

# Influence of the distillation processes from Rio de Janeiro in the ethyl carbamate formation in Brazilian sugar cane spirits

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## Abstract

Ethyl carbamate (EC) or urethane, a potentially carcinogenic substance found in significant amounts in distilled fermented beverages, has become the main technical obstacle to the exportation of Brazilian sugar cane spirits (*cachaças*). In this evaluation, the EC levels were measured in samples of recently distilled beverages and of finished products, collected at 28 production plants using different distillation systems, from various regions of the state of Rio de Janeiro. The results obtained through GC–MS, using selective monitoring of ions with  $m/z$  62 and 74, showed that approximately 45% of the products and raw materials are above the maximum allowed value of 150  $\mu\text{g/l}$ , with an EC average value of 160  $\mu\text{g/l}$ . The lower values were those for samples from alembics using low temperature and high reflux rate distillation, which emphasized the role of these two variables on the EC levels.

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**Keywords:** Ethyl carbamate; GC–MS; Brazilian sugar cane spirits; Distillation processes; Reflux; Temperature

## 1. Introduction

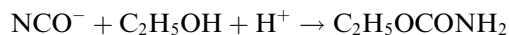
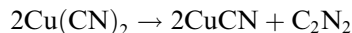
The results of laboratory tests performed in mice and rats, injected with up to 0.1 mg of ethyl carbamate (EC) per kg of body weight, showed a greater incidence of malignant tumors and leukemia. These tests were conducted to establish an “acceptable daily intake” (ADI) of 0.3 mg per kg of body weight. In 1985, various studies conducted in Canada detected the presence of EC in wines and distilled beverages and established a maximum value of 150  $\mu\text{g/l}$  in distilled beverages, based on the consumption patterns and on the ADI evaluation (Riffkin, Wilson, Howie, & Müller, 1989). The United States organized internal voluntary programmes to lower the levels of EC for whisky to 125  $\mu\text{g/l}$ , after 1989 (Canas, Joe, Diachenko, & Burns, 1994) and, today, impose this value as the maxi-

imum limit for all imported distilled beverages. Recently, Brazil established a five year limit for sugar cane distilleries to attain the same maximum acceptable level as Canada (DOU, 2005).

EC occurs naturally in many foods and beverages as a result of their microbiological activity. In table wines, the levels are usually around 10–50  $\mu\text{g/l}$  (Canas et al., 1994; Riffkin et al., 1989), as a result of the enzymatic degradation of arginine into urea, which reacts with ethanol to produce EC (Monteiro, Eugene, & Bisson, 1989; Zietsman, Viljoen, & van Vuuren, 2000). The EC levels increase with product overheating during the distillation of spirits, especially in the distillation of some beverages rich in cyanogenic glycosides, such as amygdalin in some stone fruit brandies for which concentrations over 1500  $\mu\text{g/l}$  have been reported (Hesford & Schneider, 2001; Schehl, 2005). Similar to several other agricultural raw materials, sugar cane (*Sacharum officinarum*) is also considered a cyanogenic crop (Aresta, Boscolo, & Franco, 2001).

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One chemical pathway proposed for the EC formation involves the oxidation of cyanide ( $\text{CN}^-$ ) to cyanate ( $\text{NCO}^-$ ) catalyzed by Cu(II) ions (Aresta et al., 2001; Beattie & Polyblank, 1995; Mackenzie, Clyne, & McDonald, 1990) followed by reaction with ethanol (Aresta et al., 2001; Taki, Imamura, Takebe, & Kobashi, 1992) according to the reaction scheme below:



Although EC precursors and its origin in sugar cane spirits have not yet been well established, another possible mechanism involves the formation of isocyanic acid, which is released directly from the thermal decomposition of urea present in the wort. Aresta et al. (2001), have reported other possible mechanisms, most of them involving cyanide, copper and cyanate.

According to Riffkin et al. (1989), who used an experimental copper alembic to produce whisky “low wines”, the amount of EC formed in the first two hours after distillation, in the presence of 0.8 mg/l of copper, represents about 20% of the EC’s final concentration. Approximately 80% of EC were formed within 48 h in the fresh distillate. By using an Amberlite IRC ion exchange resin a complete inhibition of the EC formation was also verified in fresh distillates when copper ions were suppressed.

Double distillation has been the most common procedure for removing ECs from distilled spirits, because of their low volatility in alcoholic solutions (Riffkin et al., 1989). Nevertheless, this procedure generally leads to losses in ethanol yield and aroma. Boscolo (2001), analyzed 84 samples of *cachaças* from various Brazilian regions, with EC levels varying from 42 to 5689  $\mu\text{g/l}$  and an average value of 904  $\mu\text{g/l}$ . Only 13% presented EC levels below the maximum international limit established (150  $\mu\text{g/l}$ ). They reported the occurrence of smaller amounts of EC in Brazilian sugar cane spirits obtained from distillation systems where the descendent parts (end part of adapter and serpentine coil) are made of stainless steel. However, these systems produce spirits with sensorial defects, mainly due to the presence of sulphur compounds (Andrade-Sobrinho, Boscolo, Lima-Neto, & Franco, 2002; Faria, Loyola, Lopes, & Dufour, 2003). In alembics entirely made of copper, these off-odors usually react more effectively with this metal producing an odorless salt (Andrade-Sobrinho et al., 2002).

Andrade-Sobrinho et al. (2002) reported the presence of high levels of EC when the distillation system presents a small area made of copper in the ascendant parts. They used 126 samples of commercial *cachaças* from several states and reported an average value of 770  $\mu\text{g/l}$ . The average value of the *cachaça* samples produced using distillation columns was of 930  $\mu\text{g/l}$ , whereas those from alembics showed an average of 630  $\mu\text{g/l}$ .

A preventive action commonly reported for the reduction of the EC levels in distilled spirits consists of fitting the upper portion of the columns with either a bubble cap tray or a section packed with copper rings (Bujake, 1992) or other copper devices (Andrade-Sobrinho et al., 2002).

The efficiency of the still is a function of its design and of the operation of the column as well as of the reflux apparatus associated with the still head. This part of the still divides the condensate so that a portion (D) is taken off as a fresh distillate (P) and the remainder (L) is returned to the column as a reflux liquid. The ratio L/P is called the reflux ratio. In addition to other factors, the columns give better performance at lower throughputs (Peters, Hayes, & Hieftje, 1974) and higher temperature gradients. Under these conditions, the residence time in the column is longer, which results in an equilibrium improvement, distillations with lower temperatures for the ethanol–water vapor phase, which is richer in ethanol than the liquid phase, and in an increased reflux rate (Maia, 2000; Peters et al., 1974). Thus, the present research is concerned with the evaluation of the influence of various small- and medium-scale distillation processes (apparatus and distillation procedure) in the EC formation in Brazilian sugar cane spirits.

Although the EC levels have been previously measured in several commercial Brazilian distilled beverages, this is the first national report documenting surveys carried out employing sugar cane freshly distilled samples taken directly from stills.

## 2. Materials and methods

### 2.1. Selection of producing sites and sampling of *cachaças*

The EC concentration was measured and evaluated for 34 sugar cane spirit samples from 28 main producers (total of 30 different distillation systems) from various regions of the Rio de Janeiro State. The first 17 samples consisted of freshly distilled fractions collected in glass flasks from 13 different distillation systems (two producers have two alembics) and four of them were recollected afterwards. Samples from alembics were collected in the heart fractions. Seventeen samples were bottled *cachaças* acquired directly at the distilleries. The selection criteria of the 28 producing locations were their different distillation systems, their legal condition, economic importance and the quality of their facilities. These systems were divided in seven continuous distillation columns (DC); 16 alembics made entirely of copper (CA, pot still); five stainless steel alembics with a copper serpentine coil (SSC); 1 alembic made entirely of stainless steel (SSS); and one double distillation copper alembic (DDC).

Due to their low commercial importance in the Rio de Janeiro State and to their low sensorial quality, *cachaças* with added sugar were not used in this study.

Most of the alembics have thermometers fixed on the cap and on the dephlegmator, used to measure the distillation temperatures and the temperature at which the entrainment starts.

## 2.2. Reagents and standards

Capillary GC–MS analysis was performed using the external ethyl carbamate (EC) and the internal *n*-propyl carbamate (*n*-PC) standards bought from Aldrich Chemicals. The stock and the analytical solutions were dissolved, respectively, in LC grade ethanol (Merck) and 45% (v/v) ethanol. The same procedure was used to dilute the analytical grade reagents (Merck) for the analysis of congeners.

A Merck copper standard (1000 mg/l) was employed to make the analytical curves in the determination of copper by flame atomic absorption spectrometry. Reagents of analytical grade were used in the analysis of congeners.

Triple-distilled water, subsequently passed through a Milli-Q system, was used to prepare the solutions of all samples.

## 2.3. Determination of EC

Capillary GC–MS was the method employed for the analysis of EC levels according to Canas et al. (1994), however, the described clean up and extraction procedures were suppressed, as described by Hesford and Schneider (2001). Ethanol 45% (v/v) was used for all sample dilutions. The *n*-PC internal standard was added directly to the vial containing 1 ml of the sample, without any pre-treatment. A Hewlett Packard 5973 MS quadrupole detector coupled to a HP 6890 gas chromatograph (a) and a Shimadzu gas chromatograph mass spectrometer GCMS-QP2010 (b) were used. Selected ion monitoring (SIM) was applied to MS detection as the data acquisition mode for low-resolution

ions  $m/z$  62 and 74. The separation of EC from the congeners and other compounds in the *cachaças* was carried out in a HP INNOWax (30 m × 0.25 mm × 0.25 μm) capillary column (a) and in a HP-FFAP column (50 m × 0.25 mm × 0.3 μm) (b).

A split mode (5:1) was used in (a) and a splitless mode in (b). The injection port temperature was 230 °C and 250 °C, and the detector temperature was 260 °C and 220 °C, for (a) and (b), respectively. Helium was used as the carrier gas at a flow rate of 1.0 ml/min (a) and 1.2 ml/min (b). The column temperature for (a) was programmed as follows: the initial temperature was 40 °C, then it was raised at a rate of 10 °C/min up to 185 °C; and, then, from 185 °C to 230 °C at a rate of 30 °C/min, and held for 4 min at this final temperature. The temperature for (b) changed as follows: initial temperature was 90 °C, which was maintained for 1 min; then raised from 90 °C up to 150 °C at a rate of 10 °C/min; and from 150 °C up to 220 °C at a rate of 30 °C/min.

## 2.4. Determination of copper

The copper content was determined by flame atomic absorption spectrometry (FAAS) carried out using a Varian model 1475 and a Perkin–Elmer model 3300 atomic absorption spectrometers. For most of the cases, the analytical method employed was the AOAC 26.1.18 method (1995). In some cases, the analyte in the sample was concentrated by boiling, to evaporate part of the ethanol, and, subsequently, treated with 1:1 HNO<sub>3</sub>:H<sub>2</sub>O and 1:1 HCl:H<sub>2</sub>O.

## 2.5. Determination of alcoholic grade

The alcoholic grade was determined using an Anton Paar density meter, operating at 20 °C. The samples from

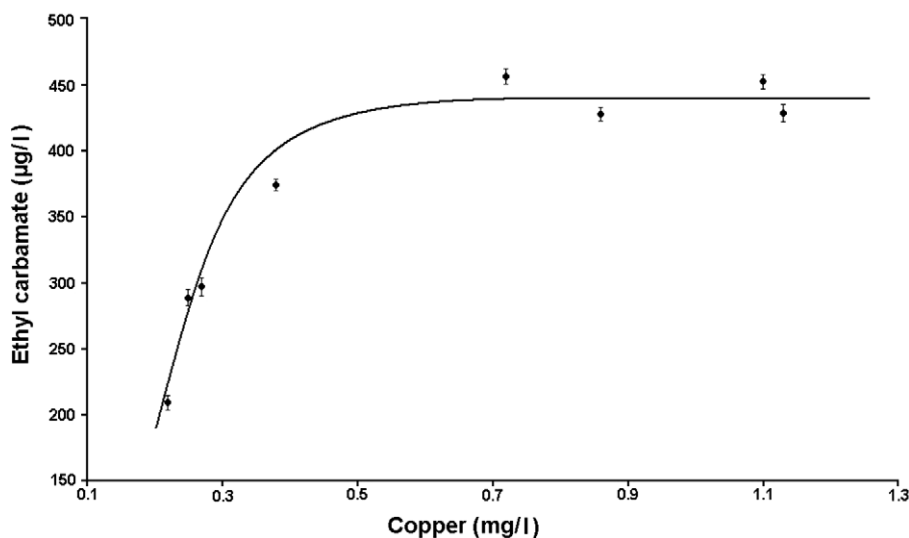


Fig. 1. EC levels as a function of copper concentration in PCA freshly distilled, eluted on Dowex Marathon C resin beds with increasing depths. The error bars show the standard deviations of EC measurements ( $n = 3$ ).

commercial *cachaças* were distilled before being injected in the density meter. An official table (DOU, 1986) was used to convert the density results to alcoholic grade (vol%) at 20 °C.

## 2.6. Selection of cationic resin and ion exchange apparatus

The Dowex Marathon C cationic resin (copolymer of styrene divinyl benzene sodium sulfonate) was the resin chosen due to its high selectivity towards copper ions, its commercial availability and to the homogeneity of its small beads, which improves the kinetics, resulting in higher operating capacity. First, the ion exchange resin was treated with 45 vol% ethanol (6 × 500 ml) inside the glass column (25 × 750 mm) containing a sintered glass disk of high porosity for resin retention. An additional 1000 ml of the

distillates were collected from the condenser in glass flasks and half of this volume was immediately eluted through the cationic resin producing two samples (treated and untreated). When larger volumes needed to be collected without changing an elution time of one hour, a second column with the same characteristics was used.

In order to correlate the ethyl carbamate concentration with the Cu content, 1000 ml fractions of the fresh distillate of a sample named PCA, collected in the condenser, were immediately percolated through the resin beds with various heights. These various heights were obtained by the successive addition of resin to the glass column. About 100 ml of eight samples obtained in this way were analyzed. The average elution flow rate was 17 ml/min. All of these samples were kept under refrigeration and analyzed about one month after being collected.

Table 1  
Main characteristics and EC levels of the distillation systems evaluated

Producer/sample	Distillation system/heating system	EC ± SD (µg/l)	Confidence limits (±µ)	Apparatus coupled (alembics)	Dephlegmator
EDF	D CA/DF	<LOQ	–	*	*
PTJ	D CA/DF	45	8	*	*
PLK	C CA/DF	214 ± 4	9	*	*
JLG (1st)	D CA/DF	73 ± 1	2	Pre-heater	Tubular (Cu)
JLG (2nd)	D CA/DF	298 ± 4	10	Pre-heater	Tubular (Cu)
CQE (A)	D CA/DF	142 ± 1	2	Pre-heater	Bowl jacket
CQE (B)	D CA/DF	156 ± 3	7	Pre-heater	Bowl jacket
CRD (A)	D CA/DF	95 ± 6	15	Pre-heater	Bowl jacket
CRD (B)	D CA/DF	195 ± 6	15	Pre-heater	Bowl jacket
PHM	C CA/SG	89 ± 2	5	Pre-heater	Tubular (Cu)
SLS	C CA/SG	50 ± 1	2	Pre-heater	Tubular (Cu)
BVS	C CA/SG	<LOQ	–	Pre-heater	Tubular (Cu)
SRV(1st)	C CA/SG	<LOQ	–	Pre-heater	Tubular (Cu)
BAV	C CA/SG	27 ± 2	5	Pre-heater	Tubular (Cu)
RCL	C CA/SG	30 ± 1	2	Pre-heater	Tubular (Cu)
CHV	C CA/SG	595 ± 4	10	Pre-heater + WSA	Bowl jacket
MAG	C CA/SG	232 ± 5	12	Pre-heater + WSA	*
SBH	D SSC/SG	79 ± 2	5	*	Tubular (SS)
PRI	D SSC/SG	35 ± 1	2	*	Tubular (SS)
CTS	C SSC/SG	17 ± 2	5	*	Tubular (SS)
DNC	C SSC/SG	215 ± 1	2	*	Tubular (SS)
CPT	C SSC/SG	106 ± 3	7	*	Tubular (SS)
PCA (1st)	D DC/SG	714 ± 7	17	*	*
PCA (2nd)	D DC/SG	456 ± 5	12	*	*
VMC(1st)	D DC/SG	262 ± 2	5	*	*
VMC (2nd)	D DC/SG	155 ± 2	5	*	*
BTB (1st)	D DC/SG	40 ± 3	7	*	*
BTB (2nd)	C DC/SG	61 ± 2	5	*	*
CAC	C DC/SG	323 ± 4	9	*	*
SFC	C DC/SG	252 ± 2	7	*	*
VRI	C DC/SG	216 ± 6	15	*	*
CAM	C DC/SG	607 ± 2	5	*	*
SRR	D SSS	11 ± 2	5	*	*
NFB	C DDC	17 ± 3	8		*

CA: copper alembic; SSC: alembic with stainless steel pot and copper serpentine; DC: continuous distillation column; SSS: alembic entirely in stainless steel; DDC: double distillation in a big pot still used for distillation of whisky; C: commercial *cachaças*; D: distillates; SG: steam generator; DF: direct fire; WSA = wine stripping alembic; \* – absent; (1st), (2nd) – first and second sampling; A, B – alembics A and B of the same producer; SD: standard deviation.

### 3. Results and discussion

#### 3.1. Correlation between the internal standard calibration and the standard addition curves

The linear regression for the internal standard calibration curve (a) was expressed by  $y = 0.00151x + 0.00615$  ( $R = 0.9996$ ), where  $y$  is the EC/PC peak area ratio and  $x$  the EC concentration ( $r = 0.99967$ ). Its homoscedasticity was confirmed by Cochran's test (Massart et al., 1997). The standard addition curve ( $y = 0.0153x + 0.41438$ ;  $R = 0.9981$ ) was obtained from the enrichment of an aged *cachaça* collected at the bottom of an "ipê" (a Brazilian tree of the *Bignonia* family) barrel, with a height of 5 m. The curves showed similar slopes, which indicated the absence of matrix interference. This experiment also allowed to verify the recoveries ( $n = 3$ ) at different levels, which were above 90%.

#### 3.2. Repeatability standard deviation (RSDr)

RSDr values were below 15% over the whole range of the method.

#### 3.3. Determination of detection and quantification limits

The limit of detection (LOD) was estimated initially using a dilution of a double distilled *cachaça* at a concentration level close to the blank's LOD, and using a Hewlett Packard GC–MS. LOD and LOQ values of 3.7 and 11  $\mu\text{g/l}$  were calculated by an available built-in software, through the use of the signal-to-noise (S/N) ratio of 3.sd and 10.sd (sd = standard deviation), respectively. Similarly, an LOQ = 10  $\mu\text{g/l}$  was estimated, (starting with the dilution of an analytical solution), when the Shimadzu GC–MS was used.

#### 3.4. Influence of copper concentration in the EC formation

The copper reduction efficiency is mainly a function of the height of the resin column. The greatest decrease on the EC peak area occurred when the sugar cane freshly distilled fraction from VMC was treated with a selective resin. The sample was percolated for about an hour and a reduction on the EC levels from 272 to 50  $\mu\text{g/l}$  (about 80%) was observed. This reduction was similar to that described by Riffkin et al. (1989) for freshly distilled samples, where only 20% of the EC level were produced during the first hour of reaction. The production of ethyl carbamate is discontinued after the copper source is removed.

The ethyl carbamate levels measured as a function of an increase in copper content in the experiment are shown in Fig. 1. The initial value of 150  $\mu\text{g/l}$  (in the  $y$ -axis) was determined from other experiments with various sugar cane freshly distilled samples from other producers, for which an average reduction of 65% was obtained and copper levels were reduced to less than 0.01 mg/l (Bruno, Vaitsman,

Kunigami, Sanctos, & Brasil, 2003). In the previous experiment, using another PCA fresh distillate, a maximum reduction of 67% in the ethyl carbamate levels was observed, when the concentration of copper was reduced to 0.16 mg/l. Therefore, in order to achieve a more complete reduction of EC, the copper concentration in the distillates should be around 0.15 mg/l.

About 0.8 mg/l was enough to promote a complete ethyl carbamate formation and higher concentrations of copper did not promote any additional catalytic effect.

#### 3.5. Influence of the distillation systems and of the distillation process conduction on the EC levels

As shown in Table 1, only one out of the seven medium sized continuous distillation columns produced EC levels below the maximum limit of 150  $\mu\text{g/l}$ . Ten out of the 16 copper alembics complied with this limit. The results also show that the average of EC levels in the *cachaças* from alembics was lower than the average for *cachaças* from distillation columns (Fig. 2). The results are partially in agreement with those reported by Andrade-Sobrinho et al. (2002) for *cachaças* from various states in Brazil. However, the average for *cachaças* from Rio de Janeiro was 5–6 times lower than that described by Boscolo (2001) and Andrade-Sobrinho et al. (2002). About 45% of the products and distilled fractions exceeded the maximum allowed limit of 150  $\mu\text{g/l}$ , with an average of  $160 \pm 68$   $\mu\text{g/l}$ .

At first, the lower EC levels found in pot still alembics could be explained by the reactions of cyanide with copper in the ascending parts, which produce non-volatile complexes (Andrade-Sobrinho et al., 2002; Aresta et al., 2001; Boscolo, 2001). However, two alembics, without any copper-made part or device inside the columns, produced

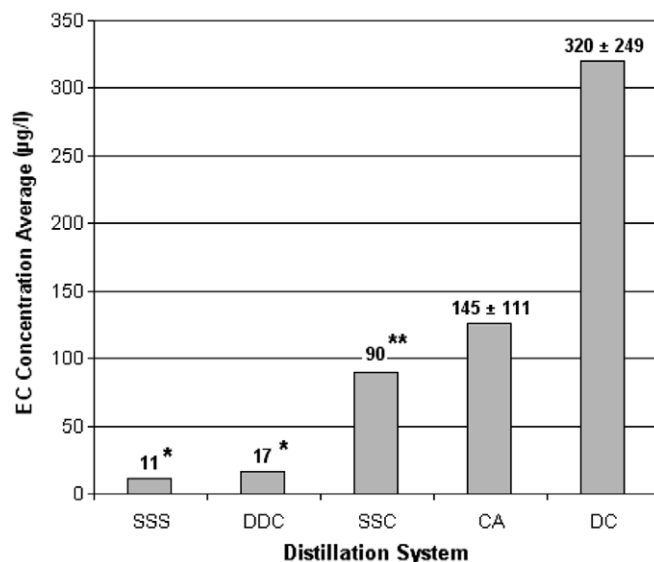


Fig. 2. EC concentration averages for each type of distillation system used for *cachaças* ( $\pm$ confidence intervals;  $P = 0.05$ ). \*One sample; \*\*non-parametric distribution.

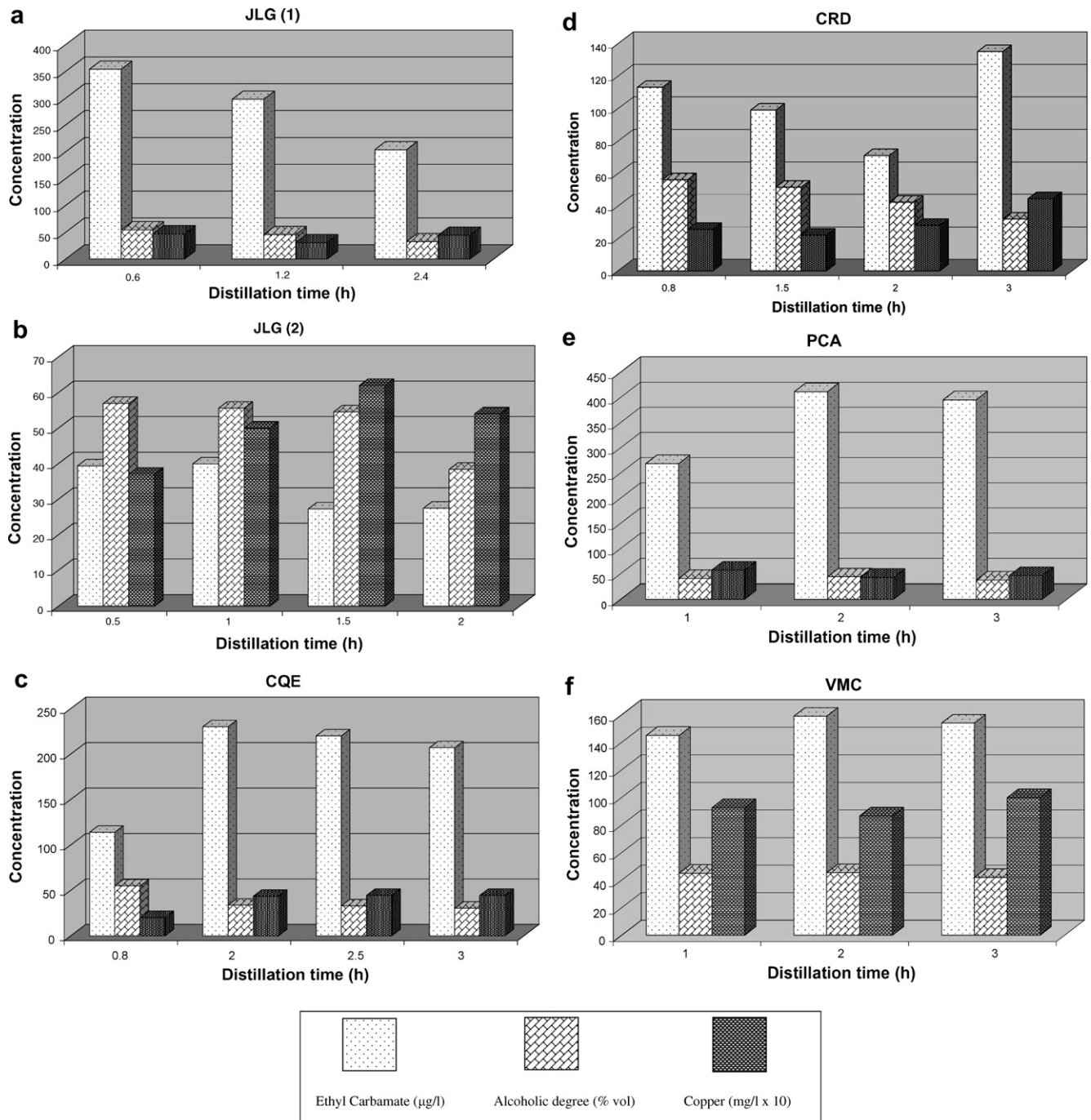


Fig. 3. Ethyl carbamate formation throughout different distillation processes. Cu levels and alcoholic grade were measured in the fractions collected at various times during the distillation process. (a) Distillation in JLG with uncontrolled reflux; (b) distillation in JLG with controlled reflux; (c)–(f) distillations in the CQE, CRD, PCA and VMC systems. Each fraction in the histogram was analyzed for ethyl carbamate, ethanol and copper.

low EC levels (<10 and 45 µg/l). These alembics are characterized by a distillation process with low flow rates and temperatures, and seemed to be favoured by their appropriate design.

A wine with an ethanol content of 8% v/v boils around 94 °C. For pot still systems, as the alcoholic content decreases, the temperature of the wine increases. However, it is possible to keep the vapour temperature lower than 80 °C in the dephlegmator by increasing the water flow rate

inside it during the distillation process. Fig. 3a and b shows that it was possible to drastically reduce the EC levels in the fresh distillate fractions from a copper alembic labeled as JLG through this control method. On the other hand, the other five alembics that produced lower ethyl carbamate levels (<LQ to 50 µg/l), with the same copper tubular dephlegmator inside the column head of JLG, have two lower sections with stainless steel or copper bubble cap trays (Fig. 4), which leads to high separation efficiencies.

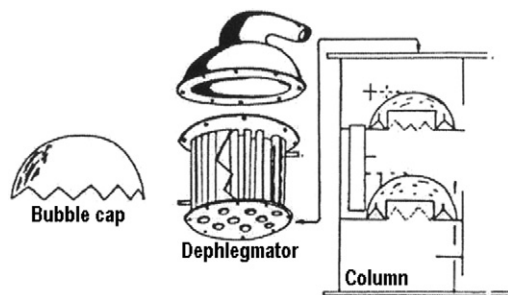


Fig. 4. Scheme of the parts from an alembic column designed to afford an efficient reflux.

In the alembics with a condensing bowl jacket around the still top, it is not possible to properly control the reflux, which, sometimes, can result in distillation temperatures higher than 90 °C and accelerate the distillation process. This is the case for the CRD, CQE and CHV alembics. The highest EC level among all alembics was found in the CHV unit (595 µg/l), which has in the bottom a wine stripping alembic producing alcohol vapors which will heat it through the bowl jacket and make the final distillation fraction.

We observed that the presence of cloudiness was very common when the temperatures in the column head were above 80–82 °C. As a result of entrainment (APV, 2005; Tham, 2006), there is an enrichment of less volatile components, such as nitrogenous compounds, into the lower parts of the distillation system (serpentine coil, etc.), which, in the presence of Cu(II) and increasing temperatures, could eventually promote the decomposition or oxidation reactions responsible for the highest EC levels formed within these systems. These reasons could explain the high EC levels found in the heart and tails from the copper alembics (with a pre-heater) CQE (Fig. 3c) and CRD (Fig. 3d), respectively.

As shown in Fig. 3a, the high EC production in the beginning of the first distillation in the JLG (1) could be related to the high initial distillation temperatures (>90 °C), which promoted the early entrainment mentioned previously and subsequent depletion of the nitrogen precursors. An expressive reduction in the EC levels was observed in the second distillation in the JLG (2) as a result of an adjustment of the reflux, which was obtained by controlling the water flow circulating in the tubular dephlegmator. In this case, the distillation temperatures stayed around 78 °C (Fig. 3b).

The lower EC levels seem to come from alembics operating with high reflux ratios, low throughputs and low distillation temperatures (<80 °C). The highest EC levels were measured for those alembics with improper reflux ratios, emphasizing that the reflux ratio plays an important role in the EC formation.

The values for the only alembic made entirely of stainless steel agree with those reported by Boscolo (2001), emphasizing the importance of copper for the EC forma-

tion. As expected, the double distilled *cachaça* sample analyzed, produced in a large alembic imported from Scotland, also showed a low EC level. Copper levels in those two systems were 0.01 mg/l and 0.02 mg/l, respectively.

Striking differences in the EC levels measured were also observed for the different continuous distillation columns. Poorly conducted rectification, usually due to the excess of vapor and of wine feeding into the columns (Belincanta, Kakuta Ravagnani, & Pereira, 2006; Kalid, 2002) could explain the high EC levels found in most of these systems. In those cases, flooding (in the trays) and entrainment can occur at the same time (APV, 2005; Kalid, 2002; Tham, 2006).

Although there is also a significant variation on the EC levels from different distillation columns, few changes occurred during the distillation processes within these systems (continuous processes) when the temperature did not vary significantly.

#### 4. Conclusion

The results indicated the great dependence of EC levels in *cachaças* on the configuration of the distillation system and on the distillation procedure itself. The EC formation is favoured by the entrainment of nitrogenous precursors during the distillation and by the high temperatures resulting from poor distillation systems i.e. with wrong design or operating with improper reflux rates. The average EC level found in the *cachaças* from the state of Rio de Janeiro analyzed was about 4–6 times lower than the Brazilian average reported in the literature.

The analytical method for determination of EC in *cachaças*, without any pre-treatment, was shown to be rapid, efficient, without interference and adequate for the quantification of EC in levels about ten times below the limit concentrations.

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