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Assessment of Ethyl Carbamate Contamination in Cachaça (Brazilian Sugar Cane Spirit)

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Abstract: Cachaça is a sugar cane spirit produced in Brazil. Ethyl carbamate (EC), a potential carcinogenic compound, may be present in cachaça above the limit established by law. The purpose of this study was to determine the concentration of ethyl carbamate in cachaças recently produced in Brazil in order to verify their compliance with the law. The concentration of ethyl carbamate was determined in 376 samples of cachaça through gas chromatography coupled to a mass spectrometer (GC-MS). The mean value of ethyl carbamate in the cachaças analyzed was 145 μg/L, and 24% of them were not in compliancy with the law (EC < 210 μg/L). However, compared to previous studies, advances have been observed regarding the adjustment of cachaças to the legal limit. Cachaças produced in large distilleries through continuous column distillation presented a mean value of 200 μg/L of ethyl carbamate. Cachaças produced in small distilleries using pot still distillation presented a mean content of 74 μg/L. Small producers have been more engaged in using good manufacturing practices to guarantee the quality of cachaças.

Keywords: sugar cane spirit; cachaça; chemical contamination; ethyl carbamate; law

1. Introduction

Cachaça is the typical and exclusive denomination of sugar cane spirit produced in Brazil, which can reach 38%–48% ABV (alcohol by volume) at 20 °C, obtained by distillation of fermented sugar cane juice [1].

The cachaça production process is not standardized, either in choosing the varieties of sugar cane, fermentation conditions, or in distillation equipment. Distillation may be carried out either in copper pot stills or in continuous columns. Aging cachaça in wooden barrels is optional.

Aside from presenting sensory properties such as an aromatic bouquet characteristic of sugarcane, flavors and pleasant aromas which satisfy the consumer, high quality cachaça must achieve certain chemical parameters so that it does not pose a health hazard if consumed moderately [2]. Low concentrations of contaminant compounds and acetic acid (the latter responsible for the acidity of cachaça) define the chemical quality of the spirit. The main compounds considered to be contaminants in cachaça are methanol, *sec*-butanol, *n*-butanol, ethyl carbamate, and copper.

Ethyl carbamate is a carcinogen in experimental animals, and is probably carcinogenic to humans (Group 2A) [3]. Its risk assessment may be conducted using the margin of exposure (MOE) approach, and the calculated lifetime cancer risk using the T25 method. The lower the MOE, the larger the risk for humans. The threshold of 10,000 is often used to define public health risks. The MOE from all alcoholic beverages was calculated to range between 400 and 2,466, indicating a great public health concern. The MOE for moderate drinkers of cachaça in Brazil ranged from 800 to 8,267. An even higher risk may exist for binge-drinkers of cachaça, with MOEs between 59 and 235. The lifetime cancer risk is at approximately 3 cases in 10,000, reaching 1 case per 1000 if alcoholic beverages with extremely

high levels of ethyl carbamate were to be consumed on a daily basis. The whole population exposure from cachaça was calculated to be around 60 to 70 ng/kg bw/day. Nevertheless, the average ethyl carbamate exposure for daily drinkers of cachaça would be 400 ng/kg bw/day. According to the risk assessment, ethyl carbamate poses a significant cancer risk for the alcohol-drinking population in Brazil, in addition to the risk associated with alcohol alone [4].

The risks for individual drinkers in Brazil appeared to be higher than the ones in Europe [5], which stated MOEs of 4,620 to 8,110 for consumers of alcoholic beverages in general, and specifically an MOE of 5,000 for consumers of spirits (125 mL daily).

In order to protect consumers' health, identity and quality standards have been established for cachaça in Brazil [1] which establish limits for non-alcoholic volatile components, as well as organic and inorganic contaminants that could be present in the spirits. In August 8th 2014, Brazilian law was revised for the maximum concentration of ethyl carbamate allowed in cachaça, 210 μg/L [6]. Before this date, the maximum value allowed was 150 μg/L.

Several types of alcoholic beverage where the production process involves microbiological fermentative activity may contain ethyl carbamate [7]. The distillation process normally contributes to an enhancement of ethyl carbamate content in distilled beverages, because of the high temperature [8] and the presence of copper in the pot stills, which catalyzes the formation of this compound [9,10]. A positive correlation between copper and ethyl carbamate content in recently distilled sugar cane spirit has been reported. Concentrations of 0.8 mg/L of copper in the distillate fraction was enough to promote complete formation of ethyl carbamate, and higher levels of the element did not show an additional catalytic effect [11].

The presence of ethyl carbamate in alcoholic beverages is important due to the toxicological aspects, since it is a potentially carcinogenic compound [12], and therefore it should be kept at the minimum possible levels. Some countries, such as Canada, the United States, France, Germany, and Switzerland have specific laws to control the presence of ethyl carbamate in alcoholic beverages [13]. Besides being a public health problem, ethyl carbamate levels above 210 μg/L in cachaças may constitute a barrier to export.

Known precursors of ethyl carbamate are cyanide, urea, citrulline, and *N*-carbamyl phosphates [8,13,14]. The main pathway of ethyl carbamate formation in sugar cane spirits involves the cyanide ion formed by enzyme degradation of cyanogenic glycosides present in sugar cane. Cyanide is oxidized to cyanate, which in turn reacts with ethanol in the presence of the copper ion, forming ethyl carbamate [10]. A correlation between cyanate and ethyl carbamate formation in sugar cane spirits has been observed [15].

Urea is also a precursor of ethyl carbamate in alcoholic beverages, through the reaction with ethanol. Its presence in cachaça comes from urea-containing soil fertilizers, or from a supplementary source of nitrogen for sugar cane juice for fermentation. The formation of ethyl carbamate in wine has been associated with high concentrations of urea during fermentation [16]. Urea may also be formed by the degradation of arginine. The thermal decomposition of urea during distillation releases cyanic acid, a precursor of ethyl carbamate in spirits [9,17]. A strong correlation between the presence of urea in fermented juice and ethyl carbamate concentrations in sugar cane spirit has been observed [15]. Supplementation of sugar cane juice with urea caused the production of higher concentrations of ethyl carbamate in cachaças [18].

In addition to the precursors, the formation of ethyl carbamate in spirits depends on factors such as the type of microorganisms involved and by-products of their metabolism, fermentation temperature, distillation process, alcohol concentration, acidity, pH, light, and period of storage after distillation [9,19–23].

Relatively high concentrations of ethyl carbamate are generally reported in cachaças [11,24–27]. A compilation of 13 studies, involving 536 samples, showed an average of 380 μg/L of ethyl carbamate in cachaças [4].

Conversely, the method of double distillation may significantly reduce the concentration of ethyl carbamate in distilled spirits. Since the boiling point of ethyl carbamate is 182–185°C, the content of this compound in distilled beverages can successfully be monitored during a well-conducted distillation process. In whisky, for instance, it was observed that only 1% of the ethyl carbamate formed during the first distillation was distilled during the second distillation [9]. The "tail" distillate fraction accumulated 15% of the ethyl carbamate formed, and the stillage retained 84% of the ethyl carbamate present in the second distillation. In cachaça, double distillation promoted reduction of ethyl carbamate from 74% to 91% in the spirit [18]. Commercial cachaças presenting the lowest concentrations of ethyl carbamate (38 to 48 μg/L) were double distilled [28]. Redistillation reduced the concentration of ethyl carbamate in 15 samples of commercial cachaça by up to 92.5% [29]. Double distillation reduced the concentration of ethyl carbamate in cachaça by 97% [30]. In another study, Alcarde [31] reported reductions of this contaminant in sugar cane spirits ranging from 94.0% to 98.5%, depending on the reflux rate during the second distillation in different types of still. In the state of Rio de Janeiro, 34 samples of cachaça collected from 28 producers were assessed, and the mean content of ethyl carbamate found was 160 μg/L [11]. The sample obtained by double distillation in a copper pot still presented only 17 μg/L of ethyl carbamate.

This study aimed to determine the concentration of ethyl carbamate in recently produced cachaças and to verify the evolution of cachaças in compliance with the national legislation since the investigation of ethyl carbamate (EC) concentration in sugar cane spirits started to be carried out in Brazil.

2. Materials and Methods

2.1. Sample Collection

During 2014 and 2015, 376 samples of cachaça were analyzed—165 in 2014, and 211 in 2015. The samples of spirits, purchased in the local markets, were produced in the Brazilian states of São Paulo (52%), Minas Gerais (12%), Ceará (9%), Pernambuco (8%), Paraíba (5%), Mato Grosso do Sul (3%), Goiás (2%), Bahia (2%), Rio Grande do Sul (2%), Rio de Janeiro (2%), Paraná (1%), Santa Catarina (1%), and Espírito Santo (1%). The number of samples of each brand ranged from three to five, depending on their availability.

2.2. Reagents and Standards

Gas chromatography grade ethyl carbamate (Sigma-Aldrich, St. Louis, MO, USA) with purity >99% was employed. High performance liquid chromatography grade ethanol (Merck, Kenilworth, NJ, USA) and ultrapure Milli-Q water (Millipore, Molsheim, France) were used for dilutions. The samples, standards, and solvents were previously filtered in Millex-HV filters (Millipore) with polyvinylidene difluoride (PVDF) membrane (13 mm diameter, 0.45 μm pore size).

2.3. Analytical Methods

Samples with no previous treatment were analyzed for ethyl carbamate in a gas chromatograph coupled to a mass spectrometer, model GCMS-QP2010 Plus (Shimadzu, Kyoto, Japan), using selected ion monitoring (SIM) acquisition (m/z = 62), equipped with a chromatography capillary column with polar phase of esterified polyethylene glycol—HP-FFAP (Hewlett Packard—Free Fatty Acids and Phenols; 49 m × 0.20 mm × 0.33 μm stationary phase film thickness). The temperatures of the injector and the detector interface were 230 °C and 240 °C, respectively. The following temperature program was used in the oven: starting with 90 °C for 2 min, increasing to 150 °C at a rate of 10 °C /min, followed by heating up to 220 °C at a rate of 40 °C /min, and kept at this temperature for 2 min. An aliquot of 2.0 μL was injected using the splitless injection mode. Helium was used as the carrier gas at 30.0 cm/s [30].

The quantification of ethyl carbamate was performed based on an external calibration curve ($C = 174.5 \times A - 38.9$; $r^2 = 0.99$) constructed using six points of standard concentrations. The detection limit (DL = 1.6 μg/L) and quantification limit (QL = 5.1 μg/L) were calculated based on the signal-to-noise ratio of the chromatograms [32].

3. Results

The median values of ethyl carbamate in cachaças analyzed in 2014 was 137 μg/L, and the value for the cachaças analyzed in 2015 was 52 μg/L (Table 1). Nevertheless, a marked difference is noted between the mean values and the medians, indicating a skewed distribution of analytical results.

Table 1. Means and medians of the concentration of ethyl carbamate (μg/L) in samples of cachaça analyzed during the years 2014 and 2015.

	Mean	Median
2014 (165 samples)	171	137
2015 (211 samples)	124	52
Total (376 samples)	145	89

Mean = arithmetic mean; median = 50th percentile.

The maximum value observed was 975 μg/L for the cachaças analyzed in 2014, and 790 μg/L for those analyzed in 2015. The minimum value found was <DL for both years. According to the Brazilian legislation, 29% of the samples analyzed in 2014 were not within the law (EC < 210 μg/L). Regarding the cachaças analyzed in 2015, 21% of the samples were not compliant with the law (Table 2).

Table 2. Percentage of samples of cachaça within the concentration ranges for ethyl carbamate (μg/L).

	Percentage of samples within the concentration range for ethyl carbamate				Samples not in compliance with law (%)
2014	<150	150–<210	210–<300	>300	>210
(165 samples)	55.7	15.8	15.2	13.3	28.5
2015	<150	150–<210	210–<300	>300	>210
(211 samples)	72.0	7.1	6.2	14.7	20.9
Total	<150	150–<210	210–<300	>300	>210
(376 samples)	64.9	10.9	10.1	14.1	24.2

Even with the increase of the maximum limit allowed for ethyl carbamate in cachaças (from 150 to 210 μg/L) recently established by Brazilian law [6], a representative part of cachaças (24%, Table 2) still present ethyl carbamate content above the maximum legal limit.

Cachaças produced in large distilleries through continuous column distillation (209 samples analyzed) presented a median value of 148 μg/L of ethyl carbamate (Table 3). Cachaças analyzed in 2014 (70 samples) produced in small distilleries using pot still distillation presented a median value of 73 μg/L of ethyl carbamate, and those analyzed in 2015 (97 samples) presented 15 μg/L.

Table 3. Mean and median of the concentration of ethyl carbamate (μg/L) in cachaças produced from small distilleries (pot still distillation) and large distilleries (column distillation) during the years 2014 and 2015.

	Small distilleries (pot still distillation)		Large distilleries (column distillation)	
	Mean	Median	Mean	Median
2014	129	73	200	160
2015	34	15	201	120
Total	74	30	200	148

Mean = arithmetic mean; median = 50th percentile.

4. Discussion

Since the analyses of ethyl carbamate in cachaça began, advances have been observed regarding the adjustment of these spirits to the legal limit (Table 4). In 2001, around 80% of cachaças were not in compliance with Brazilian law. In 2015, only 30% of cachaças presented contents of ethyl carbamate above the maximum legal limit. Concerning the cachaças analyzed in the present study, 24% were not compliant with the law. Nevertheless, around 14% of the samples analyzed still presented ethyl carbamate contents above 300 μg/L (Table 2), which is much higher than the maximum legal limit of 210 μg/L.

Table 4. Literature data on assessment of ethyl carbamate contamination in cachaça indicating the evolution of the percentage of samples not in compliance with Brazilian law over the years. LD: limit of detection.

Reference study	Sample characterization	Collection sites	*N*	Minimum (μg/L)	Mean (μg/L)	Maximum (μg/L)	Percentage above 210 μg/L
Boscolo, 2001 [33]	Brazilian market	Different states, Brazil	84	42	904	5,589	84
Andrade-Sobrinho et al., 2002 [34]	Column/pot still, Brazilian market	Different states, Brazil	126	13	770	5,700	76
Bruno et al., 2007 [11]	Column/pot still, experimental and Brazilian market	Rio de Janeiro State, Brazil	34	<LD	160	714	35
Baffa Júnior et al., 2007 [24]	Brazilian market	Minas Gerais State, Brazil	22	5	1,206	12,376	82
Labanca et al., 2008 [25]	Pot still, Brazilian market	Minas Gerais State, Brazil	71	33	893	2,609	91
Nóbrega et al., 2009 [26]	Pot still, Brazilian market	Paraíba State, Brazil	25	55	221	700	40
Nóbrega et al., 2011 [27]	Column/pot still, Brazilian market	Pernambuco State, Brazil	33	<LD	181	532	42
Borges et al., 2014 [21]	Pot still, experimental	Minas Gerais State, Brazil	30	44	141	320	27
Present study, samples 2014	Brazilian market	Different states, Brazil	165	<LD	171	975	29
Masson et al., 2014 [35]	Pot still, Brazilian market	Minas Gerais State, Brazil	71	23	191	980	30
Riachi et al., 2014 [36]	Column/pot still, Brazilian market	Different states, Brazil	657	<LD	257	1,206	42
Present study, samples 2015	Brazilian market	Different states, Brazil	211	<LD	124	790	21
Bortoletto and Alcarde , 2015 [37]	Brazilian market	Different states, Brazil	268	4	221	1,365	39

Earlier studies have indicated that most of the cachaças produced in Brazil presented a higher ethyl carbamate content than the upper permitted limit of 210 μg/L. Ethyl carbamate contents ranging from 42 to 5,589 μg/L, with a mean value of 904 μg/L, were observed in 84 samples of cachaça from several regions of Brazil [33]. Only 16% of these samples presented ethyl carbamate contents below the maximum legal limit of 210 μg/L. Contents ranging from 13 to 5,700 μg/L of ethyl carbamate, with a mean value of 770 μg/L, were determined in 126 samples of cachaça (63 from the Southeast Region, 39 from the Northeast Region, 22 from the South Region, and 2 from the Midwest Region of Brazil) [34]. Only 24% of these samples had ethyl carbamate contents below 210 μg/L.

Other studies in Brazil also found high contents of ethyl carbamate in commercial sugar cane spirits and cachaças. Twenty-two brands of cachaça from Zona da Mata Mineira, in the state of Minas Gerais, registered contents of this contaminant ranging from 5 to 12,376 μg/L, with a mean value of 1,206 μg/L [24]. Among the 22 samples analyzed, only four (18%) presented ethyl carbamate contents below 210 μg/L. Ethyl carbamate content above the maximum legal limit was found in 35% of 34 samples of cachaça collected from 28 different producers in the state of Rio de Janeiro [11]. The content of ethyl carbamate ranged from 33 to 2,609 μg/L, with a mean value of 893 μg/L, in 71 samples of sugar cane spirits produced in the state of Minas Gerais [25]. Only 9% of the samples presented contents below the maximum legal limit. The contents of ethyl carbamate ranged from 55 to

700 μg/L, with a mean value of 221 μg/L, in 25 cachaças from 19 different distilleries in the state of Paraíba; 40% of the samples exceeded the legal limit [26]. A study with 33 cachaças from 19 different distilleries in the state of Pernambuco found that the maximum content of ethyl carbamate in the samples analyzed was 532 μg/L, and 14 samples (42%) exceeded the limit of 210 μg/L [27].

A compilation of 24 studies showed that 42% of 657 cachaças analyzed presented ethyl carbamate contents above 210 μg/L [36]. A similar value (39%) was found analyzing ethyl carbamate content in 268 cachaças produced all over Brazil [37]. Ethyl carbamate levels were determined in 30 cachaças produced in the State of Minas Gerais by spontaneous fermentation or selected *Saccharomyces cerevisiae* strains, and only eight samples (27%) presented ethyl carbamate contents above 210 μg/L [21]. The ethyl carbamate concentrations in samples of cachaça produced in the state of Minas Gerais varied from 22 to 980 μg/L, and 70% of the samples analyzed had concentrations equal to or below the maximum legal limit [35]. A similar value was found in the present study.

In the present study, cachaças produced in large distilleries through continuous column distillation presented concentrations of ethyl carbamate (median 148 μg/L, N = 209) higher than those produced in small distilleries (median 30 μg/L, N = 167). These results show the concern of the small producers in decreasing the level of the contaminant in cachaça using good manufacturing practices (GMP) in order to guarantee the quality of the production process and aiming to control the quality of the spirit.

Cachaças from different regions of Brazil presented a mean value of 930 μg/L for those distilled in columns (69 samples) and 630 μg/L for those distilled in pot stills (34 samples) [34]. In the state of Rio de Janeiro, 32 samples of cachaça collected from 28 producers were assessed, and the mean content of ethyl carbamate found was 160 μg/L [11]. The 10 samples obtained by distillation in a continuous column presented a mean value of 320 μg/L of ethyl carbamate, and the 17 samples obtained by simple distillation in a copper pot still presented a mean value of 145 μg/L ethyl carbamate. The five samples obtained by distillation in a stainless steel pot still with a coiled copper pipe presented a mean value of 90 μg/L of the contaminant.

In a literature review (comprising 13 studies) on the occurrence of ethyl carbamate in cachaças purchased in the Brazilian market, a total of 536 analytical results provided an average of 380 μg/L of the contaminant in the samples analyzed [4]. However, taking into account the weighting of data based on the production of cachaças in pot still (38%) and column still (62%), the average content of this contaminant was 450 μg/L. Column still cachaça (mean 490 μg/L, N = 101) had a higher content of ethyl carbamate than pot still cachaça (380 μg/L, N = 275).

Thirty-three cachaças (13 from pot stills and 20 from column stills) produced in the state of Pernambuco presented averages of 60 μg/L for pot still cachaças, and 257 μg/L for those distilled in columns [27].

According to the compilation of 24 studies [35], single distilled pot still cachaças (35 samples) presented an average concentration of ethyl carbamate of 75 μg/L. On the other hand, the average concentration of ethyl carbamate in cachaças distilled in continuous columns (214 samples) was 493 μg/L.

The risks for individual drinkers in Brazil appeared to be higher than the ones in Europe [38]. The evaluation of levels of ethyl carbamate in European spirits during the years 2010–2012 indicated that 14% of the analytical results were above the target value of 1 mg/L indicated in Commission Recommendation 2010/133/EU. The highest occurrence levels of ethyl carbamate were found in the group "spirits made from stone fruits" (mean 698 μg/L; median 214 μg; N = 1945). Although the mean occurrence was below the target value of 1 mg/L, 15 % of the results were above this value.

In the group "spirits not from fruit" the mean occurrence was 55 μg/L (median = 38 μg/L; N = 20), which is lower than in the previous group by almost a factor of 13. The mean occurrence in the subgroup "rum" was 65 μg/L (N = 4). In the subgroup "vodka and vodka-like spirits", a mean occurrence of 66 μg/L (median = 50; N = 10) was observed. The subgroup "whisky" presented a mean of 30 μg/L (median = 20 μg/L; N = 6). All of the analytical results for the group "spirits not from fruit" were below the target value of 1 mg/L.

Considering the group "spirits not from fruit", in which cachaça could be included, the mean occurrence of 145 μg/L (median = 89 μg/L; $N = 376$) found in the present study was roughly three times the mean value presented by rum, vodka, and whisky.

5. Conclusions

The quality of cachaça depends on two main factors: sensory properties agreeable to consumers, and chemical composition that does not pose risks to their health. Among the 377 samples of cachaça analyzed during the years 2014 and 2015, 27.8% were not in compliance with the limit of ethyl carbamate established by Brazilian law. Furthermore, based on the high standard deviation among the samples analyzed, cachaça presents a great heterogeneity concerning the concentration of the contaminant in the spirit. This suggests that producers continue to face difficulties in using GMPs aiming to ensure consistency in the production process. However, since the analyses of this contaminant have started, advances have been observed regarding the adjustment of ethyl carbamate levels in cachaças to the legal limit.

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