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Determination of polycyclic aromatic hydrocarbons in cachaça by HPLC with fluorescence detection

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Abstract

Cachaça is a distilled alcoholic beverage obtained from sugar cane fermentation. In this study, 25 brands of cachaça commercially available in Brazil were analysed for the presence of 5 PAHs (benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]-pyrene and dibenz[*a*,*h*]anthracene). The methodology involved liquid–liquid extraction with cyclohexane and dimethyilformamide–water (9:1, v/v), clean up on silica gel column and determination by high performance liquid chromatography using fluorescence detection. PAHs peak identity was confirmed by gas chromatography–mass spectrometry (GC–MS). Variable levels of summed PAHs were detected in the analysed samples, ranging from not detected to 1.94 μg/L. The results confirm the presence of PAHs in cachaça and suggest that contaminated sugar cane may be the source of PAHs in sugar cane spirits.

Keywords: Polycyclic aromatic hydrocarbon; PAH; Sugar cane spirits; Cachaça; HPLC; GC–MS

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) constitute a large class of organic compounds containing two or more fused aromatic rings. During incomplete combustion or pyrolysis of organic matter, hundreds of these substances may be released in the environment. PAHs have been found as contaminants in different food categories such as dairy products, vegetables, fruits, oils, coffee, tea, cereals and smoked meat (Camargo & Toledo, 2002, 2003; De Vos, Van Dokkum, Schouten, & Jong-Berkhout, 1990; Kazerouni, Sinha, Hsu, Greenberg, & Rothman, 2001; Kruijf, Schouten, & Van Der Stegen, 1987; Lin, Tu, & Zhu, 2005; Simko, 2002). Their presence in food originate mainly from processing and cooking (smoking, roasting, baking, frying). Foods can also be contaminated by environmental PAHs that are present in the air (by deposition), soil (by transfer) and water (by deposition and transfer) (WHO, 1998, 2005).

PAHs have attracted most attention because of their carcinogenic potential. During its 64th meeting the Joint FAO/WHO Expert Committee on Food Additives (JECFA) reviewed all information relevant to the toxicology, epidemiology, intake assessment, analytical methodology, formation, fate and occurrence of PAHs in food. Overall, the Committee concluded that 13 PAHs were clearly carcinogenic and genotoxic, including the five selected for this study: benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene and dibenz[*a*,*h*]anthracene (WHO, 2005).

Cachaça is the denomination of a typical Brazilian distilled alcoholic beverage produced from fermented sugar cane juice (Cardoso et al., 2004). In the year 2002,

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1.3×10^9 L of cachaça were produced, of which 14.8 million litres were exported. This spirit is the second alcoholic beverage most consumed in the country and the third most consumed in the world. There are almost 30,000 cachaça producers in Brazil and over 5000 cachaça brands available in the market (PBDAC, 2005).

At harvesting season, most sugar cane plantation is burnt in order to facilitate manual harvest and to protect rural workers from sharp leaves, insects and poisonous snakes (Godoi et al., 2004; Zamperlini, Santiago-Silva, & Vilegas, 2000). Studies conducted in Brazil have shown that sugar cane burning is an important source of PAHs emission and could be responsible for the presence of these contaminants in burned sugar cane and its by-products (Azevedo, Santos, & Neto, 2002; Godoi et al., 2004; Santos, Azevedo, & Neto, 2002; Serra, Pupin, & Toledo, 1995). Recent studies reported that, during harvesting season rural workers responsible for sugar cane manual harvest excrete PAHs through the urine at levels nine times higher than a control group (Bosso, 2004).

The Brazilian regulation regarding maximum levels (ML) of PAHs in food refers only to benzo[*a*]pyrene in liquid smoke (ML = 0.03 μg/kg of the final product) and drinkable water (ML = 0.01 μg/L) (Brasil, 1990). The European Union has recently established maximum level of benzo[*a*]pyrene in different foodstuff such as oils and fats (2.0 μg/kg), smoked meat, crustaceans (5.0 μg/kg), bivalve molluscs (10.0 μg/kg) and infants and baby foods (1.0 μg/kg) (CEC, 2005).

The most common analytical procedure used in the determination of PAHs in food is high performance liquid chromatography (HPLC) with fluorescence detection and this procedure has been successfully used in several food matrices (Camargo & Toledo, 2003; De Vos et al., 1990; Kruijf et al., 1987; Lage-Yusty & Coritzo-Daviña, 2005).

The objective of the present study was to determine the levels of 5 PAHs (benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene and dibenz[*a*,*h*]-anthracene) in different brands of cachaça commercially available in the Brazilian market. This beverage has not so far been considered a source of exposure to PAH in the diet.

2. Material and methods

2.1. Standards and reagents

PAHs standards were purchased from Supelco Inc. (benz[*a*]anthracene and dibenz[*a*,*h*]anthracene) and Aldrich Chemical Co. (benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene). Cyclohexane and *N*,*N*-dimethylformamide (HPLC grade) were purchased from Tedia Company Inc. Acetonitrile (HPLC grade) and anhydrous sodium sulfate were purchased from J.T. Baker, silica gel (70–230 mesh, ASTM) from Merck AS Chemical Industries. Water was obtained from a Millipore Milli-Q water purification system.

2.2. Samples

Different brands of cachaça (25 samples) produced in 7 different states of the country were purchased from supermarkets and cachaça stores in the city of Campinas, State of São Paulo (SP), in the years of 2004 and 2005. The chosen brands were those reported as the most consumed in the region of SP. The collected samples were analysed in duplicate.

2.3. Extraction and clean up

Extraction and clean up procedures were based on the method described by Speer, Steeg, Horstmann, Kuhn, and Montag (1990), adapted from Grimmer and Bohnke (1975).

A 20 ml volume of the sample was transferred into a 500 ml separating funnel and PAHs were successively extracted with three aliquots of cyclohexane (50, 35 and 35 ml) and the combined solution with three aliquots of *N*,*N*-dimethylformamide–water (9:1, v/v) (50, 25 and 25 ml). Subsequently 100 ml of a 1% sodium sulfate solution was added and re-extracted with 50, 35 and 35 ml aliquots of cyclohexane. The combined extract was washed twice with 40 ml of water, dried with anhydrous sodium sulfate and concentrated on a rotary evaporator to 5 ml at 40 °C.

The concentrated extract (5 ml) was purified by column chromatography on silica gel. A glass column (200 × 10 mm i.d.) was packed with 5 g deactivated silica gel (15% water) and 2.5 g anhydrous sodium sulfate on the top. The 5 ml extract was applied to the top of the column and eluted with 85 ml cyclohexane. The first 10 ml was discarded and the 10–85 ml fraction was concentrated to about 1 ml on a rotary evaporator at 40 °C, and dried under a flow of nitrogen. The residue was then dissolved in 2 ml acetonitrile and analysed by HPLC with fluorescence detection and gas chromatography–mass spectrometry (GC–MS).

2.4. HPLC

The analysis was carried out using a Waters HPLC apparatus equipped with a Model 600 Controler pump, an in-line degasser, a Model 717 plus autosampler, a Model 474 fluorescence detector (excitation wavelength 290 nm and emission wavelength 430 nm) and a Millenium 32 data processor. For separation, a C18 column (Vydac 201 TP54, 250 × 4.6 mm, 5 μm particle size) stable at 30 °C was used. The mobile phase consisted of 75% acetonitrile and 25% water at a flow rate of 1 ml min^{-1}. The injection volume was 30 μL. Peaks were identified by comparing the retention time with that of the standard and by GC–MS.

2.5. Peak identity confirmation by gas chromatography–mass spectrometry (GC–MS)

The identification of PAHs was conducted on a Hewlett–Packard (HP) 6890 gas chromatograph interfaced with

Table 1
MS data acquisition parameters

PAH	Retention time (min)	Ions monitored[a]
B[*a*]A	26.3	114, 150, **226**, **228**
B[*k*]F	31.2	126, 224, **250**, **252**, 253
B[*b*]F	31.3	126, 224, **250**, **252**, 253
B[*a*]P	32.5	126, 224, **250**, **252**, 253
D[*ah*]A	38.5	139, 250, **276**, **278**

[a] Boldfaced ions represent the quantitation ions used to calculate percent recoveries.

Table 2
Detection limit, mean recovery (*R*) and coefficient of variation (CV) of PAHs in cachaça

PAH	Detection limit (μg/L)	*R*[a] (%)	CV (%)
B[*a*]A	0.007	70.7	14.1
B[*b*]F	0.007	80.2	12.6
B[*k*]F	0.006	96.7	12.0
B[*a*]P	0.011	74.5	11.5
D[*ah*]A	0.090	70.0	21.3

[a] Average of three different concentrations.

a HP 5973 mass spectrometer selective detector equipped with an autosampler HP 7683 and a Gerstel PTV injector. The analysis were performed by electron ionization (70 eV) and data were acquired in the single-ion monitoring mode (SIM). A HP5-MS fused capillary column (30 m × 0.25 mm i.d., 0.25 μm film coated with 95% methyl-5% phenylpolysiloxane) and helium carrier at flow rate of 1.0 ml/min were used in the separation. The PTV temperature program was as follows: 120 °C, hold for 0.05 min, 500 °C/min to 300 °C, hold for 5 min. Injections of 1 μl were performed in the splitless mode and split valve was opened after 2 min. The GC oven temperature was programmed from 120 to 280 °C at a rate of 5 °C/min with a 26 min final hold. Sample peaks were identified based on retention times on target ion chromatograms and in relative abundance of the qualifiers ions selected for each PAH in comparison with PAHs standards. The MS acquisition parameters are summarised in Table 1.

2.6. Quantification

The external standard plot method was used for quantification. Duplicate HPLC injections of 30 μl PAHs standard solutions were used to construct linear regressions lines (peak area ratios versus PAH concentration). The detection limit for each PAH was calculated following Taylor (1987) guidelines.

2.7. Recovery study

Recovery experiments were carried out by spiking samples of cachaça with three different concentrations of PAHs standard solution ranging from 0.6 to 3.0 μg/L. The spiked samples as well as the unspiked controls were analysed in duplicate. Recoveries were calculated from the differences in total amounts of each PAH between the spiked and unspiked samples. The repeatability of the method was evaluated through the coefficients of variation (CV) associated to measurements of each PAH performed during recovery tests.

3. Results and discussion

The mean recovery, CV and detection limits for benz[*a*]anthracene (B[*a*]A), benzo[*b*]fluoranthene (B[*b*]F), benzo[*k*]fluoranthene (B[*k*]F), benzo[*a*]pyrene (B[*a*]P) and dibenz[*a*,*h*]anthracene (D[*ah*]A) are presented in Table 2. Recoveries obtained for different PAH ranged from 70.0% to 96.7% and the CV ranged from 11.5% to 21.3%. These results are satisfactory for determinations at μg/L levels (Horwitz, Kamps, & Boyer, 1980; Jenke, 1996). Results reported for PAHs were not corrected for recovery.

Table 3 shows the levels of PAHs determined in different brands of cachaça and their respective producer state. Benz[*a*]anthracene and benz[*b*]fluoranthene were the most representatives PAHs, detected in 96% of the analysed samples, while dibenz[*a*,*h*]anthracene was detected in 28% of the samples. Only one cachaça sample was not contaminated with any of the 5 PAHs. Mean levels of summed PAHs were within the range of not detected to 1.94 μg/L. Samples of cachaça produced in the state of Ceará presented the highest mean levels of summed PAHs and the narrowest range of values (Fig. 1). The wider variation in the levels of PAHs observed among samples of cachaça from the other states could be related to different processing or differences on the type of sugar cane (burnt or not burnt) used in the process. Nowadays 30% of the sugar cane cultivated in the main Brazilian producer areas are mechanical harvested without prior burning (ProCana, 2005). Other possible sources of contamination are: (1) the contact of the product with coal tar: the use of reservoirs coated with coal tar to store cachaça may result in the transfer of PAHs from the tar to the beverage; (2) some cachaça brands have added sugar: the sugar used can be contaminated with PAHs, resulting in their presence in the final product (Bettin & Franco, 2005; Camargo & Toledo, 2002; Tfouni, 2005).

There is not much data available in the literature concerning PAHs content in cachaça or any other sugar cane spirit. Serra et al. (1995), analysed two samples of cachaça for the presence of B[*a*]P and B[*a*]A and reported levels of contamination similar to those found in the present study: 0.27 and 0.40 μg/L for B[*a*]P and 0.49 and 0.50 μg/L for B[*a*]A. In another study, 30 cachaça samples were analysed for the presence of 16 PAHs (Bettin, 2001; Bettin & Franco, 2005). For the same 5 PAHs, the results reported ranged from not detected (nd) to 5.0 μg/L (B[*a*]A), nd-9.0 μg/L (B[*b*]F), nd-9.0 μg/L (B[*k*]F), nd-6.0 μg/L (B[*a*]P) and nd-5.0 μg/L (D[*ah*]A). The authors also reported that cachaça produced from burnt sugar cane had higher PAHs levels than those processed with not burnt sugar cane. In the present study, the levels of the 5 PAHs in cachaça were

Table 3
Levels of PAHs in cachaça from various Brazilian Federal States

Sample	State	Mean concentration of PAHs (μg/L) (SD)					
		B[*a*]A	B[*b*]F	B[*k*]F	B[*a*]P	D[*ah*]A	∑PAHs
1	SP	0.30 (0.02)	0.45 (0.02)	nd	0.21 (0.03)	nd	0.96
2		0.32 (0.09)	0.30 (0.02)	0.01 (0.00)	0.16 (0.01)	nd	0.78
3		0.28 (0.03)	0.49 (0.10)	nd	0.23 (0.01)	0.21 (0.06)	1.21
4		0.38 (0.01)	0.49 (0.03)	0.05 (0.01)	0.30 (0.02)	0.38 (0.06)	1.59
5		0.10 (0.02)	0.03 (0.01)	nd	0.02 (0.00)	nd	0.15
6		nd	nd	nd	nd	nd	nd
7		0.04 (0.00)	0.03 (0.00)	nd	nd	nd	0.07
8	CE	0.35 (0.05)	0.75 (0.06)	0.02 (0.01)	0.36 (0.03)	0.43 (0.25)	1.91
9		0.23 (0.00)	0.63 (0.02)	nd	0.32 (0.01)	0.60 (0.02)	1.78
10		0.39 (0.01)	0.83 (0.11)	0.02 (0.00)	0.35 (0.01)	0.34 (0.17)	1.94
11	PE	0.11 (0.00)	0.23 (0.02)	nd	0.09 (0.03)	nd	0.43
12		0.09 (0.00)	0.05 (0.01)	0.04 (0.00)	0.07 (0.00)	nd	0.25
13		0.21 (0.01)	0.60 (0.05)	0.28 (0.02)	0.15 (0.00)	0.17 (0.05)	1.41
14		0.08 (0.00)	0.07 (0.00)	0.05 (0.00)	0.10 (0.00)	nd	0.30
15		0.07 (0.01)	0.07 (0.00)	0.05 (0.00)	0.10 (0.00)	nd	0.28
16	MG	0.02 (0.00)	0.53 (0.00)	nd	0.03 (0.00)	nd	0.10
17		0.11 (0.00)	0.05 (0.01)	0.05 (0.00)	0.08 (0.00)	nd	0.29
18		0.06 (0.00)	0.02 (0.00)	0.04 (0.00)	0.07 (0.00)	nd	0.20
19		0.05 (0.01)	0.04 (0.01)	0.05 (0.01)	0.08 (0.01)	nd	0.21
20		0.15 (0.01)	0.08 (0.01)	0.06 (0.00)	0.09 (0.00)	nd	0.38
21	PR	0.46 (0.02)	0.49 (0.01)	0.06 (0.00)	0.29 (0.00)	0.14 (0.04)	1.44
22		0.05 (0.00)	0.02 (0.00)	0.04 (0.03)	0.07 (0.00)	nd	0.17
23		0.12 (0.00)	0.07 (0.00)	0.04 (0.00)	0.08 (0.00)	nd	0.31
24	SC	0.12 (0.00)	0.07 (0.00)	0.05 (0.00)	0.09 (0.00)	nd	0.32
25	RS	0.04 (0.01)	0.02 (0.00)	0.02 (0.00)	0.07 (0.00)	nd	0.15

nd, not detected; SD, standard deviation; SP, São Paulo; CE, Ceará; PE, Pernambuco; MG, Minas Gerais; PR, Paraná; SC, Santa Catarina; RS, Rio Grande do Sul.

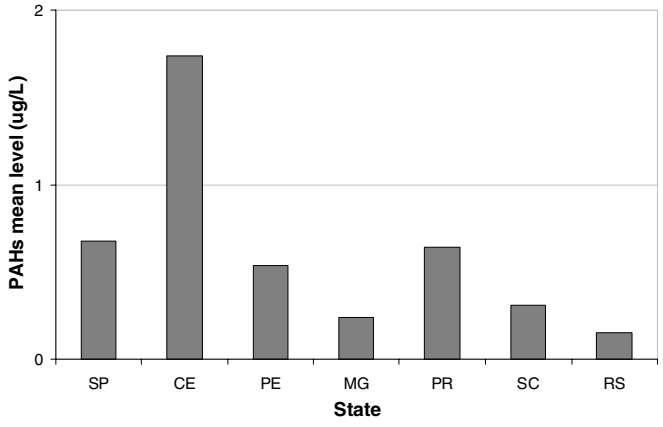

Fig. 1. Mean values of summed PAHs in cachaça from different Brazilian Federal States. SP; São Paulo; CE, Ceará; PE, Pernambuco; MG, Minas Gerais; PR, Paraná; SC, Santa Catarina; RS, Rio Grande do Sul.

relatively lower than the ones reported by Bettin (2001). At present those are the only published data on the occurrence of PAHs in cachaça in Brazil.

Taking into consideration the various origins of the cachaça available in the Brazilian market a more comprehensive study is needed in order to generate data that better reflect the extremely variety of cachaça available (over 5000 brands) as well as the influence of the different manufacture processes used by producers.

4. Conclusion

Results from previous and the present study confirm the presence of PAHs in sugar cane by-products and suggests that the practice of burning sugar cane before harvest may be the source of contamination. In Brazil, there has been an effort of the São Paulo State Government to reduce the practice of sugar cane burning: a State Regulation in force predicts a gradual elimination of the practice in the State in a period of 20–25 years (GESP, 2002).

Taking into consideration the carcinogenic potential of PAHs any measure directed to the reduction of these contaminants in the environment and in the diet is highly desirable and should be strongly stimulated.

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