the 70 samples analyzed were adulterated, since they presented results not consistent with what is expected for an EVOO sample. Among these, 14 samples showed almost all parameters altered. The inconsistent levels of sterols and fatty acids (especially C18:1ω9, C18:2ω6 and C18:2ω6*T*) suggest the presence of vegetable oil from a different origin rather than olives, while the detection of stigmastadiene indicates the presence of refined oil. So these 14 samples can be considered to be adulterated by the addition of a vegetable oil of a different origin, probably soybean oil. In the remaining five samples, the detection of stigmastadiene indicates the presence of refined oil, and the unaltered fatty acid composition indicates a possible absence of vegetable oil from another source; therefore, these samples may be

considered adulterated by the addition of refined oil. Thus, Table 2 results allow the separation of the samples into three groups: EVOO samples adulterated by addition of vegetable oil from another origin, samples adulterated by addition of refined oil, and samples possibly not adulterated.

The 19 adulterated samples were from 12 different brands and 3 countries (Portugal, Spain and Argentina). Seven of these brands presented with both lots tampered; three samples presented only one lot adulterated and for two brands, a second lot was not found for purchase and analysis. Additionally, according to the label, among the adulterated samples, 10 were packed in Brazil, one was packed in the country of origin (Spain) and the remaining 8 did not provide this information on the label. For samples packed in Brazil, the adulteration may occur during packaging of the imported product for retail, usually by addition of soybean oil [9, 10, 12].

To better illustrate the correlation between all the variables studied, a multivariate analysis was performed applying the technique of PCA using Pirouette software. A matrix of data composed by the 70 EVOO samples and 11 variables analyzed (levels of stigmastadiene, campesterol, stigmasterol, β-sitosterol, Σ sterols, K232, K270, ΔK, C18:1ω9, C18:2ω6 and C18:2ω6*T*; 70 × 11 matrix) was used. During the process, data was autoscaled and a cross-validation technique was used.

Figure 2 shows PCA score plot with the first principal component (PC1) versus PC2, which describes 71.4 and 11.2% of the total variance, respectively. PCA allowed distinguishing samples into three sets (A, B and C). Samples from A are related mainly with the variables stigmastadiene, stigmasterol, campesterol, Σ sterols, K232, K270, ΔK, C18:2ω6 and C18:2ω6*T*, which showed positive influence in these samples; samples from B are shown to be related mainly with stigmastadiene, which indicates the presence of refined oil; and samples from C are positively influenced by β-sitosterol and C18:1ω9.

The projection of the samples onto a plane defined by PC1 x PC2 presented in Fig. 2 corroborates the previous discussion, with A, B and C being formed, respectively, by EVOO samples adulterated by addition of vegetable oil from another origin, samples adulterated by addition of refined oil, and samples possibly not adulterated.

Of a total of 70 EVOO samples, 20% were in set A (addition of vegetable oil from other origin), 7% were in set B and 73% in set C. A previous study by Aued-Pimentel [9] analyzed olive oil samples collected in Brazil between the years of 1993 and 2000 and observed that 16.5% were adulterated with the addition of a vegetable oil with lower commercial value. As it may be observed, there has been a slight increase in adulteration frequency. This can be considered a cause of concern, as apparently no action has been taken to inhibit the use of this practice in this period (2002–present).

PAH and Adulteration of EVOO Samples

In order to evaluate if there is a relationship between PAH levels and sample adulteration, the results obtained (Table 1) were analyzed in combination with the evaluation of sample adulteration.

Among the 20 samples with higher levels of summed PAH (>3.00 μg/kg), 13 presented parameters indicating adulteration by addition of vegetable oil from another origin and one presented parameters indicating adulteration by addition of refined oil. Therefore, 73% of the samples considered adulterated were among the ones with higher PAH levels. Results also show that out of the 14 samples included in set A (EVOO samples adulterated by addition of vegetable oil from another origin), 13 were among the ones with higher PAH levels. These results demonstrate that, in general, samples classified as adulterated present higher PAH levels, contamination which may result from the addition of a contaminated vegetable oil. However, presenting a high level of these compounds does not necessarily mean that the sample is adulterated, as observed in samples 07 B2, 08 B2 and 10 B2. In these cases, contamination might come from environmental pollution.

To evaluate the correlation between all variables studied, PCA was performed with a matrix of data composed by the 70 EVOO samples and the 12 variables analyzed (all variables used before plus ΣPAH). Figure 2 presents the graphical representation of the principal components originated from the data. PC1 describes 68.1% of the total variance in the data, while PC2 describes 10.0%. PCA allowed distinguishing samples into three sets (A, B and C).

The inclusion of the variable ΣPAH did not modify the projection of the samples (Fig. 2). The variable ΣPAH was shown to be related mainly to the samples of EVOO tampered with low-grade vegetable oil, with the relation among other variables and samples remaining the same.

Results obtained in the present study suggest that PAH could be an additional variable that indicates the presence of vegetable oils from another source. Although samples classified as adulterated present a higher PAH level, the presence of these compounds does not necessarily mean that the sample is adulterated. In these cases, contamination is probably a result of deposition due to environmental pollution Therefore, PAH level may indicate a possible adulteration; nevertheless the use of a wider group of analysis is still necessary to establish the purity of an EVOO sample.

Conclusion

There are not many studies in the literature regarding the presence of PAH in EVOO, especially with samples commercialized in Brazil. Furthermore, in these cases, the

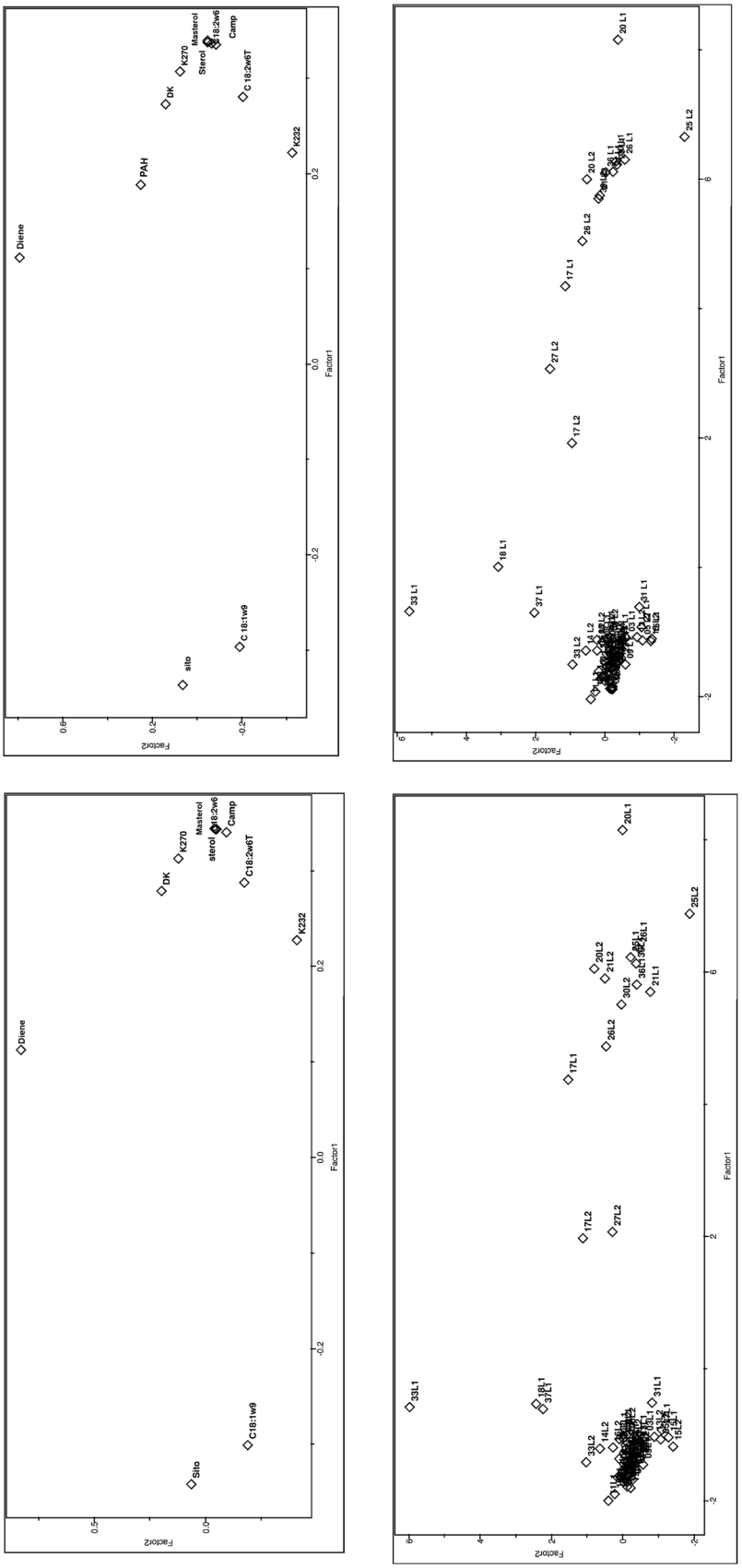

Fig. 2 Principal component analysis: loadings of original variables (above) and scores of the samples (below) obtained for EVOO adulteration evaluation (left) and for PAH and EVOO adulteration evaluation (right). Diene = stigmastadiene, Camp = campesterol, Masterol = stigmasterol, Sito = β-sitosterol, Sterol = Σ sterols, DK = ΔK, PAH = ΣPAH

presence of these compounds is commonly explained as related to environmental contamination or to the addition of refined olive oil or pomace oil. In the present study, it was shown that it can also be related to adulteration and the presence of vegetable oil from other sources rather than olives.

As 10 of the 19 samples considered adulterated were imported and packed in Brazil, there is a possibility that, in these cases, the adulteration occurred during packaging.

Results presented show a need for a better quality control of EVOO commercialized and offered to the population, in order to assure the authenticity of these products, since 27% of the samples analyzed were considered adulterated. It is expected that the Brazilian Regulation set in 2012 contributes to preventing adulteration in this class of products. Furthermore, this type of adulteration may lead to EVOO contamination by carcinogenic and genotoxic compounds, which contrasts with EVOO health benefits.

Acknowledgements This work was supported by the Fundação de Amparo à Pesquisa do Estado de São Paulo (Fapesp) under grant 2011/10966-4. N.P.L.A (Instituto de Química, UNICAMP, Campinas, SP, Brazil) and R.M.R. (Faculdade Jaguariúna, FAJ, Jaguariúna, SP, Brazil) thank CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico), Brazil for the PIBIC/CNPq program undergraduate student fellowships.

References

1. EFSA-European Food Safety Authority (2008) Polycyclic aromatic hydrocarbons in food, Scientific opinion of the panel on contaminants in the food chain. EFSA J 724:1–114
2. Rey-Salgueiro L, Martínez-Carballo E, Sonia García-Falcón M, González-Barreiro C, Simal-Gándara J (2009) Occurrence of polycyclic aromatic hydrocarbons and their hydroxylated metabolites in infant foods. Food Chem 115:814–819
3. Rey-Salgueiro L, Martínez-Carballo E, Sonia García-Falcón M, Simal-Gándara J (2009) Survey of polycyclic aromatic hydrocarbons in canned bivalves and investigation of their potential source. Food Res Int 42:983–988
4. Camargo MCR, Antoniolli PR, Vicente E, Tfouni SAV (2011) Polycyclic aromatic hydrocarbons in Brazilian commercial soybean oils and dietary exposure. Food Addit Contam Part B 4:152–159
5. Camargo MCR, Antoniolli PR, Vicente E (2012) Evaluation of polycyclic aromatic hydrocarbons content in different stages of soybean oils processing. Food Chem 135:937–942
6. IARC (2012). Monographs on the evaluation of carcinogenic risk to humans. In: a review of human carcinogens: chemical agents and related occupations (Vol. 100F). Lyon, France: IARC
7. WHO-World Health Organization. (2005) Summary and conclusions of the sixty-fourth meeting of the Joint FAO/WHO Expert Committee on Food Additives. Rome, p 47
8. Rodríguez-Acuña R, Pérez-Camino MC, Cert A, Moreda W (2008) Polycyclic aromatic hydrocarbons in Spanish olive oils: relationship between benzo(a)pyrene and total polycyclic aromatic hydrocarbon content. J Agric Food Chem 56:10428–10432
9. Aued-Pimentel S, Takemoto E, Minazzi-Rodrigues RS, Badoloto ESG (2002) Azeite de oliva: incidência de adulterações entre os anos de 1993 e 2000. Rev Inst Adolf Lutz 61:69–75
10. Aued-Pimentel S, Takemoto E, Kumagai EE, Cano CB (2008) Determinação de diferença entre o valor real e o teórico do triglicerídeo ECN 42 para a detecção de adulteração em azeites de oliva comercializados no Brasil. Quím Nova 31:31–34
11. IOC-International Olive Council (2016). Trade standard applying to olive oils and olive pomace oils. COI/T.15/NC No3/Rev. 11
12. Tfouni SAV, Padovani GR, Reis RM, Furlani RPZ, Camargo MCR (2014) Incidence of polycyclic aromatic hydrocarbons in vegetable oil blends. Food Control 46:539–543
13. Brasil. Ministério da Agricultura, Pecuária e Abastecimento – MAPA. Instrução Normativa 1, de 30 de janeiro de 2012. Regulamento Técnico do Azeite de Oliva e do Óleo de Bagaço de Oliva. Diário Oficial da União, Brasília, DF, 01 de fevereiro de 2012
14. Camargo MCR, Antoniolli PR, Vicente E (2011) HPLC-FLD simultaneous determination of 13 polycyclic aromatic hydrocarbons: validation of analytical procedure for soybean oils. J Braz Chem Soc 22:1354–1361
15. AOCS. Official Methods and Recommended Practices of the AOCS (2014) 6th Ed., Urbana, IL, USA, Official Method ch 5-91
16. AOCS. Official Methods and Recommended Practices of the AOCS (2014) 6th Ed., Urbana, IL, USA, Official Method ce 1a-13
17. AOAC International (2010) Official methods of analysis of AOAC international (2010) 18th Ed., Gaithersburg, MD, USA, Official Method 996.06
18. Hartman L, Lago RCA (1973) Rapid preparation of fatty acid methyl esters from lipids. Lab Pract 22(8):475–481
19. AOCS. Official Methods and Recommended Practices of the AOCS (2014) 6th Ed., Urbana, IL, USA, Official Method cd 26–96
20. Almeida CAS (2009) Avaliação dos principais fitosteróis em óleos vegetais e azeites. Faculdade de Engenharia de Alimentos. Universidade Estadual de Campinas, Campinas
21. CEC-The Commission of the European Communities (2011) Commission Regulation (EC) No 835/2011 of 19 August 2011. Official Journal of European Union
22. Camargo MCR, Toledo MCF (2003) Polycyclic aromatic hydrocarbons in Brazilian vegetables and fruits. Food Control 14:49–53
23. Ergönül PG, Sánchez S (2013) Evaluation of polycyclic aromatic hydrocarbons content in different types of olive and olive pomace oils produced in Turkey and Spain. Eur J Lipid Sci Technol 115:1078–1084
24. Krajian H, Odeh A (2014) Polycyclic aromatic hydrocarbons in Syrian olive oils and their likely daily intakes. Toxicol Environ Chem 96:342–352
25. Alomirah H, Al-Zenki S, Husain A, Sawaya W, Ahmed N, Gevao B, Kannan K (2010) Benzo[a]pyrene and total polycyclic aromatic hydrocarbons (PAH) levels in vegetable oils and fats do not reflect the occurrence of the eight genotoxic PAH. Food Addit Contam Part A 27:869–878