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Profiles of Polycyclic Aromatic Hydrocarbons in Brazilian Sugar Cane Spirits: Discrimination between Cachaças Produced from Nonburned and Burned Sugar Cane Crops†

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Cachaça may be contaminated by a remarkable presence of polycyclic aromatic hydrocarbons (PAHs) when the sugar cane crop used for its production is burned before harvesting. The analysis of 15 PAHs by liquid chromatography coupled to a fluorescence detector in 131 cachaça samples from burned and nonburned sugar cane crops is reported. Average contents of 21.1 and 1.91 μg L^{-1} for total PAHs were observed for cachaças originating from burned and nonburned sugar cane plantations, respectively. The main difference between these two classes of cachaças is in the quantitative profile of the most potent carcinogenic PAH, benzo[a]pyrene, which is more abundant in cachaça produced from burned sugar cane crops (4.54×10^{-2} μg L^{-1}) than in cachaça produced from nonburned crops (9.02×10^{-3} μg L^{-1}). The contents of benzo[a]pyrene in both classes of cachaça are lower than the legal limit established by the European Union (EU) at 2.00 μg L^{-1} for food products. In relation to the total PAH content suggested by the German Society for Fat Science, both cachaças from burned (21.1 μg L^{-1}) and nonburned crops (1.91 μg L^{-1}) are below the limit (25 μg L^{-1}) for total PAH content. The analytical data for PAHs, when treated through the multivariate statistical methods principal component analysis and canonical discriminant analysis, provide a very good distinction between samples produced from burned and nonburned sugar cane crops with a certainty of 98.1%.

KEYWORDS: PAHs; cachaça; burned sugar cane crop; multivariate analysis

INTRODUCTION

The production of cachaça, Brazilian sugar cane spirit, is about 2×10^8 L/year. A considerable percentage of this volume is produced from burned sugar cane crops. The same scenery is true for fuel ethanol (*1*, *2*), which has an annual production around 2×10^9 L.

Sugar cane is harvested by hand or mechanically. Hand harvesting accounts for more than half of the Brazilian production. However, when harvested by hand, the field is first set on fire aiming to facilitate the manual harvest and also to increase the sugar weight by evaporating the water in the stalk. Therefore, this common practice may lead to the formation of large amounts of soot resulting (*2*, *3*) in a substantial environmental impact and in a high incidence of respiratory diseases in the local population. Furthermore, carcinogenic and mutagenic polycyclic aromatic hydrocarbons (PAHs) (*4*−*8*) are formed during incomplete combustion or pyrolysis of organic matter and may become adhered to the sugar cane stalks resulting in contamination of the juice during processing.

Aiming to abolish environmental and food contamination, the authorities in the São Paulo state, the major producer of cachaça and ethanol in Brazil, passed a regulation (*9*) establishing 2020 as the deadline for mechanization of all sugar cane harvesting processes and banishing the burning practice.

A variety of analytical methods have been used for determining trace concentrations of PAHs in foods and beverages (*1*−*7*, *10*−*16*). These include gas chromatography (GC) with various detectors, high-performance liquid chromatography (HPLC) with various detectors, and thin-layer chromatography with fluorescence detection. Diverse detection devices used for GC quantification include flame ionization detection, MS, and Fourier transform infrared spectrometer (FT-IR). GC/MS and HPLC with UV−vis or spectrofluorimetric detectors are perhaps the most prevalent analytical methods for determining concentrations of PAHs in environmental and biological samples. Furthermore, the determination of PAHs by means of HPLC with fluorescence detector (FLD) has been widely employed (*5*−*7*, *15*, *16*) and has advantages over GC methods such as: better selectivity, ability to handle a larger amount of sample, and low detection limits.

Recently, the presences of five PAHs {benz[a]anthracene (BaA), benz[b]fluoranthene (BbF), benz[k]fluoranthene (BkF), benz[a]pyrene (BaP), and dibenz[a,h]anthracene (DBahA)} in cachaça were reported (*14*) indicating that samples produced from burned sugar cane fields had higher PAH levels than those produced from nonburned sugar cane fields. However, up to

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now, there is no extensive study on the profile of naphthalene (NA), acenaphthene (AC), fluorene (F), phenanthrene (PA), anthracene (A), fluoranthene (FL), pyrene (P), BaA, chrysene (CH), BbF, BkF, BaP, DBahA, benz[g,h,i]perylene (BghiP), and indeno[1,2,3-c,d]pyrene (IP) in cachaça produced from sugar cane harvested from fields set on fire or not.

Herein, a procedure to discriminate cachaça produced from burned sugar cane crops from nonburned crops is described. The analytical data collected for PAHs were treated by means of the multivariate statistical methods (*17*−*20*) principal component analysis (PCA) and canonical discriminant analysis (CDA) to differentiate samples from nonburned crops from the samples produced with burned sugar cane crops.

MATERIALS AND METHODS

Samples. One hundred and thirty-one (131) cachaça samples were collected in loco in the São Paulo state, Brazil. From these samples, 26 cachaças were produced from burned sugar cane crops and 105 were produced from nonburned sugar cane crops. All information regarding source and harvest procedure employed in the cane sugar field used for spirit production was provided by the manufacturer.

In addition, eight whiskey samples and four rums were further analyzed for comparison purposes. The whiskey samples were as follows: Grants (Scotland, 40% v/v), Glenfiddich (Scotland, 40% v/v), Black Label (Scotland, 43% v/v), Buchanan's (Scotland, 40% v/v), Four Roses (United States, 40% v/v), Jim Beam (United States, 40% v/v), Grand Dad (United States, 43% v/v), and Jack Daniel's (United States, 43% v/v). The rum samples were as follows: Havana Club anejo 3 años (Cuba, 40% v/v), Havana Club Silver Dry (Cuba, 40% v/v), Bacardi Carta de Oro (Brazil, 38% v/v), and Bacardi Carta Blanca (Brazil, 38% v/v).

Materials. Studied were the following PAHs: 98% NA, 99% AC, 99% F, 98% PA, 98% A, 98% FL, 98% P, 99% BaA, 95% CH, 99% BbF, 98% BkF, 97% BaP, 97% DBahA, 98% BghiP, and 98% IP, which were purchased from Sigma-Aldrich (Steinheim, Germany) and used as received. The HPLC grade solvents acetonitrile, methanol, ethanol, dichloromethane, and hexane were obtained from J. T. Baker and Tedia (Phillipsburg, NJ). Water was previously bidistilled and further deionized using a Milli-Q system Millipore (Millipore, Bedford, MA).

Individual stock solutions of PAHs (0.100 mg L^{-1}) were prepared by dissolving 0.05 g of the desired compound into 50.0 mL of methanol/acetonitrile (1:1 v/v). From stock solutions, the working solution containing PAHs ranging from 50.0 to 250 μg L^{-1} was prepared in acetonitrile and stored in amber flasks at 4 °C.

Waters Sep-Pak C18 Plus (1 g) cartridges were used for solid-phase extraction (SPE) cleanup and a preconcentration step (Milford, MA). The nitrogen (99.99%) was supplied by White Martins Praxair Inc. (Sertãozinho, SP, Brazil).

Sample Cleanup and Preconcentration. The octadecilsylane (C18) cartridge was preconditioned by sequential flushing with 6 mL of dichloromethane, 6 mL of acetonitrile, and 6 mL of water and then dried under vacuum for 45 min. After that, samples (30 mL) of added acetonitrile (9 mL) were loaded and percolated through the C18 cartridges under negative pressure and constant flow (2 mL min^{-1}). The analytes were eluted using 2 mL of dichloromethane/hexane (1:1 v/v), and the eluting solution was evaporated through dryness under a gentle N_2 stream. The residue containing the extracted PAHs was then redissolved in 300 μL of acetonitrile for further HPLC analysis.

HPLC Analysis. The preconcentrated samples were analyzed in a LC-10AD HPLC (Shimadzu, Tokyo, Japan) system coupled to a RF-551 (Shimadzu) FLD. The analytical methodology was adapted from one described by the U.S. Environmental Protection Agency for water and wastes analysis (*21*). The HPLC separation was achieved by means of a reverse phase C18 Supelcosil LC-PAH column (25 cm × 4.6 mm i.d. and 5 μm particle size) (Supelco, Bellefonte, PA), with water−acetonitrile as the mobile phase. The elution gradient was employed as follows: isocratic step, 50% acetonitrile for 5 min, reaching 100% of acetonitrile in 25 min at a constant flow rate of 0.4 mL min^{-1}.

Table 1. FLD Parameters

time (min)	λ excitation (nm)	λ emission (nm)	target compound
0–14.6	280	330	NA, AC, FA
14.61–16.0	280	365	PA
16.01–17.6	356	400	A
17.61–33.5	270	410	FL, P, BaA, CH, BbF, BkF, BaP, DBahA, and BghiP
33.51–42.0	280	500	IP

Quantitative analyses were performed using a standard addition method and were always carried out in triplicate. The detection of the 15 PAHs studied was accomplished by programming variable excitation and emission wavelengths as function of time as can be seen in **Table 1**.

Multivariate Statistical Methods. The analytical data for 131 samples were organized in a matrix form and autoscaled prior to multivariate analysis. The PCA and CDA were performed using the MINITAB Release 14 (Statistical Software, State College, PA) and JMP 5 (SAS Institute Inc., Cary, NC) software.

RESULTS AND DISCUSSION

A typical chromatogram obtained for samples of cachaça produced from nonburned sugar cane crops (**A**), burned sugar cane crops (**B**), and the standard mixture of PAHs (**C**) is depicted in **Figure 1**.

The sorption of PAHs on the SPE cartridge, which is a common problem observed during PAH analysis, was prevented by addition of acetonitrile to the sample. The addition of acetonitrile increases the solubility of PAHs in the sample and also turns (*22*, *23*) its displacement along the SPE cartridge easier by small aliquots of the eluting solvent.

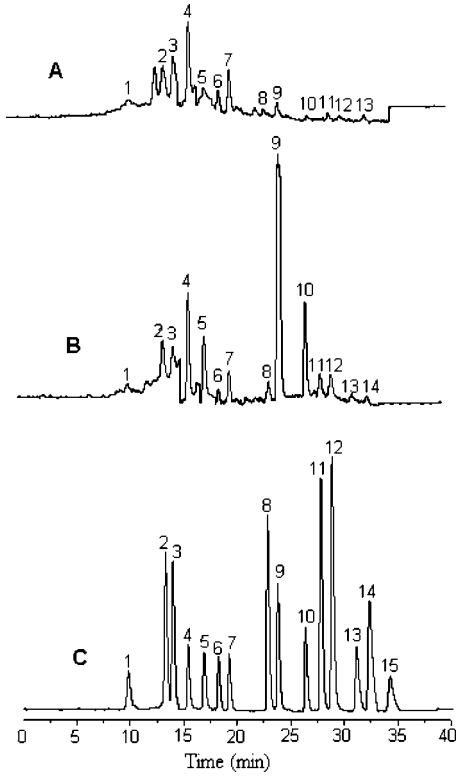

Figure 1. Typical chromatogram for cachaça produced from nonburned cane sugar crops (**A**), produced from burned cane sugar crops (**B**), and a 25 μg L^{-1} standard mixture of PAHs (**C**). Peaks: 1, NA; 2, AC; 3, F; 4, PA; 5, A; 6, FL; 7, P; 8, BaA; 9, CH; 10, BbF; 11, BkF; 12, BaP; 13, DBahA; 14, BghiP; and 15, IP.

Table 2. Percentage of Recovery Index, RSDs, Repeatability, Reproducibility, Linearity Range, Correlation Coefficient (r^2), and LODs and LOQs for the 15 Target PAHs Analyzed in Cachaça

PAHs	recovery index[a] (%)	RSD	repeatability[b] (%)	reproducibility[c] (%)	linear range (μg L^{-1})	r^2	LOD (μg L^{-1})	LOQ (μg L^{-1})
NA	86.8	9.22	90.8	96.8	38.3–690	0.992	3.58×10^{-3}	7.16×10^{-1}
AC	113	5.26	94.7	93.6	89.2–1.31×10^{3}	0.992	3.93×10^{-3}	1.25×10^{-1}
F	85.5	3.29	96.7	95.5	144–1.53×10^{3}	0.996	2.93×10^{-3}	4.74×10^{-1}
PA	106	0.56	99.4	96.9	30.9–821	0.991	2.02×10^{-3}	8.81×10^{-1}
A	94.2	1.64	98.6	94.2	37.3–590	0.999	4.20×10^{-3}	1.36×10^{-1}
FL	99.9	2.02	98.0	97.9	15.2–504	0.994	1.28×10^{-2}	5.88×10^{-1}
P	101	1.07	98.9	91.1	79.6–860	0.994	6.95×10^{-3}	1.12×10^{-1}
BaA	106	5.02	95.0	96.5	560–6.0×10^{3}	0.991	1.68×10^{-3}	8.56×10^{-1}
CH	81.5	9.10	89.8	91.5	111–1.38×10^{3}	0.997	2.24×10^{-3}	3.98×10^{-1}
BbF	96.6	9.22	90.8	96.6	165–1.95×10^{3}	0.997	5.68×10^{-3}	5.22×10^{-1}
BkF	95.5	6.15	93.8	95.5	883–9.40×10^{3}	0.995	6.56×10^{-3}	5.61×10^{-1}
BaP	85.5	6.09	93.9	95.5	765–8.70×10^{3}	0.993	5.92×10^{-3}	9.30×10^{-2}
DBahA	85.7	9.22	90.8	95.7	51.7–1.82×10^{3}	0.998	1.08×10^{-3}	8.29×10^{-1}
BghiP	93.6	9.22	90.8	93.6	34.5–2.13×10^{3}	0.996	3.22×10^{-3}	4.49×10^{-1}
IP	86.8	9.02	91.0	92.4	38.1–755	0.991	4.54×10^{-2}	7.43×10^{-1}

[a] Recovery index (%) = ($C1/C2$) × 100, where C1 = measured content and $C2$ = expected content. [b] Within-day precision data (n = 7) analysis in triplicate. [c] Day-to-day (continuous 10 days) analysis in triplicate.

Table 3. Average, Median, Maximum, Minimum, and Averages Sum of the Content PAHs (μg L^{-1}) in Samples of Cachaça Produced from Burned and Nonburned Sugar Cane Crops

	cachaça produced from burned sugar cane				cachaça produced from nonburned sugar cane			
PAHs	average	median	maximum	minimum	average	median	maximum	minimum
NA	2.73×10^{-1}	3.88×10^{-1}	2.68	3.14×10^{-2}	2.33×10^{-1}	2.45×10^{-1}	2.70	1.08×10^{-2}
AC	1.05	7.03×10^{-1}	5.62	6.93×10^{-2}	2.15×10^{-1}	1.48×10^{-1}	2.77	1.18×10^{-2}
F	1.13	8.82×10^{-1}	4.74	3.70×10^{-2}	9.70×10^{-2}	7.16×10^{-2}	6.48×10^{-1}	8.79×10^{-3}
PA	7.01	6.19	33.7	4.43×10^{-1}	6.06×10^{-1}	4.21×10^{-1}	3.13	6.06×10^{-3}
A	1.41	1.22	5.39	1.33×10^{-1}	1.29×10^{-1}	1.08×10^{-1}	1.35	1.26×10^{-2}
FL	2.07	1.80	8.95	3.94×10^{-1}	2.26×10^{-1}	1.47×10^{-1}	2.07	3.86×10^{-2}
P	2.32	2.12	15.3	3.37×10^{-1}	1.89×10^{-1}	1.49×10^{-1}	1.19	2.09×10^{-2}
BaA	4.95	1.56×10^{-1}	138	2.18×10^{-2}	3.99×10^{-2}	1.80×10^{-2}	1.12	5.03×10^{-3}
CH	7.34×10^{-1}	2.16×10^{-1}	6.75	5.07×10^{-2}	8.43×10^{-2}	7.11×10^{-2}	1.79	6.71×10^{-3}
BbF	5.27×10^{-2}	2.24×10^{-1}	5.46×10^{-1}	2.72×10^{-2}	1.21×10^{-2}	2.70×10^{-2}	3.41×10^{-1}	1.70×10^{-3}
BkF	2.05×10^{-2}	2.99×10^{-2}	7.93×10^{-2}	7.74×10^{-3}	8.62×10^{-3}	7.40×10^{-3}	4.58×10^{-2}	1.97×10^{-3}
BaP	4.56×10^{-2}	4.54×10^{-2}	3.50×10^{-1}	1.03×10^{-2}	1.68×10^{-2}	9.02×10^{-3}	5.99×10^{-1}	1.77×10^{-3}
DBahA	2.52×10^{-2}	1.64×10^{-1}	3.29×10^{-1}	7.38×10^{-2}	3.90×10^{-2}	4.35×10^{-2}	2.54	7.67×10^{-3}
BghiP			2.41×10^{-1}	$<3.22 \times 10^{-3}$	1.61×10^{-2}	5.31×10^{-2}	3.40×10^{-1}	9.64×10^{-3}
IP			1.36×10^{-1}	$<4.54 \times 10^{-2}$				$<4.54 \times 10^{-2}$
$\sum$ averages	21.1				1.91			

The analytical methodology was validated (*24*) by way of precision, repeatability, reproducibility, linearity, limits of detection (LODs) and quantification (LOQs), and recovery index. The recovery index and repeatability of the method were verified through the analysis of four PAH-fortified samples. The relative standard deviations (RSD) for recovery index, repeatability, and reproducibility are given in **Table 2**. The recovery index ranges from 81.5 ± 9% for CH to 113 ± 5% for AC, which is considered acceptable for trace analysis. The repeatability and reproducibility of the method were higher than 89.8 and 91.1%, respectively.

The method linearity was ascertained plotting the chromatogram area of each compound vs the corresponding analyte concentration. The obtained calibration plots show good linearity with correlation coefficients (r^2) higher than 0.991 for all PAHs studied (**Table 2**).

The LOD was visually evaluated by the analysis of standard solutions with known concentrations of analyte and by establishing the minimum level at which the analyte could be reliably detected. The LOQ was calculated on the basis of the standard deviation of the response and the slope obtained from the linearity plot of each PAH of the standard mixture, as described in the relevant ICH guideline (*24*). LOQs were calculated as 10 α/S, respectively, where α is the standard deviation of the y-intercept and S is the slope of regression line. The calculated values of LOQ and LOD for each PAH are reported in **Table 2**.

Table 3 shows the collected average, median, maximum, minimum, and average sum for PAH contents in the two groups of cachaças studied (produced from burned and nonburned sugar cane plantations). As observed, samples produced from burned sugar cane crops display higher median levels of PAHs (from 2.99×10^{-2} for BkF to 6.19 μg L^{-1} for phenantrene) than samples produced from nonburned sugar cane plantations (from 7.40×10^{-3} to 1.08×10^{-1} μg L^{-1}).

The high average content of NA, AC, F, PA, A, FL, P, BaA, CH, BbF, BkF, and BaP ranging from 2.05×10^{-2} μg L^{-1} (BkF) to 7.01 μg L^{-1} (PA) is observed in spirits produced from burned sugar cane crops. Also, a median content of PAHs in cachaça produced from burned sugar cane plantation is generally higher (21.1 μg L^{-1}) than those found in samples produced from nonburned sugar cane crops (1.91 μg L^{-1}). Nevertheless, five cachaça samples produced from burned sugar cane crops (#5, 9, 113, 119, and 120) display an average content for total PAH superior than the content suggested by the German Society for Fat Science (25 μg L^{-1}) (*7*).

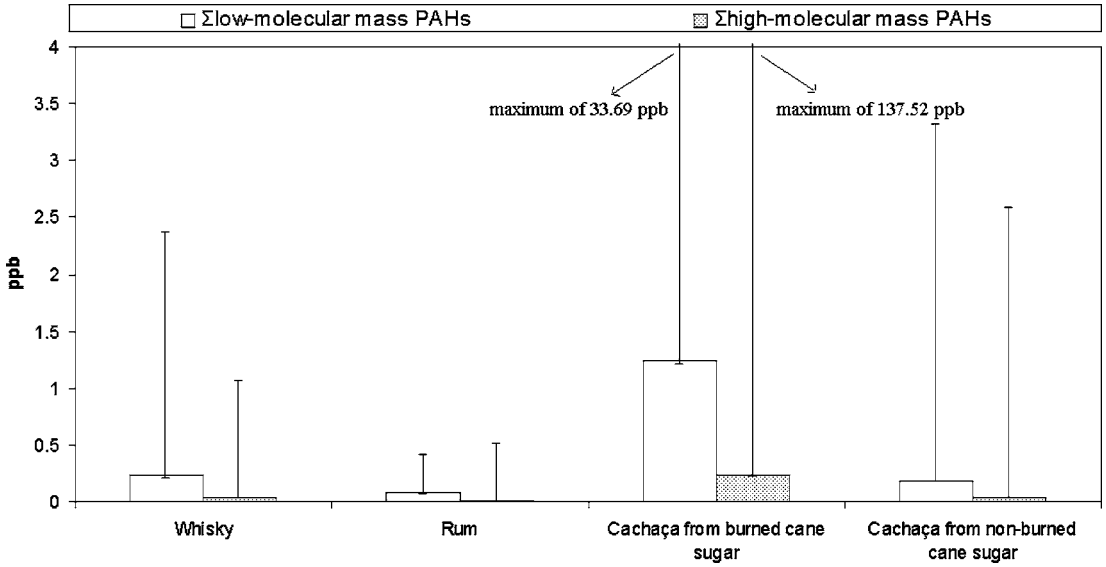

Figure 2. Median and range values for total content of high- and low-molecular mass PAHs in whiskey, rum, and cachaças (**Table 3**). The *Y*-axis bar corresponds to minimum and maximum contents.

Table 4. Average and Median Content for PAHs in Whiskey and Rum Samples (μg L^{-1})

	whiskey		rum	
PAHs	average	median	average	median
NA	8.34×10^{-1}	3.88×10^{-1}	<LOD[a]	
AC	1.63×10^{-2}	1.63×10^{-2}	<LOD[a]	
F	4.37×10^{-1}	3.37×10^{-2}	<LOD[a]	
PA	3.33×10^{-1}	2.29×10^{-1}	9.88×10^{-2}	9.78×10^{-2}
A	2.03×10^{-1}	1.49×10^{-1}	1.53×10^{-1}	9.94×10^{-2}
FL	1.67×10^{-1}	1.37×10^{-1}	5.97×10^{-2}	5.58×10^{-2}
P	1.43×10^{-1}	1.53×10^{-1}	4.61×10^{-2}	3.37×10^{-2}
BaA	4.10×10^{-2}	2.81×10^{-2}	9.11×10^{-3}	9.54×10^{-3}
CH	1.97×10^{-1}	3.75×10^{-2}	1.78×10^{-1}	1.43×10^{-2}
BbF	6.62×10^{-2}	4.16×10^{-2}	<LOD[a]	
BkF	3.67×10^{-2}	9.87×10^{-3}	1.43×10^{-2}	6.12×10^{-3}
BaP	9.09×10^{-3}	9.28×10^{-3}	4.92×10^{-3}	4.77×10^{-3}
DBahA	3.74×10^{-2}	3.74×10^{-2}	<LOD[a]	
BghiP	9.28×10^{-2}	<LOQ[a]	<LOD[a]	
IP	<LOD[a]		<LOD[a]	
∑ averages	2.61		0.564	

[a] <LOD, smaller than the LOD; <LOQ, smaller than the LOQ.

The median and range values for total content of high- and low-molecular mass PAHs in cachaça, whiskey, and rum are given in **Figure 2**.

It can be visualized in **Figure 2** that the content of PAHs with low (two and three rings) and high (four to six rings) molecular mass in the set of cachaças produced from burned sugar cane plantation shows a great variation. Moreover, the profile of low molecular mass PAHs (NA, AC, F, PA, and A) exhibits the highest concentration in this group of spirits, with a median value ranging from of 3.88×10^{-1} to 6.19 μg L^{-1}. However, it should be noted that PAHs with a high molecular mass (FL, P, BaA, CH, BbF, BkF, BaP, DBahA, and BghiP) display a median content below 2.12 μg L^{-1}. Cachaças produced from nonburned sugar cane crops show for low molecular mass PAHs a median content ranging from 7.16×10^{-2} to 4.21×10^{-1} μg L^{-1} and for high molecular mass PAHs a median content ranging from 7.40×10^{-3} to 1.49×10^{-1} μg L^{-1} (**Table 3**).

From various possible sources of PAHs in the production of whiskey and rum, some are related to the use of raw materials such as caramel and others are related to the technological process itself (*11*, *25*). The collected data for the analysis of PAHs profile in whiskey and rum samples are reported in **Table 4**. For whisky samples, the average and median content are 9.09 $\times 10^{-3}$ and 9.28 $\times 10^{-3}$ μg L^{-1} for BaP and 8.34 $\times 10^{-1}$ and 3.88 $\times 10^{-1}$ μg L^{-1} for NA, respectively.

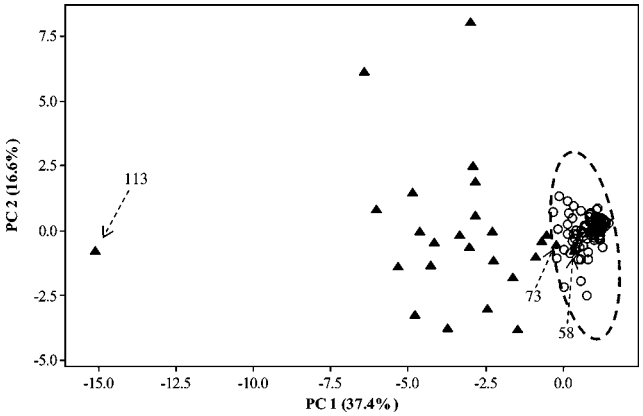

Figure 3. Score plot for cachaça produced from burned cane sugar crops (▲) and produced from nonburned cane sugar crops (○).

On the other hand, for rum samples, only seven PAHs were identified (PA, A, FL, P, BaA, CH, BkF, and BaP) with an average content ranging from 4.92 $\times 10^{-3}$ (BaP) to 1.78 $\times 10^{-1}$ μg L^{-1} (CH). The quantitative profile observed for whiskey and rum samples is similar for those observed for samples produced from nonburned sugar cane crops.

The sum of average content for PAHs in whiskey (2.61 μg L^{-1}) and rum (5.64 $\times 10^{-1}$ μg L^{-1}) samples is lower than for cachaça produced from nonburned sugar cane crops (21.1 μg L^{-1}). Furthermore, rum samples show average (from 4.92 $\times 10^{-1}$ μg L^{-3} to 1.78 $\times 10^{-1}$ μg L^{-1}) and median contents (4.77 $\times 10^{-1}$ μg L^{-3} to 9.94 $\times 10^{-2}$ μg L^{-2}) lower than of those observed for cachaça (**Table 4**).

Even though the carcinogenic/mutagenic properties of PAHs have been demonstrated (*25*), it is difficult to extrapolate toxicity data from animals to humans and to stipulate the PAH levels that may constitute a health risk for consumers (*2*, *25*). The European Union (EU) recently adopted a legal limit of 2 μg L^{-1} for BaP content, as an indicator of the presence of PAHs in foodstuffs (*26*). In spite of this situation, the German Society

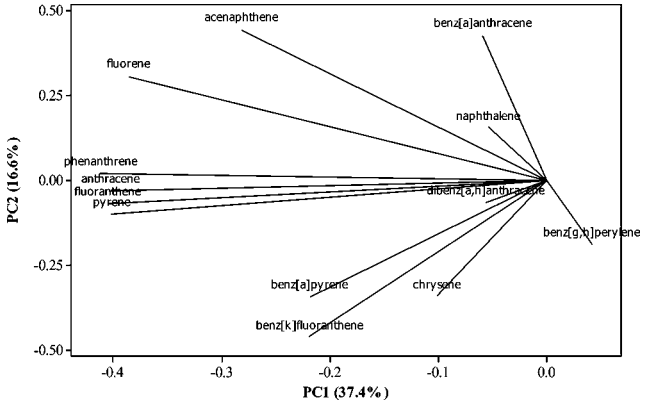

Figure 4. Loading plot for PAHs.

for Fat Science has suggested the following limits: 25 μg L^{-1} for total PAHs and 5 μg L^{-1} for high molecular mass PAHs in food products (*7*).

The average and sum of averages for PAH contents in cachaça produced from nonburned cane and burned sugar cane crops are given in **Table 3**. The levels of BaP in both types of cachaça are lower (nonburned and burned) than the limit established in EU for food products (2 μg L^{-1} for BaP). In relation to the sum of the PAH contents suggested by the German Society for Fat Science, both cachaça from burned (21.1 μg L^{-1}) and nonburned crops (1.91 μg L^{-1}) are below this limit (25 μg L^{-1} for total PAHs content). An identical situation is observed for the heavy fraction of PAHs, which has a limit fixed at 5 μg L^{-1}, and levels found for cachaça produced from burned sugar crops ranging from 2.05×10^{-2} (benz[k]fluoranthene) to 4.95 μg L^{-1} (benz[a]anthracene) and from 8.62×10^{-3} (benz[k]-fluoranthene) to 8.43×10^{-2} μg L^{-1} (CH) for cachaça produced from nonburned sugar cane crops.

The collected analytical data for PAHs were further analyzed by means of PCA using the following chemical descriptors: NA, AC, F, PA, A, FL, P, BaA, CH, BkF, BaP, DBahA, and BghiP. From the PCA analysis, the existence of two different groups was observed in the score plot of **Figure 3**: one consisting of cachaça produced from burned sugar cane crops and the other containing cachaças produced from nonburned sugar cane crops. From the score plot for the two first components (PCs), accounting for 54% of total variance of the original data, a clear separation between groups can be verified (PC1 vs PC2) suggesting the above selected compounds as promising chemical descriptors to identify cachaça produced from burned sugar cane fields. As displayed in **Figure 3** from 26 samples analyzed, only two cachaça samples (#58 and 73) were erroneously classified, most likely due to their low levels of acenaphthene (AC) and fluorene (FL) (Supporting Information).

Examination of PC loadings plot in **Figure 4** shows PC1 (37.4% of original information) correlated with higher levels of most PAHs, whereas BkF, AC, and BaA were the main contributors to PC2 (16.6% of total variance). From the main contributors for each PC, it is clear that cachaça produced from burned crops is correlated with higher levels of nearly all PAHs. On the other hand, cachaça produced from nonburned crops is strongly correlated with a low incidence of almost all PAHs except BghiP (**Table 3**).

The CDA was applied in order to classify the cachaça samples from burned and nonburned sugar cane crops. The use of NA, AC, F, PA, FL, CH, and BghiP as chemical descriptors was found optimal for classification purposes.

Employing CDA in the analytical data also provides a good separation for the two groups of cachaças. The model (**Table 5**) was constructed using 103 cachaça samples (19 produced from burned sugar cane crops and 84 from nonburned sugar cane crops), and a training group (**Table 6**) was formed by 28 cachaça samples (seven produced from burned sugar cane crops and 21 from nonburned sugar cane crops).

According to **Table 5**, only two cachaça samples produced from burned sugar cane crops were erroneously classified, both belonging to "true samples" (73 and 76), and all samples produced from nonburned cane sugar crops were correctly classified in the modeling group leading to a correct assignment of 98.1%. **Table 6** illustrates the data obtained for the training

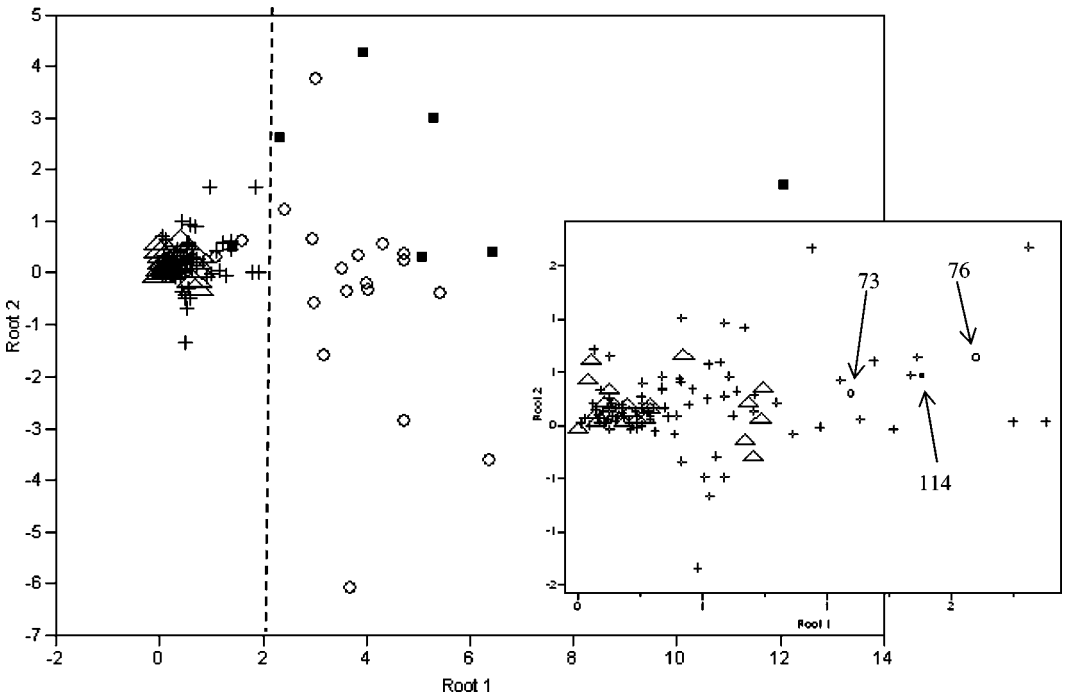

Figure 5. Score plot for CDA analysis. Group model: burned (○) and nonburned (+). Test group: burned (■) and nonburned (△). The inset is an enlargement of part of the plot.

Table 5. Classification Obtained through CDA Analysis for Two Groups of Cachaça

	prediction of sample type	
reference	burned cane sugar crops	nonburned cane sugar crops
burned cane sugar crops	17	0
nonburned cane sugar	2	84
samples	19	84
correctly assignment	17	84
percentage (%)	**89.5**	**100**
total sampling	103	
total correct	101	
% correct assignment	**98.1**	

Table 6. Classification Obtained in the CDA for Training Group

	prediction of sample type	
reference	burned cane sugar crops	nonburned cane sugar crops
burned cane sugar crops	6	0
nonburned cane sugar crops	1	21
samples	7	21
correctly assignment	6	21
percentage (%)	**85.7**	**100**
total sampling	28	
total correct	27	
% correct assignment	**96.4**	

group of CDA analysis. Only sample #114 (nonburned) was erroneously classified in this test group, and the entire samples produced from nonburned cane sugar crops were correct classified. The test of the CDA model for two classes of cachaças leads to an accuracy of 96.4%.

Figure 5 illustrates the CDA scores plot for cachaça taxonomy according to the preharvesting process on the raw material. It is observed that three samples were erroneously predicted, two in "true group" (open circle) and one in "training group" (filled square). Remarkably, the use of PAHs analysis combined with multivariate analysis could ascribe 98.1% of certainty to the taxonomy of these samples.

In conclusion, the qualitative and quantitative PAH profile of cachaças is dictated by harvest prepractice. The content of benzo[a]pyrene in both classes of cachaça is lower than the legal limit established by the EU for foodstuffs. Also, for total PAH content suggested by the German Society for Fat Science, only five samples of cachaça from burned are below the stipulated limit. The main difference between these two classes of cachaças is in the quantitative profile of the most potent carcinogenic PAH, benzo[a]pyrene, which is more abundant in cachaça produced from burned sugar cane crops (4.54×10^{-2} μg L^{-1}) than in cachaça produced from nonburned crops (9.02×10^{-3} μg L^{-1}). The quantitative analytical data for PAHs combined with PCA and CDA analysis allow the identification of cachaça produced from burned crops, with a correct assignment of 98.1%. This analytical protocol can be recommended as a useful routine method for forensic purposes.

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Supporting Information Available: Sample list, ethanol content, and complete analytical data. This material is available free of charge via the Internet at http://pubs.acs.org.

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