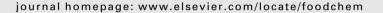
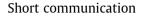
Food Chemistry 117 (2009) 693-697

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Food Chemistry





## Ethyl carbamate in pot still cachaças (Brazilian sugar cane spirits): Influence of distillation and storage conditions

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#### ARTICLE INFO

Article history: Received 20 February 2009 Received in revised form 23 March 2009 Accepted 20 April 2009

Keywords: Ethyl carbamate Cachaça Brazilian sugar cane spirit Pot still Saccharum L. Food contamination Alcoholic beverages

#### ABSTRACT

Ethyl carbamate (EC), a known genotoxic carcinogen, was studied in 25 brands of pot still cachaças from 19 distilleries in Paraíba State, Brazil. A concentration range of 55–700 µg/l was found with most brands ( $\sim$ 70%) exceeding the international EC limit for spirits (150 µg/l). Brand characteristics (colour, distillation [single or double], and bottle colouration) showed no consistent connection with EC levels. However, when EC levels of yellowish (cask matured) and colourless single-distiled cachaças from the same distillery were compared, the yellowish type was much more heavily contaminated. Eleven distilleries were visited and information regarding the distillation scale, kettle heating system, kettle's shape, and cooling system of the column was collected. A close connection between EC levels and cooling system was found, with the non-cooled and cooled columns dominating the brands with high (200–700 µg/l range) and low (55–100 µg/l range) contamination levels, respectively.

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#### 1. Introduction

According to Brazilian legislation (DOU, 2005), "cachaça" is a beverage made in Brazil from the distillation of fermented fresh sugar cane juice and bottled with between 38% and 48% alcohol by volume; it may have not more than 30 g of sugar per litre added to the end product.

The Brazilian cachaça industry generates 450,000 direct jobs and production has remained stable since 1995, with an annual rate of 1.3 billion litres, making cachaça the third most consumed spirit worldwide (ABRABE, 2009; Cachaça Official Guide, 2005).

Cachaça's production methods vary enormously in Brazil, particularly the distillation and preparation of yeasts and must (Faria, Franco, & Piggott, 2004). Distillation is generally carried out in discontinuous pot stills (alembics) or continuous column stills, producing the so-called pot still and column still cachaças. Pot still cachaças are typically un-sweetened and more expensive than column still cachaças. Although most cachaças are colourless ("white"), many yellowish cachaças also result from maturation in casks.

Ethyl carbamate (EC, C<sub>2</sub>H<sub>5</sub>OCONH<sub>2</sub>, CAS # 51-79-6), a known animal and possibly human carcinogen (IARC, in press; Zimmerli & Schlatter, 1991), occurs in many fermented foods, particularly alcoholic beverages (EFSA, 2007; Lachenmeier, Schehl, Kuballa, Frank, & Senn, 2005), where it is thought to form from the reaction between ethanol and nitrogen-containing compounds, such as urea, citrulline and hydrogen cyanide; the latter compound normally derives from cyanogenic glycosides present in raw materials (EFSA, 2007).

In 2002, an average EC level of 770 µg/l was reported from a large survey in commercial cachaças (Andrade-Sobrinho, Boscolo, Lima-Neto, & Franco, 2002), causing concern in Brazil. In 2005, following EC legislation in other countries, Brazil established a 150 µg/l EC limit for cachaça, which is coming into effect in June 2010 (DOU, 2005). Some recent surveys of commercial cachaças have continued to show very high levels of EC (Baffa Júnior, Soares, Pereira, & Melo, 2007; Labanca, Glória, & Afonso, 2008); but one has reported an apparent decline in concentration (Andrade-Sobrinho et al., 2009). However, detailed product and process description is normally scarce in these surveys, which makes finding connections between EC levels and certain types of cachaça difficult.

There is an ongoing debate about ways to control EC in cachaça, but simple aspects of its formation are not yet fully understood, such as the influence of the sugar cane plant itself, known to be cyanogenic (Jones, 1998), distillation and aging. Some interesting findings have been seen on the distillation side (Andrade-Sobrinho et al., 2009; Bruno et al., 2007), but supporting scientific data are needed to confirm them.

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In this paper we report on quantifying EC in commercial brands of pot still cachaças from Paraíba State, Northeastern Brazil; and discuss the results in light of the brands' characteristics and distillation profile.

#### 2. Materials and methods

#### 2.1. Sampling of cachaças and selecting distilleries

Duplicate samplings of 25 brands of cachaças, produced in all 19 approved pot still distilleries in Paraíba, the third major Brazilian State in pot still production of cachaça (Cachaça Official Guide, 2005), were conducted from retail outlets in Paraíba's capital, between September and October 2007. In order to obtain a valid representation of each brand's EC level, samples of different batch codes were purchased. General characteristics of the brands, including the distillation method, were obtained from local inspecting authorities and from label information. Detailed information on distillation was collected in visits to 11 distilleries, which were selected on the basis of their interest in participating in the project. According to label information, the samples contained no added sugar.

#### 2.2. Chemicals

Ethyl carbamate (99.0%), for calibration, and propyl carbamate (98.0%), internal standard, were purchased from Chem Service (West Chester, USA) and Aldrich (Milwaukee, USA), respectively. The analytical solutions were dissolved in LC grade ethanol (Merck, Darmstadt, Germany) at 40% (v/v).

#### 2.3. Analysis of ethyl carbamate

Preparation of calibration curves and EC analysis were based on Andrade-Sobrinho et al. (2002) and Reche et al. (2007),

#### Table 1

respectively. An Agilent 6873 autosampler was used to introduce, in splitless mode, 2 µl aliquots of each cachaça brand (n = 2) onto a capillary column (FFAP, 50 m × 0.2 mm i.d. × 0.3 µm film thickness; HP) installed in an Agilent 6890 gas chromatograph (GC) coupled to an Agilent 5973 mass selective detector (MS). The MS was operated in the electron impact mode with an ionisation energy of 70 eV and helium at 1.5 ml/min was used as carrier gas. The GC oven was initially kept at 90 °C (2 min), followed by an increase to 150 °C (0 min) at 10 °C/min, then up to 230 °C at 40 °C/min. The injector temperature was 250 °C, and the GC/MS interface was maintained at 230 °C. Quantification was performed in terms of single ion monitoring mode for m/z 62 mass fragment and was based on an internal standard procedure. The limits of detection and quantitation were 10 and 40 µg/l of EC, respectively.

#### 2.4. Analysis of alcoholic strength

The alcoholic strenghts (% volume at 20 °C) of the spirits were determined using a Densimat hydrostatic balance coupled to an Alcomat converter (Gibertini Elettronica, Milano, Italy).

#### 3. Results and discussion

#### 3.1. EC levels in commercial brands of pot still cachaças

Table 1 shows the EC concentrations, in increasing order, of 25 brands of pot still cachaças produced in Paraíba State, Brazil. EC was detected in all samples. The brands' EC concentration range and average value were 55–700  $\mu$ g/l and 221  $\mu$ g/l, respectively. Ethyl carbamate levels in 70% of the brands exceeded the international limit for spirits (150  $\mu$ g/l).

The average EC levels found in pot still cachaças from Paraíba State are considerably lower than the mean values reported previously for 34 samples of pot still cachaças from different parts of Brazil ( $630 \mu g/l$ , Andrade-Sobrinho et al., 2002) and for several

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Cachaça brand	$EC^{a} \pm SD^{b} (\mu g/l)$	Alcoholic strength (% vol)	Cachaça colour <sup>c</sup>	Distillation <sup>d</sup>	Bottle colour <sup>e</sup>	Distillery
01	55 ± 5	44.3	W	S	А	А
02	55 ± 5	39.9	W	S	С	В
03	60 ± 10	38.0	W	S	С	С
04	75 ± 5	37.2	W	S	С	D
05	90 ± 0	43.5	W	S	С	E
06	$100 \pm 0$	39.0	W	S	С	F
07	125 ± 15	44.4	Y	D	С	F
08	$140 \pm 30$	42.0	Y	S	С	Н
09	165 ± 15	42.4	Y	S	С	Ι
10	165 ± 5	42.0	W	S	А	J
11	$180 \pm 10$	42.0	W	S	А	K
12	195 ± 5	38.3	Y	S	С	L
13	195 ± 25	42.3	Y	S	С	А
14	200 ± 50	43.6	W	S	С	М
15	$220 \pm 20$	41.3	W	S	А	Ν
16	225 ± 45	39.2	W	S	С	0
17	230 ± 30	38.4	Y	S	С	D
18	235 ± 15	40.4	W	S	А	G
19	$240 \pm 0$	37.9	Y	S	С	Р
20	295 ± 15	36.7	Y	S	С	В
21	360 ± 60	38.9	Y	D	С	Q
22	385 ± 85	38.5	Y	S	С	Ν
23	$410 \pm 10$	40.1	W	D	С	Q
24	$420 \pm 10$	43.2	W	S	С	R
25	700 ± 100	46.9	W	S	А	S
Mean	221					

<sup>a</sup> Ethyl carbamate levels (in increasing order) of cachaça brands are the mean of duplicate sampling of different batch codes.

<sup>b</sup> SD, standard deviation.

<sup>c</sup> Cachaça colour, according to visual inspection, varied from white (colourless, W) to yellowish (pale to dark yellow, Y).

<sup>d</sup> Distillation type (single – S or double – D) of product, according to information obtained from label and local inspecting authorities.

<sup>e</sup> Bottle colour, according to visual inspection, varied from clear (colourless, C) to amber (brown, A).

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