DOI: http://doi.org/10.1590/1678-992X-2020-0281

SCIENTIA AGRICOLA

ISSN 1678-992X

Research Article

Total nitrogen content and its influence on ethyl carbamate incidence in cachaça

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Edited by: Luís Guilherme de Lima Ferreira Guido

Received September 09, 2020
Accepted April 23, 2021

ABSTRACT: Cachaça is a typical Brazilian spirit of significant economic and social relevance. The spirit may contain organic and inorganic contaminants that impair its quality, such as ethyl carbamate (EC), which is potentially carcinogenic. The study of factors that cause EC incidence is extremely important to produce cachaça with good physicochemical and sensory quality and for public health reasons. Therefore, this study aimed to analyze and quantify nitrogen (N) in sugarcane juice and correlate it with the EC levels, with copper (Cu) as precursor, in cachaça from stills, produced from the sugar of three different sugarcane varieties (RB857515, RB966928 and RB855453). We used the plantation systems without fertilization and with organic and conventional fertilization. The EC and Cu levels were below the legislation threshold and the N levels were correlated with EC formation in different planting systems.

Keywords: contaminant, legislation, spirit, sugar cane, planting systems

Introduction

Ethyl carbamate (EC) is a compound popularly known as urethane, belonging to the group of organic compounds considered as ethyl esters of carbamic acid (CH_3NO_2). EC has several commercial applications, such as solubilizer for pesticides, cosmetics, and drug constituent (Ghanayem, 2007). The margin of exposure (MOE) is applied to assess the risk of carcinogens. MOE greater than or equal to 10,000 is considered to have a low level of risk to public health. For cachaça consumers, the MOE ranges from 4,294 to 5,058 (Lachenmeier et al., 2010). Therefore, the International Agency for Research on Cancer (IARC), based on distinct studies that described EC toxicity, listed it in class 2B, the group of carcinogenic substances (Alexander et al., 2007).

Copper (Cu) is an essential heavy metal and the Food Nutritional Board (FBN) stipulates for this metal RDA (Recommended Dietary Allowance) from 1.5 to 3.0 mg d^{-1} for adults (Copper, 2001), whereas Prashanth et al. (2015) recommend trace levels around 2.0 to 5.0 mg d^{-1} for adults. Nevertheless, Cu is another inorganic contaminant found in cachaça and many studies correlate EC formation with Cu presence.

Cyanide (CN^-) is also classified as EC precursor and its formation in alcoholic beverages is linked to distillation or even after this process. Another pathway that involves the cyanide ion (CN^-) in EC formation has been related to the enzymatic action and oxidation of cyanogenic glycosides found in sugarcane (Alexander et al., 2007; Galinaro et al., 2015).

Both compounds are found in cachaça, a beverage produced from sugarcane in Brazil. Nonetheless, nitrogen (N) doses are added to promote sugarcane development. Doses of N fertilizer used in sugarcane fields in Brazil are 40 kg ha^{-1} of N_2 in sugarcane-plant and 80 kg ha^{-1} of N_2 in the ratoons, on average (Nunes Junior et al., 2005). Furthermore, each sugarcane variety responds differently to the addition of N fertilizers (Schultz et al., 2012).

This study aimed to analyze and quantify the total N in sugarcane juice used in cachaça production from three sugarcane varieties cultivated under organic, conventional and without fertilization cultivation systems, and correlate it to EC and Cu contents in cachaça from stills.

Materials and Methods

Material

The study was performed in the municipality of Araras, São Paulo State, Brazil. The location of the experiment was under the coordinates 22°30′73.61″ south latitude and 47°37′53.39″ west longitude, a local altitude of 629 m, with slightly sloping relief and humid temperate climate with dry winters and hot and wet summers. The average temperature in the period of the experiment was of 20.3 °C, with average rainfall of 60.6 mm. Three cachaças were used in the analyses, which were produced using the planting systems: organic, conventional, and without fertilization, using sugarcanes of the varieties RB857515, RB966928 and RB855453. The characteristics of the raw material and alcoholic content of the cachaças are presented in Table 1.

Table 1 – Raw material characteristics and alcoholic content of the cachaças studied.

Management System	Variety				Cachaça
	Varieties	Maturation	IUP*	Agricultural Productivity	Alcoholic content**
Organic	RB855453	Early	Short	High	43
	RB867515	Middle-late	Medium		
	RB966928	Early	Medium		
Conventional	RB855453	Early	Short		
	RB867515	Middle-late	Medium		
	RB966928	Early	Medium		
Without Fertilization	RB855453	Early	Short		
	RB867515	Middle-late	Medium		
	RB966928	Early	Medium		

Source: Silva et al. (2020). *Industrial Usage Period (IUP), is the number of days on which a cultivar presents ART above 12.5 % and sugar extraction greater than 80 kg t^{-1}; ** % in v/v of ethanol at 20 °C.

Determination of copper (Cu)

The Cu content of the cachaças was determined by atomic absorption spectrophotometry in a flame atomic absorption apparatus. The conditions for the analyses were determined according to MAPA (2005) and adapted by Silva et al. (2020).

Determination of ethyl carbamate (EC)

The EC in the cachaças was determined in a gas chromatograph coupled to a mass spectrometer with the libraries NIST 11 and NIST 11s, equipped with a split/splitless automatic injector. The conditions for the analyses were performed according to MAPA (2014a) and adapted by Silva et al. (2020).

Determination of total nitrogen

The total N was determined by sulfuric solubilization together with the semi-micro Kjeldahl method. In the analysis, the wet solubilization was used, followed by steam distillation and titration for NH_4 quantification. Sulfuric solubilization (H_2SO_4 + catalysts) transformed protein and amino acids into $N^-NH_4^+$, which was distilled and complexed in boric acid with mixed indicator, and it was titrated with a standard solution of diluted H_2SO_4. The analysis started with the transference of 500 mg of sugarcane juice to a digester tube. Pure nitric acid (4.0 mL) was added to the analysis and subsequently it rested for approximately 12 h for previous digestion. Then, it was gradually heated until reaching 120 °C and remained at this temperature until the release of NO_2 ceased completely, which is characterized by a brown steam. In this step, the organic matter (OM) was partially digested and the volume of the nitric acid was reduced to half of the initial volume. Subsequently, 2.0 mL of pure perchloric acid were added to the analysis and the temperature was gradually increased to 180 °C. This temperature was kept until white $HClO_4$ fumes were obtained and the extract became colorless. It was cooled and the volume was completed to 25 mL with ultrapure water. The whole digested extract was transferred to the Kjeldahl semi-micro distiller. A 50 mL erlernmeyer was connected, containing 10 mL of the solution of 20 g L^{-1} H_3BO_3 with the mixture of indicators (methyl red and green bromocresol) at the cooling end of the distiller. Gradually, 10 mL of 13 mol L^{-1} NaOH were added to the digested. Distillation occurred until a volume of 30 mL was reached, the flask was removed and titrated with a 0.014 mol L^{-1} H_2SO_4 solution until the color changed to pink. A blank control was performed together with the samples (Carmo et al., 2000). The result was determined by Eq. (1).

$$N\text{–}NH_4\ (g\ kg^{-1}) = (Vol_A - Vol_B) \times 1.4 \quad (1)$$

where: Vol_A is the volume of H_2SO_4 used in the sample (mL) and Vol_B is the volume of H_2SO_4 used in the blank control (mL).

Statistical analysis

To determine the contaminants (Cu and EC) and total N in the cachaças, a completely randomized design (CRD) in 3 (varieties) × 3 (managements) factorial scheme was considered, with four repetitions. Each plot had 37 m^2 with five furrows of 5.0 m long and interline spacing of 1.5 m, where the three central furrows formed the useful plot area and the two side furrows were border plants.

The concentration of these compounds in cachaça was evaluated and subjected to the univariate analysis of variance ($p \leq 0.05$) and analyzed in a rejoinder. Whenever necessary, the Tukey multiple mean comparison test was applied. The multivariate analysis was also applied and the principal component technique was used to evaluate the behavior of the factors under study and the response of the variables. Statistical procedures were conducted using R Development Core Team (R Foundation for Statistical Computing, version 4.1.0).

Results and Discussion

The total N content measured in the sugarcane juice free from coarse impurities showed that the N content was not altered among the varieties (RB867515, RB966928 and RB855453) (Table 2). These juices were used in the must preparation for inoculation and fermentation.

The Cu and EC contents were also analyzed in the cachaças correlated to the respective juices. All spirits produced from these juices presented EC contents below 210 µg L^{-1}, threshold of the Brazilian legislation. The Cu contents also remained below 5 mg L^{-1}, threshold of the Brazilian legislation (MAPA, 2005; MAPA, 2014b; Silva et al., 2020). These low concentrations derive from the processing with good hygiene practices in the production, even with the use of the Cu still in the distillation process and fractions separation in distillation (head, heart and tail) (Silva et al., 2020).

The evaluation of the total N contents in the juice showed that the interaction between sugarcane varieties and management was not significant ($p \geq 0.05$), indicating that the varieties do not depend on management (Table 2). Nevertheless, for EC, the interaction between variety and management was significant ($p \geq 0.05$), indicating that the varieties depend on the management. These factors can be analyzed independently for each variable (Table 3).

Sugarcane varieties respond differently to N fertilization in the soil (Schultz et al., 2012). In this study, the varieties did not present significant differences in terms of total N in the juices; nonetheless, all management systems differed from each other (Table 2). The same occurred for the EC contents (Table 3). The highest contents of both products were detected in the juices derived from the sugarcane cultivated using the conventional management, followed by the organic and without fertilization.

For total N, these differences are related to N availability in the soil, absorbed by the plant, corroborating the results. In this sense, juices derived from fertilized soil (conventional and organic), the total N contents were, respectively, 5.60 and 3.96 times superior (Table 2) to the juice derived from the sugarcanes cultivated without the addition of fertilizer. This demonstrates N availability in the soil and, consequently, nutrient absorption and metabolization by the plant, as well as the influence of fertilization on sugarcane composition.

Table 2 – Mean content of the total nitrogen concentration (mg kg^{-1}) in the sugarcane juice used in the must, analysis of simple effect of the varieties and of the managements.

Varieties	Means	Management	Means
RB 867515	350.50$^{a(\pm 196.63)}$	Conventional	523.75$^{a(\pm 58.84)}$
RB 855443	319.00$^{a(\pm 194.43)}$	Organic	370.91$^{b(\pm 57.47)}$
RB 966928	318.75$^{a(\pm 182.85)}$	Without Fertilization	93.58$^{c(\pm 10.80)}$

Same lowercase letters in the columns indicate that the means do not differ from each other ($p > 0.05$), results obtained by the Tukey test.

Table 3 – Mean content of the ethyl carbamate concentration (µg L^{-1}) in the cachaças because of the development of the double interaction (variety × management).

Management	Variety			Mean
	RB867515	RB966926	RB855453	
Conventional	46.70$^{Cb(\pm 0.85)}$	114.20$^{Aa(\pm 1.25)}$	55.27$^{Ba(\pm 0.77)}$	72.04$^{a(\pm 19.95)}$
Organic	77.60$^{Aa(\pm 0.26)}$	46.77$^{Bb(\pm 0.87)}$	31.95$^{Cb(\pm 0.55)}$	52.11$^{b(\pm 31.25)}$
Without Fertilization	31.05$^{Ac(\pm 1.55)}$	22.47$^{Bc(\pm 0.99)}$	31.25$^{Ab(\pm 0.55)}$	28.26$^{c(\pm 47.02)}$

For management, the same lowercase letter in the columns indicate that the means do not differ from each other. For the variety, the same capital letters in the lines indicate that the means do not differ from each other ($p > 0.05$), results obtained by the Tukey test. Source: Silva et al. (2020).

For the Cu contents, interaction between variety and management was significant, indicating that these factors are dependent on each other. The results of the developments are presented in Table 3. Copper is a contaminant usually found in cachaça and several studies correlate its presence with the EC formation, having an N source (urea) as one of the pathways proposed for its formation (Alexander et al., 2007; Galinaro et al., 2015). If EC can be formed by Cu and total N, the Cu in the beverage and the existence of a total N source in the juice (must) are indicative of EC formation in cachaça due to the influence of these sources.

The varieties RB867515 and RB855453 did not differ from each other in terms of the mean Cu content using the conventional management; however, the variety RB966928 differed from all other varieties and presented the lowest value. The same occurred for the organic management. Nevertheless, considering the management without fertilization, the varieties RB867515 and RB966928 presented the lowest mean Cu concentrations and did not differ from each other; nonetheless, the variety RB855453 differed from the others. In this study, no direct correlation of the sugarcane varieties with Cu and total N contents was evidenced (Tables 2 and 3).

The correlations between the response variables were obtained (Table 4), revealing that only EC concentration presented a significant correlation, 0.5739, with N_2 content, indicating that the cachaças with higher (lower) total N contents presented higher (lower) EC concentrations. This fact can be visualized in the map of circular variables (Figures 1A and 1B): the smaller the cosine between the vectors of the variables, the higher the correlation between them, corroborating the results obtained by the univariate analyses. The closer the vectors are to the 90° cosine, right angle, the lower the correlation for variables, in other words, greater independence.

In this study, a relation between total N and EC incidence was observed and the managements with the highest total N concentrations in the juice (conventional, organic and without fertilization, respectively), presented higher EC values in the beverage. According to Bruno et al. (2007), a content of 0.8 mg L^{-1} is sufficient for Cu to act as an EC precursor and promote complete EC formation in spirits. The Cu contents in the beverages indicate sufficiency to act as precursor, although they did not influence quantitatively in EC production.

Table 4 – Diagonally, standard deviation; in the inferior triangular part, the correlation coefficient and the *p*-value of the Pearson's linear correlation test (in parenthesis) of the variables: ethyl carbamate, copper and total nitrogen.

Variables	Ethyl carbamate	Copper	Total nitrogen
Ethyl carbamate	27.8385	-	-
Copper	0.0740 ($p = 0.6681$)	0.9950	-
Total nitrogen	0.5739 ($p = 0.0003$)	–0.0115 ($p = 0.9470$)	186.4692

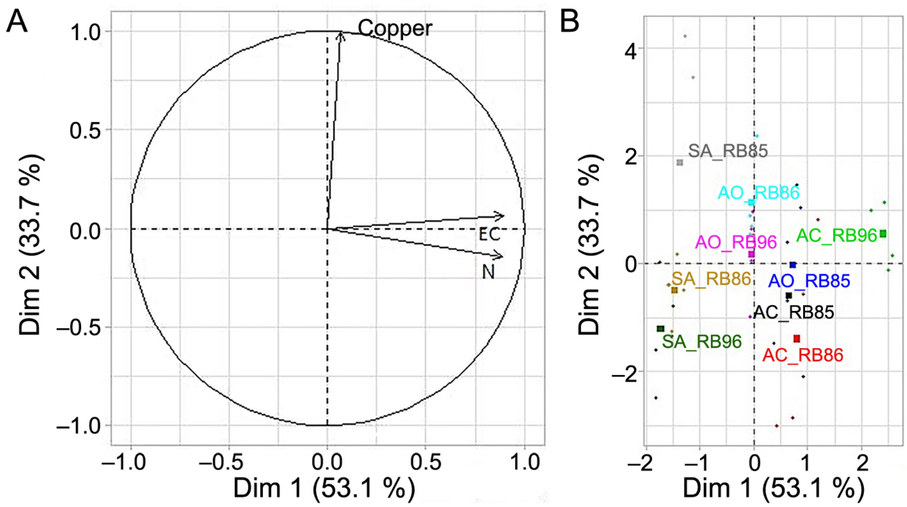

Figure 1 – Principal Component Analysis (PCA), two-dimensional map: A) relation of copper, ethyl carbamate and total nitrogen (variables); and B) of the treatments (management and variety) considering the three variables (copper, ethyl carbamate and total nitrogen).

Conclusion

Copper and ethyl carbamate were identified in the cachaças at concentrations below the threshold established by the Brazilian legislation. Higher total N contents in the juices and EC in the cachaças were exhibited by conventional and organic management systems. A correlation between the total N incidence in these juices and EC in the beverages was evidenced.

The relation of Cu in the beverage, total N in the juice and EC incidence in cachaça should be further studied for product commercialization and for public health reasons.

Authors' Contributions

Conceptualization: Silva, J.H.N.; Oliveira, A.L.; Verruma-Bernardi, M.R. **Data acquisition:** Silva, J.H.N.; Belluco, A.E.S. **Data analysis:** Silva, J.H.N.; Verruma-Bernardi, M.R.; Oliveira, A.L.; Medeiros, S.D.S. **Design of the methodology:** Verruma-Bernardi, M.R.; Oliveira, A.L.; Medeiros, S.D.S.; Medeiros, S.R.R. **Writing and editing:** Silva, J.H.N.; Verruma-Bernardi, M.R.; Oliveira, A.L.; Medeiros, S.D.S.; Medeiros, S.R.R.; Belluco, A.E.S.

References

Alexander, J.; Gudjón A.A.; Benford, D.; Cockburn A.; Jean-Pierre C.; Dogliotti, E.; Di Domenico A.; Férnandez-Cruz, M.L.; Fürst, P.; Fink-Gremmels, J.; Galli, C.L.; Grandjean, P.; Gzyl, J.; Heinemeyer, G.; Johansson, N.; Mutti, A.; Schlatter, J.; Leeuwen, R.V.; Peteghem, C.V.; Verger, P. 2007. Ethyl carbamate and hydrocyanic acid in food and beverages scientific opinion of the panel on contaminants. European Journal Food Safety Authority 551: 1-44.

Bruno, S.N.F.; Vaitsman, D.; Kunigami, C.N.; Brasil, M.G. 2007. Influence of the distillation processes from Rio de Janeiro in the ethyl carbamate formation in Brazilian sugar cane spirits. Food Chemistry 104: 1345-1352.

Carmo, C.D.S.; Araujo, W.S.; Bernardi, A.C.C.; Saldanha, M.F.C. 2000. Methods of Analysis of Plant Tissues Used at Embrapa Solos = Métodos De Análise De Tecidos Vegetais Utilizados Na Embrapa Solo. Embrapa Solos, Rio de Janeiro, RJ, Brazil (Circular Técnica) (in Portuguese).

Copper, I.O.M. 2001. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. The National Academies Press, Washington, DC, Brazil.

Galinaro, C.A.; Ohe, T.H.; Silva, A.C.; Silva, S.C.; Franco, D.W. 2015. Cyanate as an active precursor of ethyl carbamate formation in sugar cane spirit. Journal of Agricultural and Food 33: 7415-7420.

Ghanayem, B.I. 2007. Inhibition of urethane-induced carcinogenicity in Cyp2e1-/- in comparison to Cyp 2e1+/+ mice. Toxicological Sciences 95: 331-339.

Lachenmeier D.W.; Lima M.C.P.; Nóbrega I.C.C.; Kerr-Corrêa, F.; Kanteres, F. 2010. Cancer risk assessment of ethyl carbamate in alcoholic beverages from Brazil with special consideration to the spirits cachaça and tiquira. BMC Cancer 10: 266.

Ministério da Agricultura, Pecuária e Abastecimento [MAPA]. 2014a. Work Instruction IT n° LABV 377, of December 20, 2014. IT LABV 377, Rev. 01 - Determination of Ethyl Carbamate in Alcoholic Beverages Distilled by GC-MS = Determinação de carbamato de etila em bebidas alcoólicas destiladas por CG-EM MAPA, Brasília, DF, Brazil (in Portuguese).

Ministério da Agricultura, Pecuária e Abastecimento [MAPA]. 2014b. Work Instruction IT n° 28, of August 18, 2014. Change The Sub-Item 5.1.2 of the Annex to Normative Instruction n° 13, of June 29, 2005 = Instrução de Trabalho IT n° 28, de 18 de agosto de 2014. Alteração do Subitem 5.1.2 do Anexo à Instrução Normativa n° 13, de 29 de junho de 2005 . Diário Oficial da União, Brasília, August 11, 2014, Section 1, p. 1 (in Portuguese).

Ministério da Agricultura, Pecuária e Abastecimento [MAPA]. 2005. Normative Instruction n° 13, of June 29, 2005. Technical regulation for setting identity and quality standards for spirit sugar cane and cachaça = Instrução Normativa n° 13, de 29 de junho de 2005. Regulamento técnico para fixação de padrões de identidade e qualidade para aguardente de cana-de-açúcar e cachaça. Diário Oficial da União, Brasília, Brasília, June 30, 2005, Section 1, p. 11 (in Portuguese).

Nunes Junior, D.; Pinto, R.S.A.; Trento, F.E.; Elias, A.I. 2005. Agricultural Indicators in the Sugarcane Sector: Harvest 2003/2004 = Indicadores Agropecuários do Setor Canavieiro: Safra 2003/2004. Idea, Ribeirão Preto, SP, Brazil (in Portuguese).

Prashanth, L.; Kattapagari, K.K.; Chitturi, R.T.; Baddam, V.R.; Prasad, L.K. 2015. A review on role of essential trace elements in health and disease. Journal of Dr. NTR University Health Sciences 4: 75-85.

Schultz, N.; Morais, R.F.; Silva, J.A.; Baptista, R.B.; Oliveira, R.P.; Leite, J.M.; Boddey, R.M. 2012. Agronomic evaluation of sugarcane varieties inoculated with diazotrophic bacteria and fertilized with nitrogen. Pesquisa Agropecuária Brasileira 47: 261-268 (in Portuguese, with abstract in English).

Silva, J.H.D.N.; Verruma-Bernardi, M.R.; Medeiros, S.D.S.; Oliveira, A.L. 2020. Monitoring the content of ethyl carbamate and copper in organic and conventional cachaça. Scientia Agricola 77: e20190027.