



## Analytical Methods

## Polycyclic aromatic hydrocarbons (PAHs) in sugarcane juice

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## ABSTRACT

Sugarcane juice is a common beverage in many Brazilian cities. At harvesting season most sugarcane plantation is burnt and this procedure has been shown as an important source of PAHs emission. In the present study 80 samples of sugarcane juice collected from two Brazilian cities, in two different periods, were analysed for the presence of four PAHs: benz(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene and benzo(a)pyrene. PAHs were detected in 50% of the samples. The samples collected between harvests presented mean sums of PAHs of 0.013 µg/kg and 0.012 µg/kg, while the samples collected during harvest presented mean sums of 0.053 µg/kg and 0.055 µg/kg. A higher concentration and incidence of PAHs in the juices collected in the harvest period was verified, corroborating the burning of the crops as a source of sugarcane juice contamination.

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## 1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are formed during incomplete combustion or pyrolysis of organic matter. These compounds have attracted most attention because of their carcinogenic potential. During its 64th meeting the Joint FAO/WHO Expert Committee on Food Additives (JECFA) concluded that 13 PAHs were clearly carcinogenic and genotoxic, including the four selected for this study: benz(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene and benzo(a)pyrene (WHO, 2005, summary and 47p.).

PAHs have been found as contaminants in different food categories and their presence 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).

Brazil is the world's largest sugar producer: in the last 2006/2007 season 420 million tons of sugarcane were processed, producing 30 million tons of sugar (Procana, 2008). São Paulo (SP) state is responsible for 60% of the production, and the harvesting season goes from May to November. At this period the crops are usually burnt in order to facilitate manual harvest and increase the sugar content by weight due to water evaporation (Zamperlini, Santiago-Silva, & Vilegas, 2000). Several studies conducted in Brazil suggest that sugarcane burning is an important source of PAHs emission and has been associated to the presence of these contam-

inants in burnt sugarcane and its derived products (Azevedo, Santos, & Aquino Neto, 2002; Bettin & Franco, 2005; Camargo & Toledo, 2002; Godoi et al., 2004; Magalhães, Bruns, & Vasconcellos, 2007; Santos, Azevedo, & Aquino Neto, 2002; Serra, Pupin & Toledo, 1995; Tfouni, Machado et al., 2007; Tfouni & Toledo, 2007; Tfouni, Vitorino et al., 2007b).

Sugarcane juice is a common beverage in many Brazilian cities; it is commercialized by street vendors who extract the juice from the cane by using mills. Study conducted by Tfouni et al. (2007b) showed that sugarcane juices collected at sugar mills, prior to sugar production, had the presence of PAHs, and the levels of these compounds were higher in juices obtained from burnt sugarcane than in samples obtained from unburnt sugarcane.

In this manner, the objective of the present study was to determine the levels of four PAHs in sugarcane juice samples collected in two Brazilian cities, Ribeirão Preto, SP and Campinas, SP, during harvest time and in the season between harvest cycles. The results will help verify if seasonal and geographical variations, and the practice of burning sugarcane crops before harvest, will influence the PAHs levels in the samples.

## 2. Material and methods

## 2.1. Samples

Samples were collected from 20 different location sites in two cities of the State of São Paulo: Ribeirão Preto and Campinas. Sampling was realized in two periods of 2007: during harvest time (September and October) and between harvests (March and April).

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The total of 80 samples were analysed in duplicate for the presence of 4 PAHs (benz(a)anthracene (BaA), benzo(b)fluoranthene (BbF), benzo(k)fluoranthene (BkF) and benzo(a)pyrene (BaP)).

## 2.2. Standards and reagents

PAHs standards were purchased from Supelco Inc. (BaA and BaP) and Aldrich Chemical Co. (BbF and BkF). Cyclohexane and *N,N*-dimethylformamide (HPLC grade) were purchased from Tedia Company Inc. Acetonitrile (HPLC grade) and anhydrous sodium sulphate 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.

## 2.3. Extraction and clean up

Extraction and clean up procedures were based on the method described by Tfouni et al. (2007b) adapted from Grimmer and Bohnke (1975). In a separating funnel, 100 ml of *N,N*-dimethylformamide–water (9:1, v/v) and 100 ml of a 1% sodium sulphate aqueous solution were added to 25 g of the sample. PAHs were successively extracted with three aliquots of cyclohexane (50, 35 and 35 ml). The combined extract was washed twice with 40 ml of water, dried with anhydrous sodium sulphate and concentrated on a rotary evaporator to 3 ml at 40 °C. The concentrated extract (3 ml) was purified by column chromatography with silica gel, as follows: a glass column (200 × 10 mm i.d.) was packed with 5 g deactivated silica gel (15% water) and 2.5 g anhydrous sodium sulphate on the top. The 3 ml extract was applied to the top of the column and eluted with 85 ml of 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.

## 2.4. HPLC

The analysis was carried out using a Shimadzu HPLC apparatus equipped with a LC-20AT pump, a Rheodyne 7725i injector, and a RF-10A xl fluorescence detector (excitation wavelength 290 nm and emission wavelength 430 nm). A C18 column (Vydac 201 TP54, 250 × 4.6 mm, 5 µm particle size) at 30 °C was used and the mobile phase consisted of 75% acetonitrile and 25% water at a flow rate of 1 ml/min. The injection volume was 20 µl.

## 2.5. Quantification

The external standard plot method was used for quantification. Duplicate HPLC injections of 20 µl PAHs standard solutions in acetonitrile were used to construct linear regressions lines (peak area ratios versus PAH concentration). The concentration range of the standard curves was from 0.1 to 30 µg/kg. The limit of detection (LOD) for each PAH was calculated following Taylor (1987) and INMETRO (2007) guidelines. For this purpose, seven independent analysis of a sample of sugarcane juice spiked with PAHs at a level of 0.05 µg/kg were performed. The LOD were calculated from the standard deviation of these determinations. Peaks were identified by comparing the retention time with that of the standards and by re-injecting the extract in the chromatograph with the addition of standard solution.

## 2.6. Recovery study

Recovery experiments were carried out by spiking a sample of sugarcane juice with three different concentrations of PAHs standard solution ranging from 0.3 to 1.0 µg/kg. The spiked samples

as well as the unspiked controls were analysed in five replicates. 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. Results reported were not corrected for recovery.

## 2.7. Statistical analysis

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

## 3. Results and discussion

Mean recovery, CV and LOD for BaA, BbF, BkF and BaP are presented in Table 1. Recoveries obtained for different PAH ranged from 90.8% to 105.2% and the CV ranged from 6.7% to 15.2%. These results are satisfactory for determinations at µg/kg levels (Horwitz, Kamps, & Boyer, 1980; Jenke, 1996).

Fig. 1 presents a chromatogram that shows the separation of the 4 PAHs peaks in a sugarcane juice sample.

Tables 2 and 3 present PAHs levels determined in the sugarcane juice samples from the cities of Campinas and Ribeirão Preto, respectively. Out of 80 analysed samples, PAHs were detected in 40, of which 23 were from Campinas and 17 from Ribeirão Preto.

According to Tables 2 and 3, 37.5% of the samples collected between harvests presented PAHs levels above the limit of detection, with sum levels ranging from 0.006 to 0.033 µg/kg. As for samples collected during harvest, 62.5% had PAHs detected with sum levels ranging from 0.008 to 0.290 µg/kg, these levels are higher than those detected in between harvests samples.

The most representative PAH was BbF, which was detected in 39% of the samples, while BkF was detected in only two of the analysed samples.

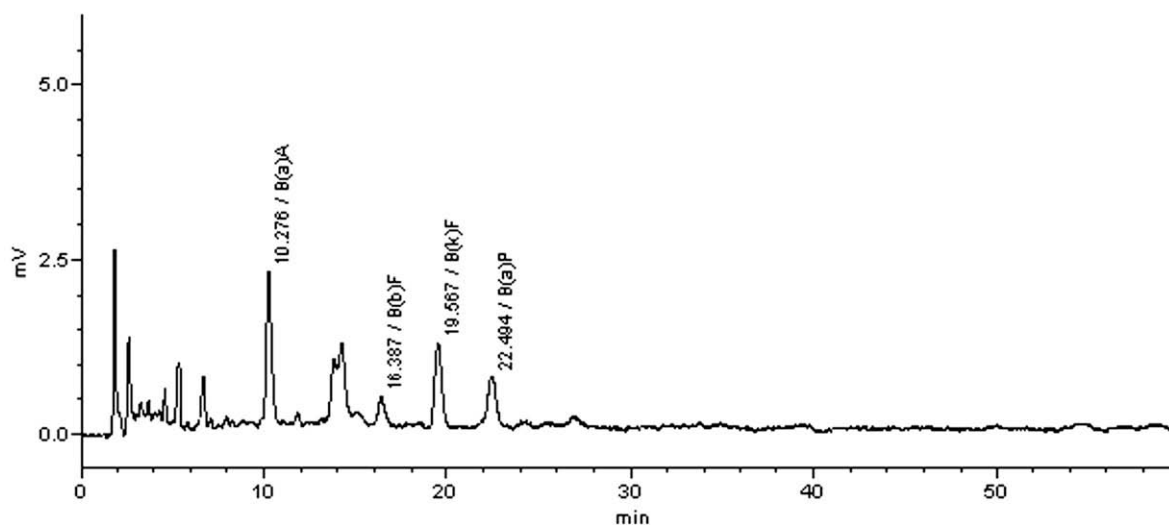
Tables 2 and 3 also show that the presence of PAHs in sugarcane juice is similar in both cities studied. For samples collected between harvests the mean sum of PAHs was 0.013 µg/kg in Campinas and 0.012 µg/kg in Ribeirão Preto. The samples collected during harvest presented mean sums of 0.053 µg/kg in Campinas and 0.055 µg/kg in Ribeirão Preto. The levels of PAHs in the samples from Campinas collected between harvests were statistically lower than the ones collected during harvest (Tukey, *p* < 0.05), period which the crops are burnt. In addition, results show that there is a higher incidence of PAHs in the juices collected during sugarcane harvest.

**Table 1**  
LOD, recovery (*R*) and coefficient of variation (*CV*) for PAHs in sugarcane juice.

HPA	LOD (µg/kg)	Spike level (µg/kg)	<i>R</i> (%) <sup>a</sup>	<i>CV</i> (%)
BaA	0.009	0.3	90.8	15.2
		0.5	103.3	9.8
		1.0	105.2	13.3
BbF	0.006	0.3	94.5	6.9
		0.5	96.7	9.7
		1.0	100.9	10.7
BkF	0.004	0.3	95.1	6.7
		0.5	95.5	8.8
		1.0	99.5	10.7
BaP	0.005	0.3	93.5	7.5
		0.5	96.3	9.1
		1.0	101.9	11.2

BaA: benz(a)anthracene, BbF: benzo(b)fluoranthene, BkF: benzo(k)fluoranthene, BaP: benzo(a)pyrene.

<sup>a</sup> *n* = 5.



**Fig. 1.** HPLC chromatogram of sugarcane juice sample (unspiked). Conditions: Column C18 (Vydac 201 TP54). Detection by fluorescence: 290 nm (excitation) and 430 nm (emission). Mobile phase: acetonitrile:water (75:25, v/v). Flow rate: 1.0 ml/min. BaA: benz(a)anthracene, BbF: benzo(b)fluoranthene, BkF: benzo(k)fluoranthene, BaP: benzo(a)pyrene.

As street vendors usually use unburnt sugarcane for juice production, the presence of PAHs in the product is probably due to environmental contamination. The compounds present in the atmosphere are deposited in the sugarcane surface, retained in the wax layer and then transferred to the juice during crushing. Other studies had already showed the presence of PAHs in food due to environmental contamination by deposition (Camargo & Toledo, 2003).

In Brazil, there is no legislation regarding levels of PAHs in sugarcane juice. There are only maximum BaP levels established for

smoke flavourings and drinkable water, where the maximum limit for the last is 0.7 µg/l (Brasil, 2004). When using the maximum limit permitted for water for comparison, one can see that the values presented on Tables 2 and 3 are considerably low.

There are only few data on the literature regarding the presence of PAHs in sugarcane juice.

Zuin et al. (2006) analysed 6 sugarcane juice samples for the presence of BaP. The compound was detected in 4 samples and the levels were a little higher than the ones found in the present study, ranging from 0.050 to 0.112 µg/l. However, the authors did not specify the sampling period.

The levels found in the present study are also lower than the ones reported by Tfouni et al. (2007b), where juice produced from unburnt sugarcane had mean values of 0.32 µg/kg for the sum of 5 PAHs (BaA, BbF, BkF, BaP and dibenzo(a,h)anthracene). This difference may be due to the fact that in Tfouni et al. (2007b) study the sugarcane juice samples were collected from a sugar mill, where the sugarcane was not previously washed and cleaned, while the street vendors that commercialize the juice usually wash and clean the sugarcane before extracting the juice.

Although there are differences in the PAHs levels in both studies, both show a relation between the burning of the crops with the PAHs presence and their higher levels in sugarcane juice.

In this manner, the present results reinforce the ones presented in previous studies where the burning of the crops is related to PAHs formation, emission and presence in sugarcane and its derived products (Azevedo et al., 2002; Bettin & Franco, 2005; Camargo & Toledo, 2002; Godoi et al., 2004; Magalhães et al., 2007; Santos et al., 2002; Serra et al., 1995; Tfouni, Machado et al., 2007; Tfouni & Toledo, 2007; Tfouni, Vitorino et al., 2007). In addition, the practice of burning the crops can result in the presence of PAHs in the soil and groundwater of areas with sugarcane plantation, as studied in burnt woodland areas (García-Falcón, Soto-González, & Simal-Gándara, 2006; Rey-Salgueiro, García-Falcón, Soto-González, & Simal-Gándara, 2004).

#### 4. Conclusion

Both cities studied presented sugarcane juice with similar levels of PAHs. Although, samples collected at harvest period presented levels of PAHs four times higher than samples collected between harvests. A higher concentration and incidence of PAHs in the

**Table 2**  
PAHs levels in sugarcane juice samples collected in Campinas, SP.

PHA	Between harvests		Harvest	
	n	Mean* (range) (µg/kg)	n	Mean* (range) (µg/kg)
BaA	1	0.010	12	0.034 (0.010–0.097)
BbF	10	0.011 (0.007–0.020)	9	0.020 (0.009–0.049)
BkF	1	0.006	1	0.007
BaP	1	0.007	9	0.011 (0.005–0.022)
Σ PAHs	10	0.013 a (0.007–0.033)	13	0.053 b (0.019–0.126)

BaA: benz(a)anthracene, BbF: benzo(b)fluoranthene, BkF: benzo(k)fluoranthene, BaP: benzo(a)pyrene.

n = Number of samples where the PAH was detected (>LOD from Table 1).

a, b – Significant statistical difference between the mean values compared in the row at significance level of  $p < 0.05$  (Tukey).

\* Mean of n samples.

**Table 3**  
PAHs levels in sugarcane juice samples collected in Ribeirão Preto, SP.

PAH	Between harvests		Harvest	
	n	Mean* (range) (µg/kg)	n	Mean* (range) (µg/kg)
BaA	2	0.017 (0.013–0.021)	8	0.047 (0.010–0.264)
BbF	3	0.008 (0.006–0.012)	9	0.025 (0.008–0.046)
BkF	0	–	0	–
BaP	0	–	4	0.017 (0.008–0.032)
Σ PAHs	5	0.012 a (0.006–0.021)	12	0.055 a (0.008–0.290)

BaA: benz(a)anthracene, BbF: benzo(b)fluoranthene, BkF: benzo(k)fluoranthene, BaP: benzo(a)pyrene.

n = Number of samples where the PAH was detected (>LOD from Table 1).

a, a – No significant statistical difference between the mean values compared in the row at significance level of  $p < 0.05$  (Tukey).

\* Mean of n samples.

juices collected in the harvest period corroborates the burning of the crops as a source of sugarcane juice contamination.

Additionally, the washing and cleaning procedures employed by the street vendors before sugarcane crushing seem to be effective in reducing the PAHs levels transferred to the juice commercialized and consequently consumed by the population.

Despite that PAHs presence was detected and that the market indicates an increase in the products consumption, the levels of PAHs found in this study may be considered low when compared with BaP limit for drinkable water. In this manner, there is no apparent risk to the consumers regarding sugarcane juice intake.

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