

Copper(II) Catalysis in Cyanide Conversion into Ethyl Carbamate in Spirits and Relevant Reactions

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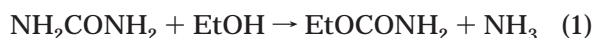
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The role of copper(II) species in the oxidation of inorganic cyanide to cyanate and in the conversion of cyanate or urea into ethyl carbamate was investigated. The oxidation process has been shown to be independent from the dissolved oxygen. Elemental analysis and infrared spectroscopy have shown the formation of a mixed copper carbonate/hydroxide in the process of oxidation of cyanide to cyanate in water/ethanol. The complexation to Cu^{II} of cyanate formed upon cyanide oxidation makes the former more susceptible to nucleophilic attack from ethanol, with conversion into ethyl carbamate. Comparatively, urea has a minor role with respect to cyanide in the formation of ethyl carbamate. Therefore, the urea present in some samples of Brazilian sugar cane spirit (cachaça) has been shown to have almost no influence on the ethyl carbamate content of cachaças, which comes essentially from cyanide. Fe^{II,III} affords results similar to those found with Cu^{II}. Some suggestions are presented to avoid ethyl carbamate formation in spirits during distillation.

Keywords: Cyanate; cyanide; urea; ethyl carbamate; spirits; alcoholic beverages

INTRODUCTION

Ethyl carbamate or urethane (molecular formula H₂NCOOC₂H₅; CAS Registry No. 51-79-6) is the ethyl ester of carbamic acid and is industrially obtained by the direct reaction of urea with ethanol (eq 1) (1). Ethyl



carbamate naturally occurs in fermented food products (2) such as bread, cheese, yogurt, and alcoholic beverages at micrograms per liter levels. Alcoholic beverages, mainly spirits such as whiskey, brandy, grappa, and cachaça (Brazilian sugar cane spirit), show the highest ethyl carbamate content, which in some cases reach levels as high as several hundreds of micrograms per liter.

Because ethyl carbamate has been shown to be a potential carcinogen when administered to animals (3), in 1985 regulations to limit its amount in beverages were established in Canada (4). The ethyl carbamate level is restricted to 30 µg/kg in table wines, 100 µg/kg in fortified wines, 150 µg/kg in distilled spirits, and 400 µg/kg in fruit brandies and liqueurs. These guidelines have been used as a reference in other countries, which do not have a specific legislation on this issue. However, the food industry is paying more and more attention to keeping the ethyl carbamate level in their products to as low as possible.

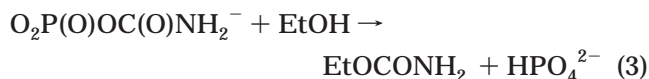
Research about the genesis of ethyl carbamate in food began in the early 1970s (5). Its formation from diethyl carbonate, a food additive used for controlling microbial activity, by reaction with ammonia present in wine was proposed (eq 2).



This is not the only way to form ethyl carbamate. As a matter of fact, ethyl carbamate may be formed following different pathways, according to the nature of the food/beverage considered and the precursors available in the raw material.

In fact, in later studies the formation of ethyl carbamate in wine was correlated to the concentration of urea and the concentration increased substantially with increasing temperature (6). The majority of the urea produced in wine comes from the arginase-catalyzed degradation of arginine (7). High urea levels can occur in wines produced from grapes with high arginine contents. Such grapes tend to come from vineyards heavily fertilized or displaying high vigor (8).

Carbamyl phosphate (CP) produced by yeast (*Saccharomyces cerevisiae*) can react with ethanol to generate ethyl carbamate in wine (eq 3). CP comes from the



arginine synthesis catalyzed by carbamyl synthase involving ATP, CO₂, and ammonia (9). Intermediates such as O₂P(O)OC(O)NH₂⁻ are easily formed also in vitro (10).

Azodicarbonamide, used as a blowing agent in beer bottle cap liners and as a bread improver, has been also suggested as an ethyl carbamate precursor (11). Interestingly, the ethyl carbamate level in these food products does not reach quantities >10 ppb. Therefore, such routes are unlikely to generate the whole amount of ethyl carbamate found in spirits.

The formation of ethyl carbamate in spirits can occur before, during, and after the distillation process (12). The formation before the distillation probably follows the same pathway discussed above and proposed for

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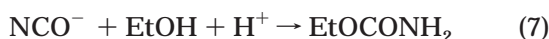
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wines, but its contribution to the final amount is not significant, because ethyl carbamate has a boiling point of 185 °C and the distillation process removes almost all ethyl carbamate formed (13).

Cyanide ion has been indicated as the most important ethyl carbamate precursor in spirits (12). It is formed by enzymatic action and thermal cleavage of cyanogenic glycosides such as epiheterodendrin in barley (13) and amygdalin in stone fruits (1). Sugar cane (*Saccharum officinarum* L.), the raw material for Brazilian cachaça, is classified as cyanogenic crop, but its cyanide source is yet unknown (14).

Two chemical pathways have been proposed to be most likely to occur for the formation of ethyl carbamate from cyanide. The first is based on the complexation of cyanide to Cu^{II} followed by its oxidation to cyanogen, with subsequent disproportionation to cyanate and cyanide (15). Cyanate may react with ethanol to form ethyl carbamate (Scheme 1). Copper most likely is released from the distillation apparatus, upon corrosion.

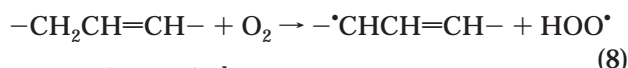
Scheme 1



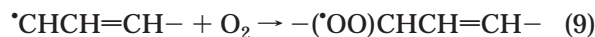
Different copper(I) cyanide species [CuCN, Cu(CN)₂⁻, Cu₂(CN)₃⁻, and Cu₃(CN)₄⁻] were detected in the pot still apparatus (13), supporting the idea that the formation of ethyl carbamate could start during the distillation process. The second pathway is based on self-oxidation under UV light of unsaturated compounds present in alcoholic beverages (16), which produce free radicals (organic or hydroperoxides), which catalyze the oxidation of cyanide to cyanate (Scheme 2).

Scheme 2

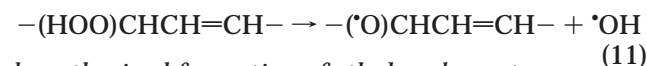
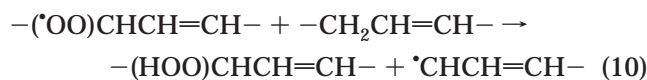
initiation period



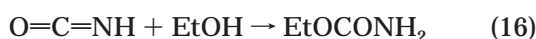
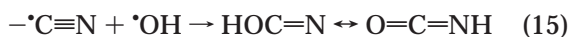
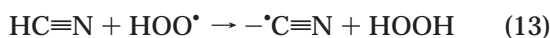
propagation period



self-oxidation period



hypothesized formation of ethyl carbamate



The factors influencing ethyl carbamate formation from cyanide are pH, light, ethanol content, tempera-

ture, vicinity of carbonyl groups in organic molecules, and concentration of Cu^{II} ions in the beverage (1, 17). Some authors have proposed pathways other than CN⁻ for the formation of ethyl carbamate in spirits. The reaction of proteins with ethanol catalyzed by Cu^{II} ions is an example (18), but attempts to reproduce such experiments were unsuccessful. Another proposal considers cyanic acid released directly from thermal decomposition of urea present in the must (19).

In fact, cyanate and its tautomeric form isocyanate have been indicated as intermediates of ethyl carbamate formation from the reaction of ethanol with urea (eq 16), cyanide, and CP (20). However, a first-order kinetics was reported for the decomposition of isocyanate and zero-order kinetics for the formation of ethyl carbamate, indicating an intermediate reaction for converting cyanate into ethyl carbamate (19). However, all ethyl carbamate in the distillate is formed at ~24–48 h after the distillation (17, 21).

Several factors still require research to elucidate the reaction that generates ethyl carbamate from nitrogen precursors in spirits and the role of metal ions (Cu, others). The aim of the present study is twofold. First was to ascertain if O₂ (which may start the radical mechanism, Scheme 2) dissolved in the beverage has any effect on ethyl carbamate formation, and second was to elucidate the role of Cu^{II} ions as catalyst for the conversion of cyanate into ethyl carbamate, independent of the source of cyanate (inorganic or organic cyanide).

MATERIALS AND METHODS

The conversion of cyanide into ethyl carbamate solution under an atmosphere of nitrogen, carbon dioxide, or oxygen was carried out in 20 mL hermetic vials containing 5 mL of an aqueous ethanol (30% EtOH) solution of CuSO₄ (20 mM). Three solutions were saturated with N₂, CO₂, and O₂, respectively, and then 260 μL of KCN (1.94 M) was added. The vials were incubated for 8 h at 80 °C, and then the solutions were spiked with cyclohexanol (internal standard) and analyzed for ethyl carbamate by GC-FID, as described below.

After the solutions had been heated, a precipitate was formed in all vials. To verify the presence of cyanate groups, these precipitates were separated, washed with diethyl ether, and dried under vacuum at ambient temperature. An FT-IR Perkin-Elmer 1710 spectrophotometer was used to record the infrared spectra.

The Cu^{II} catalysis in the oxidation of cyanide into cyanate and subsequent conversion of the latter into ethyl carbamate was demonstrated by carrying a check assay in the absence of Cu^{II}. In this case no reaction was observed.

The influence of Cu^{II} on the reaction of ethanol with cyanate was studied using 20 mL vials charged with 5 mL of aqueous ethanol (30%) solution, KOCN (6.7 mM), with CuSO₄ (0.7, 1.4, 2.7, 6.7, and 13.4 mM). The solutions were incubated for 4 h at 80 °C and then spiked with cyclohexanol (internal standard) and analyzed for ethyl carbamate by GC-FID. The same procedure was performed with FeSO₄·7H₂O and FeCl₃, respectively, in concentrations of 3.0, 6.0, and 12.0 mM.

The effect of Cu^{II} ions in the reaction of ethanol with urea was investigated using 20 mL vials charged with 5 mL of aqueous ethanol (30%) solution of urea (30 mM) and 0.0, 10, and 20 mM CuSO₄, respectively. The vials were incubated for 4 h at 80 °C, and then the solutions were spiked with cyclohexanol (internal standard) and analyzed for ethyl carbamate by GC-FID.

Because of the difference between the two matrices analyzed by GC-FID (synthetic solutions and commercial cachaças), two distinct procedures for the analysis have been implemented using an HP-5890 gas chromatograph. The synthetic solutions were analyzed using a capillary column Altech Heliflex AT-1000 (30 m × 0.2 mm × 0.33 μm) with the following oven

Table 1. Ethyl Carbamate Content Determined in the Reaction between Aqueous Ethanol (30%) and the Product of KCN (0.10 M) Oxidation in the Presence of CuSO₄ (20 mM) under an Atmosphere of O₂, N₂, or CO₂^a

atmosphere	ethyl carbamate content (ppm)
O ₂	15.8 ± 0.8
N ₂	13.1 ± 1.4
CO ₂	16.4 ± 0.6

^aEach value is the average of five measurements.

temperature program: 70 °C for 1 min and then a temperature increase of 25 °C/min to 185 °C. The commercial cachaças were analyzed using two capillary columns with different polarities connected in a serial arrangement: HP-ULTRA-1 (25 m × 0.2 mm × 0.33 μm) and HP-FFAP (50 m × 0.2 mm × 0.33 μm); the temperature program was from 70 to 185 °C at an increase of 1 °C/min and then at 30 °C/min to 230 °C. In both methodologies the temperature of the injection and detection blocks was kept at 250 °C, He was used as carrier gas, and head column pressure was 200 kPa with splitless mode injection (2.0 μL samples were injected).

The cachaça samples were concentrated by the addition of 50 mL of the beverage in a separator flask with 25 mL of aqueous solution of KCl (300 g/L) followed by two successive extractions with methylene chloride/ether (4:1). The organic phases were combined and evaporated under vacuum at 40 °C to 5 mL and injected in the gas chromatograph. The recovery was better than 95%, and the reproducibility was 96% (*n* = 5).

Cyanide was analyzed with the modified König reaction (22) using pyridine and barbituric acid. Urea has been analyzed using the phenolic colorimetric method (23). Standard solutions of ethyl carbamate were prepared at 10, 50, 100, 300, 500, 800, and 2000 ppb and used for calibration and quantifying the species in real samples by GC-FID. The limit of detection is 20 ppb. GC-MS was also used as control (fragment *m/z* 62) of the nature of the target compound. The GC and GC-MS were equipped with the same column (see above). The quantification of commercial samples has been performed without internal standard to avoid coelutions with other compounds.

RESULTS AND DISCUSSION

Table 1 reports the amount of carbamate formed from cyanide under O₂, N₂, or CO₂ atmosphere in the presence of Cu^{II}. There is not a significant difference in the amount of ethyl carbamate formed in the reaction of ethanol with oxidized cyanide in the presence of Cu^{II}. This result confirms that the oxygen dissolved in solution does not contribute significantly to the direct oxidation process of cyanide. From this result it is proposed that cyanate is formed according to reaction 17 more than reaction 18. This rules out a substantial contribution of the radical mechanism (eqs 8–16), supports a coordination chemistry for the oxidation of cyanide, and confirms the key role of the metal ions.



The precipitate resulting from each of the three reactions was isolated and characterized as copper cyanate mixed with carbonate/hydroxide derivatives. The infrared spectra (Figure 1) show that there is a split band nearby 2200 cm⁻¹, which is attributed to the ν_{as} of the NCO⁻ in the cyanate–Cu^{II} complexes (24).

The weaker intensity of the band nearby 2200 cm⁻¹ in the product isolated from the solution saturated with CO₂ (Figure 1B) can be explained on the basis of the

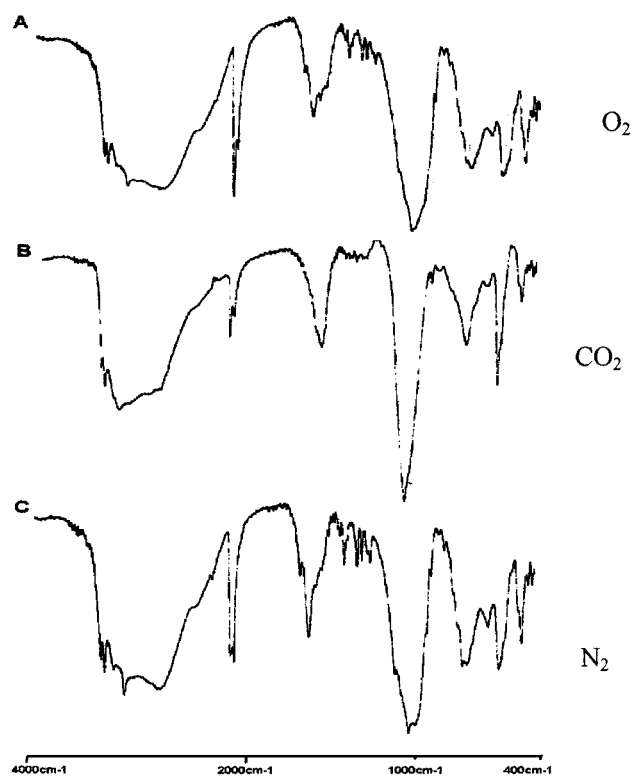


Figure 1. IR spectra of the precipitate from the reaction between KCN and CuSO₄ in aqueous ethanol (30%) solution under an atmosphere of O₂ (A), CO₂ (B), or N₂ (C).

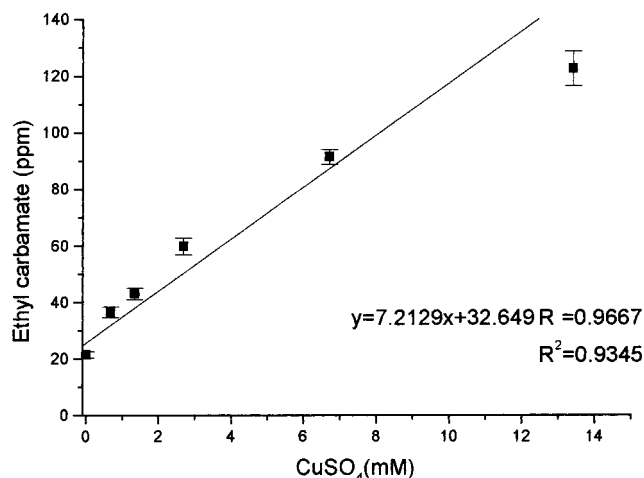


Figure 2. Influence of Cu^{II} on ethyl carbamate formation in aqueous ethanol solution (30%) of KCN (6.7 mM) (4 h at 80 °C). Each value is the average of three measurements.

influence of pH of this solution (pH 4.25) on the conversion of cyanide into cyanate. The other two solutions have a higher pH (pH 6.45) and consequently a higher concentration of the hydroxyl ions necessary for cyanide oxidation.

This finding clearly shows that the oxidation of cyanide to cyanate does not need oxygen and, most probably, does not imply the formation of cyanogen, which is not formed, not even in traces, in the solution.

Although it has been suggested that the inorganic cyanate, independent of its source, plays a central role in the ethyl carbamate formation in alcoholic beverages, the Cu^{II} influence on the cyanate conversion in ethyl carbamate has not yet been established. Figure 2 clearly shows that ethyl carbamate formation from ethanol and cyanate is dependent on the Cu^{II} concentration.

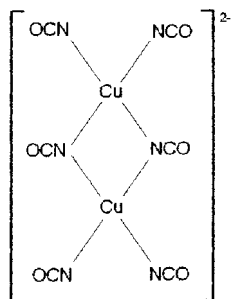


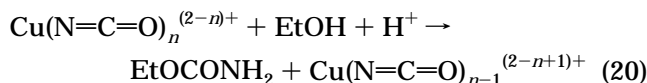
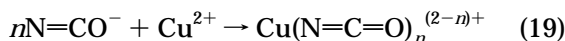
Figure 3. Structure of a cyanate-copper(II) complex.

Table 2. Influence of Copper Species on the Reaction of KOCN (12.1 mM) with Aqueous Ethanol (30%) (4 h at 80 °C)^a

copper species [mM]	ethyl carbamate formed (ppm)
no catalyst	83.0 ± 2.9
CuCO ₃ [0.0202]	94.4 ± 3.7
Cu(OH) ₂ [0.0195]	82.1 ± 7.1
CuCl/O ₂ [0.0360]	313 ± 14
CuCl/N ₂ [0.0181]	321 ± 16
Cu(OOCCH ₃) ₂ [0.0228]	342 ± 23

^a Each value is the average of three measurements.

Such an effect can be explained by considering that the coordination of cyanate to Cu^{II} occurs through the nitrogen atom, forming a binuclear structure (Figure 3). In such a way, the carbon atom tends to be more positive, favoring the nucleophilic attack from the oxygen of ethanol. Equations 19 and 20 show the formation of the carbamate from cyanate mediated by Cu^{II}.

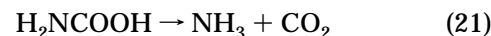


If the solution is heated for a long time, a dark precipitate is formed in the vial. It has been isolated and analyzed by infrared spectroscopy and elemental analysis (C, H, N, Cu, and K). Analytical and spectral IR data show it to be CuCO₃·Cu(OH)₂. The band at 2200 cm⁻¹ is absent in the infrared spectrum of the solid. According to our results, neither copper species (carbonate or hydroxide) is a good catalyst for the ethyl carbamate formation (Table 2), explaining why the catalytic potential of copper decreases with time *in vitro*. However, such deactivation is not a limiting step in the ethyl carbamate formation in spirits because their average Cu^{II} content is ~5 ppm, whereas that of the cyanate, when detected, is ~15 ppb (21).

As discussed above, oxygen is not directly implicated in the oxidation of cyanide into cyanate. Most likely it is implicated in the subsequent oxidation of Cu^I into Cu^{II}, a reaction well-known to occur in several industrial processes catalyzed by Cu^{II} (25). However, Cu^{II} is implicated in both the oxidation of cyanide into cyanate and in the conversion of the latter species into urethane. The catalytic effect of Cu^{II} is demonstrated by the fact that a solution of KCN in aqueous ethanol is converted into KOCN first and then into urethane in the presence of Cu^{II}, whereas no reaction is observed in the absence of Cu^{II}.

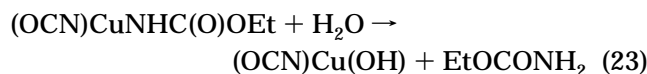
We have observed that when copper sulfate or copper acetate is added to a solution of cyanate in aqueous

ethanol, a gas is evolved during the heating procedure. The gas was analyzed as CO₂, by using a gas chromatograph equipped with a packed column of Chromosorb S-II and a thermal conductivity detector. This finding can be explained by considering the formation of carbamic acid (eq 21), which is very unstable and decomposes into CO₂ and NH₃ (26). The same result has been observed using Fe^{III}, indicating that coordination to a metal center has an important role in the activation of the cyanate to undergo a nucleophilic attack.

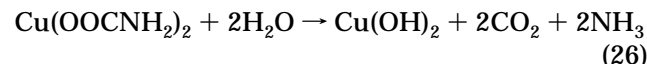
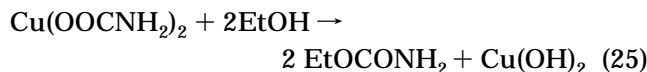
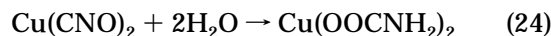


Two reaction pathways by which cyanate could be converted into ethyl carbamate can be proposed according to our results. Ethanol may attack the isocyanate (path A) to form the carbamic unit (eq 22), which undergoes an attack by water to form ethyl carbamate (eq 23). The other pathway (B) deals with the attack by water to isocyanate to form a carbamate moiety, which by ethanolysis may afford the urethane (eqs 24 and 25) or by reaction with water may produce NH₃ and CO₂ (eq 26).

Path A



Path B



The route represented by path B seems to be most likely to occur as it can explain both the formation of urethane and the observed evolution of CO₂.

We have also investigated the reaction between urea and ethanol in the presence of Cu^{II}. Urea reacts with ethanol to afford urethane (eq 1). To gain an insight into the copper involvement, the reaction was carried out using different quantities of Cu^{II}. Figure 4 shows that the amount of ethyl carbamate formed depends on the concentration of Cu^{II}.

It is worth recalling that urea has been detected at millimolar levels in several commercial cachaças samples (23) as an effect of an incorrect distillation.

According to our results a correlation between urea concentration and ethyl carbamate content (Figure 5) is difficult to find.

Conversely, a better relationship can be demonstrated between the cyanide and ethyl carbamate contents in the same samples (Figure 6). These findings disclose that among all factors which cause ethyl carbamate formation in cachaça, urea is not the predominant one. Cyanide is most likely to be the source of ethyl carbamate. However, the chemical profile of cachaça is not too different from that of other spirits such as whiskeys and grappas (27). Therefore, that cyanide is the main precursor of ethyl carbamate in cachaças is in agreement with what has been shown for other distillates.

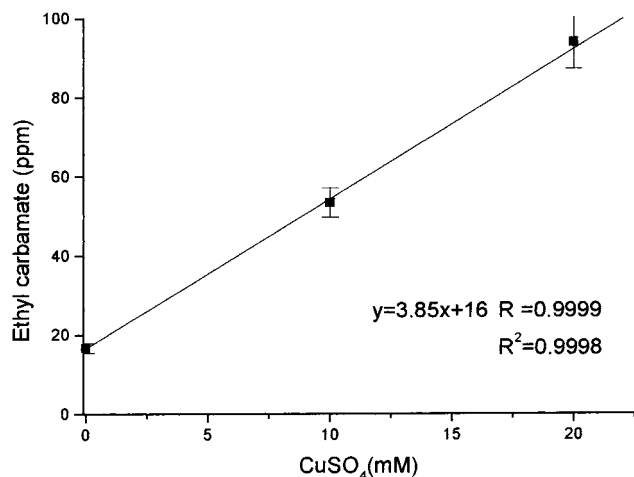


Figure 4. Influence of the Cu^{II} on the reaction of urea (30 mM) with aqueous ethanol (30%) to form ethyl carbamate (4 h at 80 °C). Each value is the average of three measurements.

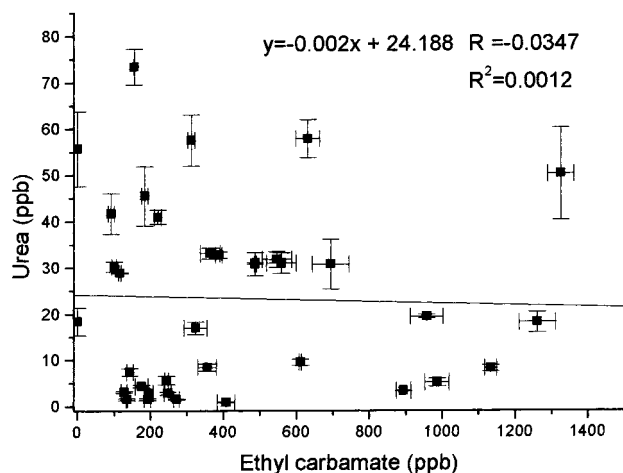


Figure 5. Relationship between ethyl carbamate and urea concentration in commercial Brazilian sugar cane spirit.

Also iron ions are frequently found in cachaça at levels of ~1 mg/L (28). We have investigated their effect on the reaction between ethanol and cyanate. Both Fe^{II} and Fe^{III} increase in the same order the formation of ethyl carbamate with respect to Cu^{II} alone as can be shown in Figure 7. The sources of iron ions in distilled beverages are the stainless steel still apparatus and mainly the water used to dilute the distillate.

For technical and economic matters, the largest Brazilian distillers, responsible of the production of ~1 billion liters/year of cachaça, use a continuous distillation process in stainless steel columns with copper condensers. This condition is ideal for the formation of ethyl carbamate in the beverage because the volatile cyanide generated accumulates in the condensed product together with the copper released from the condenser. Noteworthy, the ethyl carbamate average level in the cachaças we have analyzed (Figures 5 and 6) is ~500 ppb, a relatively high value if compared to the internationally accepted limit of 150 ppb in whiskeys. This can be the effect of uncontrolled distillation.

Many preventive actions to avoid the ethyl carbamate formation in alcoholic beverages have been proposed, among which are (i) the use of enzymes such as urease (29) and rhodanese (20) to destroy urea and cyanide, respectively, (ii) the addition of patented copper and silver salts (30) to precipitate the cyanide in the must,

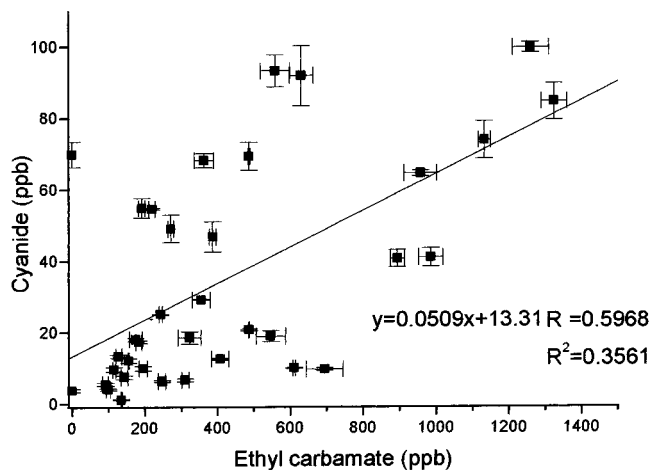


Figure 6. Relation between the concentration of ethyl carbamate and cyanide in commercial Brazilian sugar cane spirit.

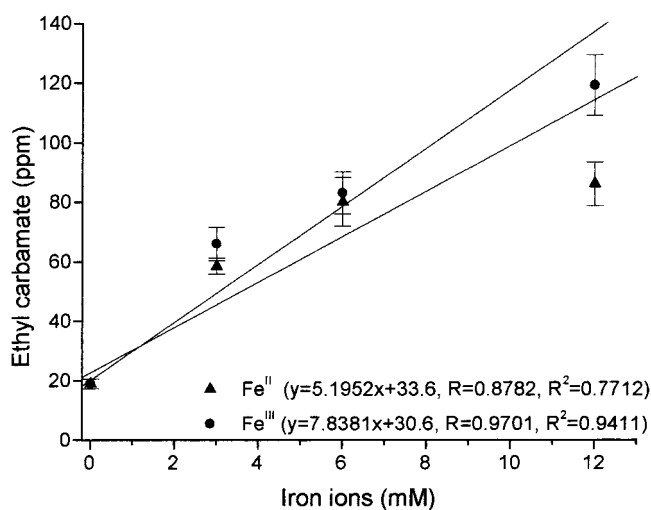


Figure 7. Influence of Fe^{II,III} ions on the reaction of cyanate (6.2 mM) with aqueous ethanol (30%) to form ethyl carbamate (4 h at 80 °C).

(iii) elimination of Cu^{II} by treatment of the beverage with cationic exchange resins (17), or even (iv) such nonpractical procedures as the storage of bottles at low temperature and in darkness (31). These actions are not economically viable. It is possible to attain the same result with other operational methods at a much lower cost.

For example, two modifications could be made to the pot still apparatus avoiding the co-presence of cyanide and Cu^{II} in cachaça or in other spirits. First, the copper condenser could be changed for one made of stainless steel and, second, the columns could be packed with copper pieces to fix volatile cyanides present in the vapor. Organoleptic properties of the beverages must be considered because they could be changed, given that an excessive packing can increase the grade of rectification of the distillation process, decreasing the volatile compounds content in the beverage.

To increase the internal copper surface in columns, with a minimum increase in the grade of rectification, it may be recommended to use flat copper pieces disposed vertically, which avoids the retention of the condensed liquid. The still columns with high inner copper surface have another positive effect, that is, removing the sulfur taste from beverages. In fact, the use of metallic salts, mainly copper, to precipitate CN⁻

in the must can generate environmental problems because the waste of the distillation is used as sewage sludge in sugar cane cultures. A similar problem has already been reported to exist in the vicinity of Scottish whiskey distilleries, where a high level of copper in soil (32, 33) was detected. The use of water rich in iron salts in the production process must be avoided in order to avoid the enhancement of the catalytic activity of copper by iron ions.

ACKNOWLEDGMENT

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