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Benzo(a)pyrene in olive oils on the Brazilian market

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Benzo(a)pyrene (B(a)P), the best known of the carcinogenic PAHs, has been found at variable concentrations in several foods. In this study 40 samples of various olive oils available in Brazil were analysed for B(a)P. The analytical method involved extraction by liquid–liquid partition, clean-up on silica gel and analysis by high performance liquid chromatography with a fluorescence detector. Benzo(a)pyrene was found in almost all samples, at levels up to 164 μg/kg. The lowest levels of B(a)P were detected in olive oils imported from the major European countries. Olive oils imported from Europe but packed in Brazil and olive oil blended with soybean and corn oils showed relatively higher levels of B(a)P, ranging from 0.9 to 9.7 μg/kg and from 2.2 to 9.2 μg/kg, respectively.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAH) are a group of compounds that have been the subject of much concern in recent years due to their toxic potential. They are known as highly stable contaminants present in many foods (Speer *et al.*, 1990). This contamination can be a result of sorption from a contaminated environment or from food preparation methods. In the specific case of vegetable oils, it has been suggested that the main sources of contamination are: (a) contamination of plant material, primarily through the air; (b) drying the plant material with smoke before extraction; and (c) contamination through the extraction solvent (Welling & Kaandorp, 1986; Larsson *et al.*, 1987).

The determination of B(a)P and other PAH in vegetable oils has been accomplished in Europe and the USA (Howard *et al.*, 1966; Ciusa *et al.*, 1970; Swallow, 1976; Joe *et al.*, 1979; Speer & Montag, 1988; Speer *et al.*, 1990; Menichini *et al.*, 1991; Gertz & Kogelheide, 1994). In general the detected levels of B(a)P in the olive oils analysed were in the range of a few μg/kg, although higher levels up to 60 μg/kg have been cited by Morgante (1973).

Remarkable amounts of 'light' PAHs (3 – 4 aromatic rings) have been found in olive oils. Gertz & Kogelheide (1994) for example, detected 54.9 μg/kg of 'light' PAHs in a sample of olive oil, while the sum of 'heavy' PAHs (5 or more aromatic rings) determined was 0.2 μg/kg. Compared to sunflower oil, lesser amounts of 'light' PAHs (15.9 μg/kg) have been detected by the same authors. The relatively high levels of 'light' PAHs found in olive oils can be attributed to the fact that cold-pressed native vegetable oils are, by definition, unrefined and untreated and therefore, alkali, bleaching and deodorization treatments are not allowed (Speer *et al.*, 1990).

Polyaromatic hydrocarbons have been removed from vegetable oils during the refining process by subsequent treatment with active carbon or by steam-distillation processes. Biernoth & Rost (1967) used these treatments on samples of crude coconut oil and observed a decrease of total PAHs from 1923 to 13 μg/kg.

At the moment there is no maximum level for B(a)P or any other PAH in oils in Brazil and the same seems to occur for many countries. The only references are from the German food industries that recommend their own limits for refined fats and oils: the content of 'light' PAHs (phenanthrene, anthracene, pyrene, benz(a)-anthracene, chrysene, triphenylene, pyrene, fluoranthene) should not exceed 25 μg/kg, and the sum of 'heavy' PAHs (benzo(a)pyrene, benzo(e)pyrene, benzo(a)-fluoranthene, perylene, indeno(1,2,3-cd)pyrene, dibenzo-(ah,ac)anthracene, benzo(bjk)fluoranthene, benzo(ghi)-perylene) should be below 5 μg/kg (Speer *et al.*, 1990).

The goal of this work was to determine the level of benzo(a)pyrene in olive oils available on the Brazilian market and to estimate the potential dietary intake of this contaminant derived from the consumption of olive oils in Brazil.

MATERIALS AND METHODS

Samples

All commercial olive oils samples were purchased from retail outlets in the metropolitan area of Campinas, SP. The samples were collected during different periods of the year.

Recovery studies

Blank controls (without the oil) were analysed for the presence of B(a)P and no interference peaks were observed. In order to verify the method validation, fortifications were made during the analysis by spiking the oils with B(a)P at differents levels in the extraction step.

Extraction

Extraction and clean-up procedures were conducted according to Speer *et al.* (1990), as described below.

Ten grams of olive oil were weighed in a beaker (fortifications were done at this stage). After adding 50 ml cyclohexane the material was transfered into a 250-ml separating funnel, and the flask rinsed with a total of 50 ml cyclohexane. The cyclohexane was extracted with portions of 50, 25 and 25 ml dimethylformamide–water 9:1 (v/v), then the extract was diluted with 100 ml of a 1% sodium sulfate solution and re-extracted with portions of 50, 35 and 35 ml cyclohexane. This solution was washed twice with 40 ml distilled water, dried with anydrous sodium sulfate (~3.0 g) and concentrated on a rotary evaporator to 2 ml at 40°C.

Clean-up

Five grams of Silica gel (E. Merck) for adsorption chromatography (with a content of 12% water) were packed into a 200×10 mm i.d. glass column. The prepared extract (2 ml) on the column was eluted with 85 ml of cyclohexane. The first 10 ml was discarded, and the pooled 10–85 ml fractions concentrated to about 1 ml, and dried under a gentle flow of nitrogen. The residue was dissolved in 2 ml acetonitrile.

High performance liquid chromatography

The HPLC system consisted of a Waters 6000 A pump, injector Valco AH 60, Varian Fluorichrom II fluorescence detector (excitation filter 220 I, emission filter 4-76). A 10-μl loop was used for injection. The mobile phase was a mixture of acetonitrile : water (70:30, v/v). The column used for the analysis was a C_{18} Spheri 5 (5 μm, 25 cm × 4.6 mm i.d., Applied Biosystem) with a pre-column of the same type. The temperature was kept constant at 32°C using a Waters oven model 1122.

The peak of benzo(a)pyrene in the samples was identified by comparing the retention time with that of the standard and quantified by comparing the integrated peak areas with that of an external standard (purchased from Sigma). Peak identity was confirmed by a Waters 991 photo diode array detector, under the same conditions as described above.

Table 1. Recovery of benzo(a)pyrene in olive oils

Fortification (μg/kg)	Recovery (%)
0.53	77.4
1.05	93.3
2.1	115.7
2.1	114.3
2.1	106.8
2.1	97.8
5.25	76.7
5.25	91.7
5.25	76.1
5.25	89.3
5.25	84.4
5.25	79.3
5.25	81.8
5.25	82.7

$\bar{x}$ = 90.5 ; standard deviation = 13.6 ; variation coefficient = 15.0%.

RESULTS AND DISCUSSION

Table 1 shows the recoveries achieved by the analytical methodology. An average recovery of 90.5% was obtained, ranging from 77.4 to 115.7%, with a variation

Table 2. Benzo(a)pyrene levels (μg/kg) in olive oil from different origins

Brand	Samples			
	a	b	c	$\bar{x}$
1	0.6	n.d.	n.d.	0.2
2	n.d.	1.2	—	0.6
3	n.d.	n.d.	—	
4	7.1	1.5	2.7	3.8
5	n.d.	n.d.	n.d.	
6	1.1	0.9	0.9	1.0
7	9.7	3.8	2.3	5.3
8	4.3	n.d.	—	2.2
9	n.d.	—	—	
10	1.8	164.4	154.7	107
11	18.0	10.3	—	14.2
12	n.q.	n.q.	—	
13	n.d.	0.5	—	0.2
14	n.d.	n.d.	—	
15	2.3	2.4	3.4	2.8
16	2.2	2.3	—	2.3
17	9.2	9.1	—	9.2
				$\bar{x}$ = 10.9

Values are averages of two determinations; n.d.: not detected (< 0.5 μg/kg); n.q.: not quantified (coeluted peak); — : not available; 1–3, olive oils imported from Europe; 4–9, olive oils imported from Europe but packed in Brazil; 10–14, olive oils imported from Argentine; 15–17, olive oils blended with corn or soybean oils.

Table 3. Levels of benzo(a)pyrene (μg/kg) in different olive oils

Olive oil	*n*	Minimum value	Median	Maximum value
European packed at the origin	7	0.6	0.6	1.2
European packed in Brazil	15	0.9	2.3	9.7
Argentine	10	0.5	10.3	164.4
Blended with vegetable oils	7	2.2	2.3	9.2

n: number of samples.

coefficient of 15.0%. No background for B(a)P was observed in the method when using a blank (no sample matrix).

The limit of detection was 0.5 μg/kg, below the referencel limit of 1 μg/kg set by some European countries for the presence of B(a)P in smoked meat (Larsson *et al.*, 1987; Salagoity *et al.*, 1990). A detection limit ranging from 0.02 to 0.5 μg/kg has been cited in the literature (Swallow, 1976; Hopia *et al.*, 1986; Speer & Montag, 1988; Speer *et al.*, 1990).

Table 2 shows the levels of B(a)P determined in the olive oils. As can be observed, variable levels of B(a)P were found in the samples (n.d. to 164 μg/kg). This range of figures can be atributed mainly to the origin of the olive oils and their composition. Olive oils imported from Europe, packed at the origin of production (samples 1–3) showed levels of B(a)P (n.d. to 1.2 μg/kg) similar to those cited in the literature (0.2–2.2 μg/kg) (Howard *et al.*, 1966; Swallow, 1976; Joe *et al.*, 1979; Hopia *et al.*, 1986; Welling & Kaandorp, 1986; Stijve & Hischenhuber, 1987; Speer & Montag, 1988; Speer *et al.*, 1990; Menichini *et al.*, 1991; Gertz & Kogelheide, 1994). On the other hand, olive oils imported from Europe but packed in Brazil (samples 4–9) showed a wide range of contamination by B(a)P (n.d. to 9.7 μg/kg). These relativly higher levels can be atributed to a possible blend of the olive oil with vegetable oils (such as corn or soybean oil) previously contaminated with B(a)P and other PAHs. Adulteration of olive oils with soybean, palm and coconut oil has been detected in Brazil by Soares & Amaya (1981).

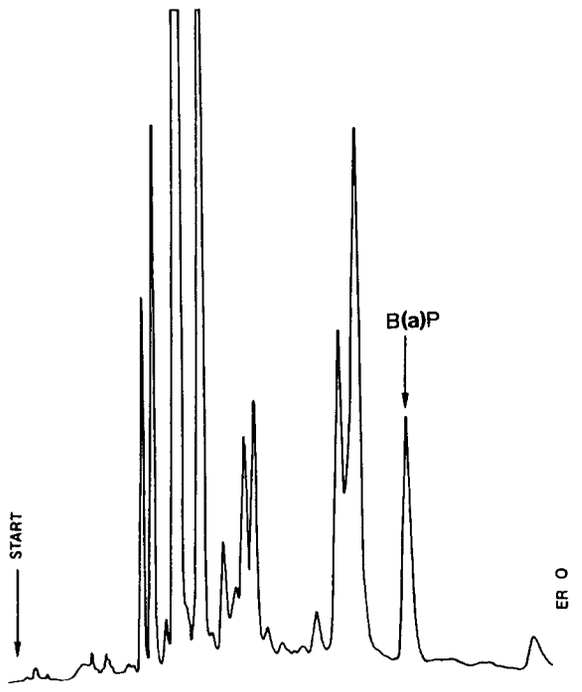

Fig. 1. HPLC chromatogram of olive oil from Argentine. Conditions: mobile phase acetonitrile : water (7:3, v/v), flow 1 ml/min, fluorescence detector, column C18, temperature of the column 32°C. B(a)P, benzo(a)pyrene.

Olive oils imported from Argentine (samples 10–14) also showed variable levels of contamination by B(a)P (n.d. to 164.4 μg/kg). Levels up to 60 μg/kg of B(a)P have been found in virgin Italian olive oils from plants exposed to industrial emissions of pitch condensate (Corradetti *et al.*, 1988). The sample number 12 was the only one which showed a coeluted peak with benzo(a)-pyrene; therefore, quantification was not possible, even after changing the chromatographic conditions.

Olive oils blended with soybean (samples 14 and 15) and corn oils (sample 17) showed B(a)P levels varying between 2.2 and 9.2 μg/kg. These relatively higher levels of contamination could be attributed to a previous contamination of the soybean and corn oil with B(a)P and other PAHs.

Table 3 presents a summary of the results obtained. The data show that the levels of B(a)P in the olive oils packed in Brazil are very similar to those found in olive oils blended with corn and soybean oils, suggesting its contamination with vegetable oils, as mentioned before.

A typical chromatogram of the samples analysed is shown in Fig. 1. In the beginning of the chromatogram various peaks corresponding to 'light' PAHs can be observed. The presence of these compounds can be explained by the fact that this kind of oil is not refined, and therefore the steps of clarification and deodourization that usually eliminate the 'light' PAHs do not exist. High levels of fenanthrene, anthracene, benzo(a)-anthracene, pyrene and fluoranthene have been detected in olive oils (Ciusa *et al.*, 1970; Mariani & Fedeli, 1984; Speer *et al.*, 1990; Menichini *et al.*, 1991).

In Brazil the yearly *per capita* consumption of olive oils is around 100 ml (Gerlach, 1992), much lower than the consumption in Italy, for example (9.4 kg, Menichini *et al.*, 1991). The relatively low Brazilian consumption of olive oils can be partially attributed to the fact that most of the consumers cannot afford to include this product in their diet due the economical restrictions. Only a small part of the population is responsible for most of the olive oil consumed in Brazil, thus increasing the *per capita* intake. Based on these considerations, two approaches can be made: (a) the

annual consumption is 100 ml *per capita* (based on all population); and (b) the consumption is 2000 ml *per capita per year* (average value estimated for individuals who consume olive oil regularly in the meal). Considering a median level of 2.4 μg/kg of B(a)P in the olive oils, as determined in this work, yearly consumptions of 0.22 μg and 4.32 μg would correspond to situations (a) and (b), respectively. In countries like the Netherlands (De Vos *et al.*, 1990) and England (Dennis *et al.*, 1983) daily intakes of B(a)P from the total diet have been estimated at levels of 0.12–0.29 and 0.25 μg B(a)P, respectively, which correspond to yearly intakes of 43.56–72.6 and 90.7 μg of B(a)P, respectively, much higher than the ones estimated in Brazil.

Based on the data obtained and in face of the present dietary habit of Brazilians, olive oils do not seem to be an important source of polycyclic aromatic hydrocarbons in the diet. Nevertheless, considering the high levels of contamination detected in some brands of the olive oils analysed, it is recommended that a fiscalization program should be initiated in order to avoid the exposure of Brazilian consumers to excessive amounts of polyaromatic hydrocarbons in their diet.

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