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A study of the ethyl carbamate level in cachaça samples

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Abstract

Cachaça is the typical and exclusive denomination of Brazilian sugar cane spirit. In this study, analysis of ethyl carbamate (EC) was carried out in 120 cachaças samples acquired at different regions from the Rio de Janeiro city (north, south, west and downtown). In total, 47% of samples exhibited EC contents above 150 mg L^{-1}. Mean value found in the west region was significantly ($P<0.05$) lower than those from other regions. Although the mean value of the samples from the downtown region was higher than that from northern and southern regions, there were no significant differences ($P<0.05$) between the three regions. Cachaças were classified, as follows: (A) very high concentration (301-800 mg L^{-1}); (B) high concentration (151-300 mg L^{-1}); standardized concentration (0-150 mg L^{-1}) (C). West region presented 3% of samples classified in the upper concentration limit (A), downtown region presented the greatest percentage (27%). In the high concentration class (B), the regions showed similar distribution percentage (ca 30%). In the class (C), west region showed the highest percentage (67%) while downtown region presented the lowest representativeness (43%). A small number (4%) of commercial multi-distilled cachaça exhibited very low contaminant level (<5 µg L^{-1}). Consumers of cachaça sold in the Rio de Janeiro city continue very exposed to the EC.

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Introduction

Brazilian sugarcane spirits, commonly called “aguardentes”, are bottled commercial beverages between 38% and 54% alcohol by volume at 20°C. The trade name “cachaça” is applied only to the spirit made in Brazil, with alcoholic content varying from of 38% to 48% (v/v) (Brazil Ministry of Agriculture, Livestock and Supply, 2005). The annual production of cachaça reaches 1.3 billion liters, making it the third most consumed spirit worldwide (ABRABE, 2009). There is a concern with the presence of potentially carcinogenic substances in beverages. This is the case of ethyl carbamate (EC) or urethane. In animal experiments, it has been shown to cause dose-dependent increases in liver and lung tumours (Beland *et al*., 2005). Some evidences have also shown that it may contribute to the carcinogenicity in regular drinkers of certain types of alcoholic beverages (EFSA, 2007; IARC, 2010). EC occurs naturally in many fermented products as a result of the enzymatic degradation of nitrogenous compounds to urea which reacts with ethanol to form EC (Monteiro *et al*., 1989; Riffikin *et al*., 1989). In spirits, the most probable mechanism involves oxidation of cyanide to cyanate that is catalyzed by cuprous ion. The coordination complex between cyanate and cupric ion is susceptible to nucleophilic attack of the oxygen in ethanol, favouring EC formation (Aresta *et al*., 2001; Taki *et al*., 1992). Presence of cyanogenic glycosides in some crops (e.g. sugarcane) and stone-fruits is possibly the major source of cyanide (Aresta *et al*., 2001; Lachenmeier *et al*., 2009).

In 1985, high levels of EC were detected in different studies and thus Canadian authorities established an upper limit of 150 µg L^{-1} for spirits, based on the consumption patterns and virtually safe dose (Riffkin *et al*., 1989). This value has also been established for spirits in United States, Czech Republic and France (EFSA, 2007). The current Brazilian legislation directly determines the same maximum value for sugar cane spirits (Brazil Ministry of Agriculture, Livestock and Supply, 2005). There have been several studies on the level of EC in cachaça from various Brazilian regions (Andrade–Sobrinho *et al*., 2002; Bruno *et al*., 2007; Labanca *et al*., 2008; Lachenmeier *et al*., 2009; Andrade–Sobrinho *et al*., 2009; Nóbrega *et al*., 2009). Analysing previous data from different states it was observed that cachaça contains high mean values of EC (overall mean 330 µg L^{-1}) (Lachenmeier *et al*., 2010). A summary of findings from our recent review showed that 45% of the sugarcane spirits from different Brazilian States exceeded the allowed maximum limit, with some

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products reaching values above 1200 µg L^{-1} (Riachi *et al*., 2014). On the other hand, analyses of levels of EC in commercial cachaças from Rio de Janeiro city are scarce. A single study carried out with cachaça from various regions of the state of Rio de Janeiro showed that approximately 45% of the spirits were not under the recommended standard (Bruno *et al*., 2007).

The city of Rio de Janeiro has six millions of habitants and thus is an important consumer market. Additionally, this metropolis will be one of the 12 host cities for the 2014 FIFA World Cup Brazil and will host the 2016 Games of the XXXI Olympiad. These competitions are expected to attract hundreds of thousands of people among participants, visitors and tourists. The monitoring of EC in cachaça will help decision-makers make better decisions toward decreasing the level of contamination in order to protect public health. The aim of this study was to determine the content of EC in cachaças sold in the top five supermarket chains located in the city of Rio de Janeiro.

Materials and Methods

Materials

One hundred and twenty samples of cachaça from twenty nine different brands were acquired in the top five supermarkets from four regions (north, south, west and downtown) located in the Rio de Janeiro city. The goal was to analyze brands commonly commercialized in each region. It also would allow monitoring a same brand in different regions. Thirty samples were purchased in each region. For each region, seven samples were aged cachaças. Samples of the same brand were acquired from different lots. All samples were analyzed in three parallels. All the spirits were bought in 2013 and analyzed in the same year of purchase. Urethane (external standard) was from Sigma (St. Louis, USA). Methyl carbamate (internal standard) was from Aldrich (Sheboygan, USA). Absolute ethanol was from Carlo Erba (Milan, Italy). All reagents were from analytical grade.

Sample preparation

EC solutions were prepared in 40% ethanol at concentrations of 20, 30, 50, 100, 300, 400, 600 and 1200 µg L^{-1}. Methyl carbamate (500 µg L^{-1}) was added to each external standard solution and each spirit sample. Samples were introduced to the GC/MS without any pre-treatment as described by Hesford and Schneider (2001). Given the background level of 5 µg L^{-1} as described previously (Caruso *et al*., 2010) individual value below the reporting level has been considered as zero for calculation purposes. Both the linear equation (y = 10.832x + 185.43) and the correlation (R = 0.9989) were determined by the linear regression analysis.

GC/MS

EC analysis was based on previous studies (Labanca *et al*., 2008; Nóbrega *et al*., 2009). A Shimadzu gas chromatograph quadrupole mass spectrometry GCMS-QP2010 (Kyoto, Japan) equipped with a 30 m x 0.25 mm i.d. SupelcowaxTM 10 fused-silica polar capillary column (Supelco, Milford, USA) with a film thickness of 0.25 µm was used. The instrument was operated in the electron ionization mode at 70 eV, taking scans from 20 to 300 m/z in a 1 s cycle. Helium was used as the carrier gas at a flow rate of 1.5 mL min–1. The injector and detector temperatures were 250 and 230^{O}C respectively. Two microliters of the sample were injected in the splitless mode. The column temperature was programmed as follows: the initial temperature was 90^{O}C (2 min), followed by an increase to 150^{o}C at 10^{o}C min-1, then up to 230^{o}C at 40^{O}C min–1. The GC/MS with selected-ion monitoring (SIM) was carried out by monitoring m/z 62 and 75 mass fragments and was based on an internal standard method.

Statistical analysis of data was performed by one way analysis of variance (at the 0.05 significance level) (OriginPro version 8.1.10.86).

Results and Discussion

The sample size was considered adequate to obtaining a reasonable estimate from the level of contamination of the cachaça samples commercialized in the Rio de Janeiro city. As mentioned by Hesford and Schneider (2001), there is the possibility of both methyl carbamate and EC formation in the injector from dirty. Pure ethanol solution was injected into GC/MS for monitoring the formation of carbamate derivatives in the injection chamber. Neither methyl carbamate nor EC were detected. Table 1 shows the average and standard deviation for EC content in each sample and region. The mean EC values of the samples from the west region were significantly ($P<0.05$) lower than those of the samples from the other three regions (downtown, south and north). A possible explanation for this might be that west region has many kinds of brands with higher added value. Distinct regions sell different brands, so the differences could be related more with the brands than the regions. The use of non standardized production practices also could explain the different values of a same brand in different regions. Although the mean

Table 1. EC content of cachaças sold in Rio de Janeiro city

Downtown	
Sample (non aged)	**EC (μg L^{-1}) Mean and SD**
A	431.34 ± 13.97
A2	303.20 ± 21.23
A3	395.39 ± 27.18
A4	497.01 ± 31.65
A5	413.37 ± 17.63
B	494.42 ± 22.28
D	268.12 ± 15.17
D2	268.89 ± 12.39
E	65.75 ± 2.51
E2	81.94 ± 7.36
E3	64.43 ± 8.03
F	131.11 ± 9.13
F2	140.96 ± 5.94
G	< 5.00
H	260.45 ± 13.55
K	270.02 ± 14.08
K2	304.82 ± 21.42
K3	225.71 ± 12.55
L4	183.86 ± 5.59
Mean	252.67 ± 148.79
Sample (aged)	**EC (μg L^{-1}) Mean and SD**
C	< 5,00
E4	104.34 ± 2.11
E5	95.39 ± 5.13
I	64.12 ± 4.04
I2	57.84 ± 17.99
J	12.24 ± 3.99
J2	11.41 ± 2.93
K4	608.80 ± 27.11
L	202.14 ± 9.44
L2	189.12 ± 13.38
L3	196.14 ± 7.36
Mean	140.14 ± 172.46
Total mean (non aged + aged samples)[a]	211.41 ± 164.44

North Zone	
Sample (non aged)	**EC (μg L^{-1}) Mean and SD**
A	281.69 ± 18.75
A2	292.49 ± 10.55
A3	334.37 ± 11.11
A4	354.80 ± 37.18
B	470.26 ± 15.02
D	257.96 ± 16.74
E	42.95 ± 1.26
E2	68.86 ± 2.14
F	171.24 ± 5.81
G	< 5,00
L2	290.27 ± 32.75
N	41.50 ± 15.22
N2	44.33 ± 6.99
N3	52.12 ± 6.24
P	< 5.00
P2	< 5.00
Q	72.68 ± 4.37
S	137.73 ± 33.92
T	226.20 ± 12.51
U	176.01 ± 7.74
V	63,23 ± 2,48
X	237.71 ± 24.84
Mean	172.21 ± 136.97
Sample (aged)	**EC (μg L^{-1}) Mean and SD**
C	33.32 ± 58.45
G2	93.08 ± 16.60
J	53.78 ± 5.41
J2	24.15 ± 3.45
J3	51.57 ± 3.14
L	191.18 ± 5.54
N4	218.08 ± 46.29
R	525.66 ± 8.33
Mean	148.85 ± 168.64
Total mean (non aged + aged samples)[a]	160.24 ± 142.61

South Zone	
Sample (non aged)	**EC (μg L^{-1}) Mean and SD**
A	701.55 ± 58.64
A2	93.63 ± 2.30
A3	340.25 ± 10.94
A4	332.96 ± 4.99
A5	408.60 ± 8.40
A6	212.94 ± 12.07
A7	683.43 ± 20.55
A8	216.39 ± 16.31
E	112.71 ± 13.46
E2	121.76 ± 4.66
E3	113.94 ± 4.74
E4	71.42 ± 2.93
E5	87.89 ± 7.74
E6	117.33 ± 3.78
E7	86.96 ± 6.58
F	223.5 ± 6.22
G	< 5.00
G2	12.82 ± 20.54
L2	299.84 ± 15.44
L3	248.88 ± 24.90
L4	235.25 ± 7.87
L5	300.27 ± 5.68
N	270.95 ± 3.52
Mean	240.61 ± 179.39
Sample (aged)	**EC (μg L^{-1}) Mean and SD**
I	81.48 ± 4.00
I2	81.29 ± 3.89
J	29.63 ± 3.16
J2	101.17 ± 1.47
L	317.14 ± 8.19
M	102.00 ± 18.37
O	424.38 ± 26.91
Mean	172.52 ± 159.04
Total Mean (non aged + aged samples)[a]	214.35 ± 174.84

West Zone	
Sample (non aged)	**EC (μg L^{-1}) Mean and SD**
A	189.95 ± 6.29
A2	72.62 ± 3.36
A3	176.29 ± 38.37
A4	192.38 ± 3.58
B	153.24 ± 9.24
B2	219.03 ± 4.29
D	229.59 ± 15.86
D2	225.83 ± 10.29
E	201.19 ± 2.52
E2	20.12 ± 1.28
E3	25.38 ± 4.20
F	52.52 ± 13.04
G	< 5.00
H	101.14 ± 17.91
L	30.98 ± 1.82
L2	52.24 ± 3.61
N2	12.79 ± 3.86
P	< 5.00
X	123.05 ± 5.15
W	31.13 ± 5.97
Z	5.81 ± 1.46
Mean	101.20 ± 85.20
Sample (aged)	**EC (μg L^{-1}) Mean and SD**
I	< 5.00
K	170.38 ± 4.99
N	110.62 ± 1.62
AA	< 5.00
AA2	< 5.00
BB	< 5.00
R	301.17 ± 60.60
Y	26.46 ± 2.02
CC	112.77 ± 4.04
Mean	80.16 ± 104.62
Total Mean (non aged + aged samples)[b]	94.89 ± 90.12

All regions	
Sample	**EC (μg L^{-1}) Mean and SD**
Non aged	190.70 ± 151.56
Aged	131.17 ± 148.37
Total mean	170.22 ± 152.80

< 5.00 = samples with values below 5 μgL-1 were considered zero for calculus purpose; EC = ethyl carbamate; SD = standard deviation; Each sample (same bottle) was analyzed in three parallels; Letter followed by different numbers (e.g. A1, A2 etc) means different lots of the same brand; Means with different superscripts are significantly different at p<0.05

value of the samples from the downtown region was higher than that from northern and southern regions, there were no significant differences ($P<0.05$) between the three regions. There was no significant ($P<0.05$) difference between aged and non aged samples which accorded with previous reports (Andrade-Sobrinho et al., 2002). A small number (4%) of commercial multi-distilled cachaças has been analyzed and it was found a very low content of EC (<5 µg L^{-1}). This may be attributed to the fact that boiling point of EC (182−185°C) is higher than temperatures generally used in the distillation process performed under properly controlled conditions. Typically, the higher the number of controlled distillation carried out, the lower the level of EC in the final distillate. However, other authors obtained levels above 150 µg L-1 in 70% of the redistilled samples. They found no difference between single and double distillation (Nóbrega *et al.*, 2009).

In previous work (Caruso *et al.*, 2010), samples were distributed into three groups according to the level of EC. This classification was adopted with small variation in the present study, as follows: standard concentration (0-150 µg L^{-1}); high concentration (151-300 µg L^{-1}); very high concentration (301-800 µg L^{-1}). Lower percentage (3%) of samples containing very high amount was encountered in the west region. Conversely, downtown region presented the highest percentage (27%). In the high concentration range, the distribution percentage was similar between regions (ca 30%). In regard to standardized concentration limit, west region showed the highest percentage (67%) whereas downtown region presented the lowest percentage (43%). High levels of contamination could be due to overheating and low reflux rate by not using cooling devices (e.g. a dephlegmator) and reflux systems (e.g. bubble cap trays) during distillation (Bruno *et al*., 2007; Nóbrega *et al*., 2009; Riachi *et al*. 2014). Another practice that also promotes increase of the level of contaminant is the use of a single distillation.

Conclusion

In spite of the availability of knowledge and technology for the production of good-quality cachaça, the result reported here indicated that about 47 % of cachaça samples presented EC contents above 150 µg L^{-1} which was similar to the value of 45 % reported in 2007 (Bruno *et al*., 2007). The fact is that reduction of EC in cachaça has been null in the last years and therefore consumers of cachaça sold in the Rio de Janeiro city continue to be very exposed to the contaminant. Brazilian authorities must require the use of standardized production practices that result in high-quality sugar cane spirit. Preliminary data suggested that the intake of multi-distilled cachaça would be more recommended for carioca consumer safety.

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