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On the role of higher alcohols in the characterization of cachaça

Sobre o papel dos álcoois superiores na caracterização da cachaça

Sobre el papel de los alcoholes superiores en la caracterización de cachaça

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Abstract

There is a growing interest in chemical markers for the identification and certification of cachaça as a cane spirit produced in Brazil. It is known that the higher alcohols that are usually analyzed (propyl alcohol, Isobutyl alcohol and isoamyl alcohol) occur in all alcoholic beverages (fermented and distilled), but the relative proportions can vary markedly according to the peculiarities of the raw material and the production process. In this work, the contents of higher alcohols in 300 samples of alembic cachaça were compared, 220 from the state of Minas Gerais and 80 from other states, as well as three samples of industrial cachaça and 14 samples of whiskeys of various brands. The typical range of total higher alcohols in cachaça was 180-360 mg/100 mL ethanol. Cachaça containing higher alcohol concentrations greater than 360 mg/100 mL ethanol do not comply with Brazilian legislation. However, cachaças with higher alcohols concentrations below 180 mg/100 mL ethanol, as was found in one of the industrial cachaças, signify adulteration, for example, by mixing with fuel alcohol. The C4/C5 ratio varied less than the C3/C5 ratio, being consistently within the range of 0.20-0.50. In the whiskeys analyzed, the concentrations of higher alcohols were in the range of 160 and

270 mg/100 mL. Therefore, this parameter would not assist in differentiating between cachaça and whiskey. But the C4/C5 ratio was consistently different, being always greater than 0.50 for the whiskeys. Thus, the routine analysis of higher alcohols provides useful information both for tracking possible fraud and for assessments related to the identity or origin of cachaça.

Keywords: Higher alcohols; Cachaça; Markers.

Resumo

Existe um interesse crescente em referenciais químicos para a identificação e certificação da cachaça como aguardente de cana produzida em território brasileiro. Sabe-se que os álcoois superiores usualmente analisados (n-propanol, Isobutanol e álcool isoamílico) ocorrem em todas as bebidas alcoólicas (fermentadas e/ou destiladas), mas as proporções relativas podem variar acentuadamente conforme peculiaridades da matéria-prima e do processo produtivo. Nesse trabalho, foram comparados os teores de álcoois superiores em 300 amostras de cachaça de alambique, sendo 220 de Minas Gerais e 80 de outros estados, assim como três amostras de cachaça industrial e 14 amostras de uísques de várias marcas. Os resultados permitiram estabelecer 180-360 mg/100 mL etanol como a faixa típica de álcoois superiores totais na cachaça. Cachaças com álcoois superiores acima de 360 mg/100 mL etanol não atendem à legislação brasileira. Mas cachaças com álcoois superiores abaixo de 180 mg/100 mL etanol, conforme encontrado em uma das cachaças industriais, sinalizam adulteração, por exemplo, por mistura com álcool combustível. A relação C4/C5 mostrou menos variação que a relação C3/C5, situando-se consistentemente na faixa de 0,20-0,50. Nos uísques analisados, os teores de álcoois superiores situaram-se na faixa de 160 e 270 mg/100 mL; portanto, esse parâmetro não auxiliaria na diferenciação entre cachaça e uísque. Mas a relação C4/C5 foi consistentemente diferenciada, sempre acima de 0,50. Assim, a análise rotineira dos álcoois superiores fornece informações úteis tanto para rastreamento de possíveis fraudes como para avaliações afetas à identidade/origem da cachaça.

Palavras chaves: Alcoois superiores; Cachaça; Marcadores.

Resumem

Existe un interés creciente en referencias químicas para la identificación y certificación de la cachaza como aguardiente de caña producida en territorio brasileño. Se sabe que los alcoholes superiores habitualmente analizados (n-propanol, Isobutanol y alcohol isoamílico) se encuentran en todas las bebidas alcohólicas (fermentadas y destiladas), pero las proporciones relativas pueden variar notablemente según las peculiaridades de la materia prima y el proceso de producción. . En este trabajo se comparó el contenido de alcoholes superiores en 300 muestras de cachaça tranquila, 220 de Minas Gerais y 80 de otros estados, así como tres muestras de cachaça industrial y 14 muestras de whiskies de diversas marcas. Los resultados permitieron establecer 180-360 mg/100 mL de etanol como el rango típico de alcoholes superiores totales en la cachaça. Las cachaças con alcoholes superiores por encima de 360 mg/100 mL de etanol no cumplen con la legislación brasileña. Pero las cachaças con alcoholes superiores por debajo de 180 mg/100 mL de etanol, como las que se encuentran en una de las cachaças industriales, señalan adulteración, por ejemplo, al mezclarlas con alcohol combustible. La relación C4/C5 mostró menos variación que la relación C3/C5, estando consistentemente en el rango de 0,20-0,50. En los whiskies analizados, los niveles de alcoholes superiores estuvieron en el rango de 160 y 270 mg/100 mL; por lo tanto, este parámetro no ayudaría a diferenciar entre cachaça y whisky. Pero la relación C4/C5 se diferenciaba constantemente, siempre por encima de 0,50. Por lo tanto, el análisis de rutina de alcoholes superiores proporciona información útil tanto para rastrear posibles fraudes como para evaluaciones relacionadas con la identidad o origen de la cachaza.

Palabras clave: Alcoholes superiores; Cachaça; Marcadores.

1. Introduction

In alcoholic fermentation, yeasts secrete small amounts of various alcohols with more than two carbon atoms, referred to as "higher alcohols", "fusel alcohol" or "fusel oil". Among the ~~higher alcohols~~, propyl, isobutyl and isoamyl alcohols generally occur in greater proportions. Several other alcohols are also secreted in much smaller quantities, including dihydroxyl alcohols (ethyleneglycol, propanediol), aromatic alcohols (phenylethanol) and mixed (hydroxypropanone and acetoin) structures. Glycerin (propanetriol) is the secondary compound that occurs in the greatest concentration, reaching 10% of the ethanol content. Countless bacteria also secrete these higher alcohols, such as lactic acid bacteria. In addition, typical sewage bacteria, such as Clostridium acetobutylicum, and other bacteria referred to as solvogenic, secrete peculiar structures such as 1-butanol and 2-butanol (Pietruszka et al., 2010).

Alcohols are potentially toxic to the human organism, although they do not represent a health risk in the concentrations in which they occur in distilled beverages (Patocka & Kuca, 2012). In cachaça, the sugarcane spirit produced in Brazil, the legislation establishes the limit of 360 mg (100 mL ethanol)$^{-1}$ for the sum of the levels of propyl, isobutyl and isoamyl alcohols (Brasil, 2005). Among the higher alcohols that are obtained through alcoholic fermentation, only phenylethanol has a prominent effect on the aroma (NG et al., 2012; Uranukul Et al., 2019).

Aliphatic structures are not endowed with relevant aromas. However, they are important factors in the sensory perceptions of "body" and "depth". In addition, being inherent to the fermentation metabolism, their occurrence and contents integrate criteria for the characterization of numerous fermented foods and beverages (Kostik et al., 2014).

The formation of higher alcohols occurs via specific metabolic pathways, which can occur to a greater or lesser extent and reflect the effect of numerous factors, such as the composition of the raw material, genetic specificity of yeast, pH and temperature, nutritional supplementation (amino acids and ammonium salts) and even the use (or not) of certain aseptic agents, such as sulfite (Petruska Et Al., 2010; Cravo, 2017; Nishimura et al., 2018). In particular, it is known that the formation is practically null under the aerobic conditions adopted in the growth of yeasts. In the fermentative phase, the use of microaeration permits the intervention in the intracellular redox balance. Thus, there is a decrease in the production of higher alcohols and an increase in the secretion of the corresponding acid structures (Hazelwood Et al., 2008).

The growing appreciation of cachaça on the international market has driven interest in deepening its chemical parameters, seeking references for its characterization, certification of origin and differentiation from other distilled beverages. New parameters have been proposed, and traditional parameters have been reevaluated. In this work, a survey was made regarding the levels and relative proportions of the higher alcohols of cachaça produced in different regions of the country.

2. Material and Methods

LABM (Laboratório Amazile Biagioni Maia - Belo Horizonte) routinely analyzes the samples of cachaça that are sent by the producers for the purposes of quality control. It also conducts analyses to support research and consulting. From an authorized consultation of the analysis records, the concentrations of ethanol and higher alcohols in cachaças registered with the Ministry of Agriculture and analyzed in the year 2019 were extracted and compiled. The survey included sample analysis records of whiskeys from various sources, which were sent to LABM by a beverage importer, as well as samples of industrial cachaça acquired by

LABM in the local commerce. All the samples were analyzed under the supervision of two of the authors using a previously validated methodology based on inter-laboratory assays coordinated by the Rede Metrológica RS (Rede Metrológica RS, 2019a; 2019b; 2020). The samples consisted of 220 cachaças from the state of Minas Gerais, 80 cachaças from ten other Brazilian states, three samples of cachaça of industrial origin, and 14 whiskey samples (13 Scotch and one American bourbon).

Alcoholic content was determined using previously calibrated INCOTERM brand alcoholmeters (Porto Alegre, RS) with a decimal scale (ranges 30.0-40.0 and 40.0-50.0 percentage by volume). Calibrated thermometers, with a range of -10.0ºC to 50.0°C (divisions of 0.05ºC) were also employed. The samples were previously distilled according to a standardized procedure (ABNT, 1997).

Higher alcohols were determined by gas chromatography using the following analytical standards: absolute ethyl alcohol (99.9%; Merck; Darmstadt, Germany), 1-Propanol (≥99,5%), 2-Methyl-1-propanol (≥99,0%), 2-Methyl-1-butanol (≥98,0%) and 3-Methyl-1-butanol (98%), all of chromatographic grade.

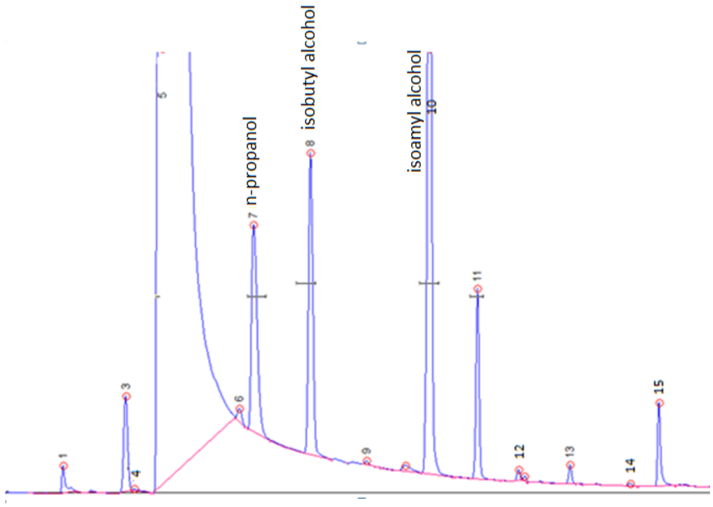
Equipment: A GCROM Generation 8000 gas chromatograph equipped with a flame ionization detector (GC-FID) and a Cwax 20M chromatographic column (30 m x 0.53 mm x 1.0 µm film thickness) from Ohio Valley (Marietta, USA).

Analytical conditions: Oven temperature program: 3 min at 35°C; ramp from 35°C to 80°C at 5.0°C/min), 3 min at 80°C; ramp from 80ºC to 165 °C at 6.1°C/min. The injector temperature was 140°C; detector temperature, 180°C; injection volume, 2 µL in split mode (1:1). The carrier gas was nitrogen (6.0 mL/min); split ratio, 1:1 Quantification of peaks utilized calibration curves previously prepared with standard solutions for each component. Assays were performed in duplicate (BRASIL, 2005).

3. Results and Discussion....

A typical chromatogram that illustrates the location of the peaks of the propyl, isobutyl and isoamyl alcohols is presented in Figure 1. The peaks are very clear, and they were most pronounced among all the traceable and quantifiable secondary components in this analytical procedure.

Figure 1. Chromatographic profile of a cachaça sample.



Source: Authors.

The percentages of samples from Minas Gerais arranged according to the concentration ranges of the propyl, isobutyl and isoamyl alcohols are presented in Figure 2. To avoid overlap of the values, successive ranges begin with a decimal unit (0.1) and end in whole units (example: 20.1 to 40). For interpretation purposes, we will always considered whole numbers (in the example: 20 to 40).

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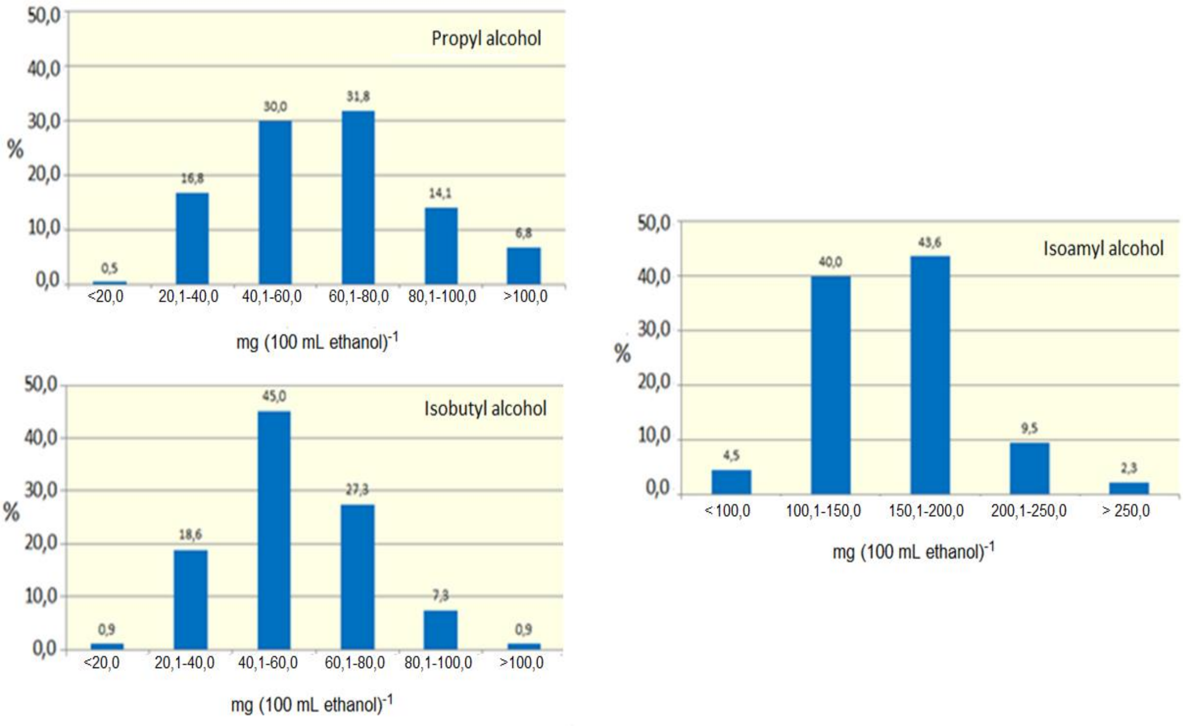
Figure 2. Percentages of cachaça samples from Minas Gerais according to the range of each higher alcohol (220 samples)



Source: Authors.

The concentrations of propyl alcohol fell predominantly between 40 and 80 mg (100 mL ethanol)$^{-1}$, totaling 61.8% of the samples (Figure 1). The number of cachaças within the range from 20 to 100 mg (100 mL ethanol)$^{-1}$ totaled 92.7% of the samples. In the same ranges, and in a similar manner, the concentrations of isobutyl alcohol totaled 72.3% and 98.2%, respectively. The concentration of isoamyl alcohol ranged from 100 to 200 mg (100 mL ethanol)$^{-1}$, which represented 83.6% of the samples. In the range of 100 to 250 mg/100 mL, 93.1% of the samples were located. Over 90% of the cachaças in Minas Gerais fall within the range of 20 to 100 mg (100 mL ethanol)$^{-1}$ for propyl and isobutyl alcohols and in the range of 100 to 250 mg (100 mL ethanol)$^{-1}$ for isoamyl alcohol (Table 1).

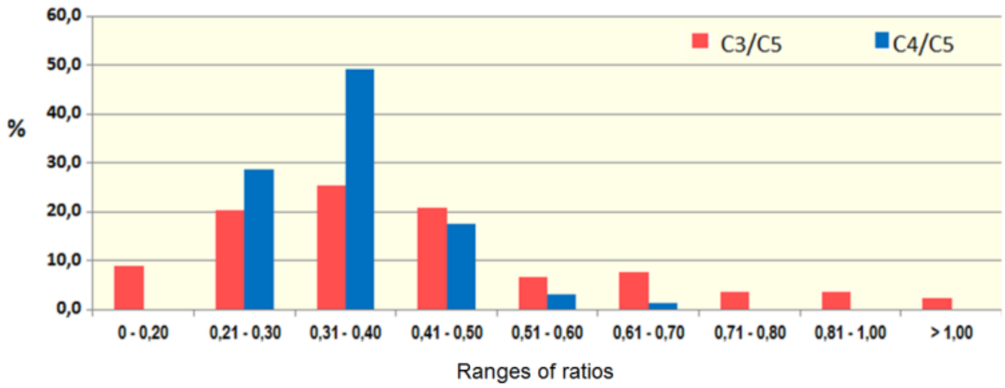
Table 1. Concentration ranges of higher alcohols in cachaças from the state of Minas Gerais.

Higher alcohol	Concentration range [mg (100 mL ethanol)$^{-1}$]	Representativeness (% of the samples)
Propyl alcohol	20 – 100	92,7
Isobutyl alcohol		98,2
Isoamyl alcohol	100 – 250	93,1

Source: Authors.

Another reference used for higher alcohols is the order of magnitude of the specific ratios of the higher alcohols: C3/C5 (propyl/isoamyl alcohol) and C4/C5 (isobutyl/isoamyl alcohol). If adulteration is suspected, for example, these ratios can integrate criteria to attest the beverage's authenticity (MARTIN et al., 1995). The calculated values for the 220 samples from Minas Gerais are shown in Figure 2

Figure 3. Percentages of occurrence of the intervals of C3/C5 and C4/C5 ratios in 220 samples of cachaça from the state of Minas Gerais.



Source: Authors.

The C3/C5 ratios varied over a wide range, from less than 0.20 to greater than 1.00. Most of the samples, however, were between 0.20 and 0.50 (66.8%). The C4/C5 ratios were more homogeneous, varying strictly between 0.21 and 0.70. Moreover, they fell between 0.21 and 0.50 in 95.4% of the samples. The data for the state of Minas Gerais were compared with the results of samples from ten other Brazilian states. Initially, the total alcohol content was compared (Figure 4).

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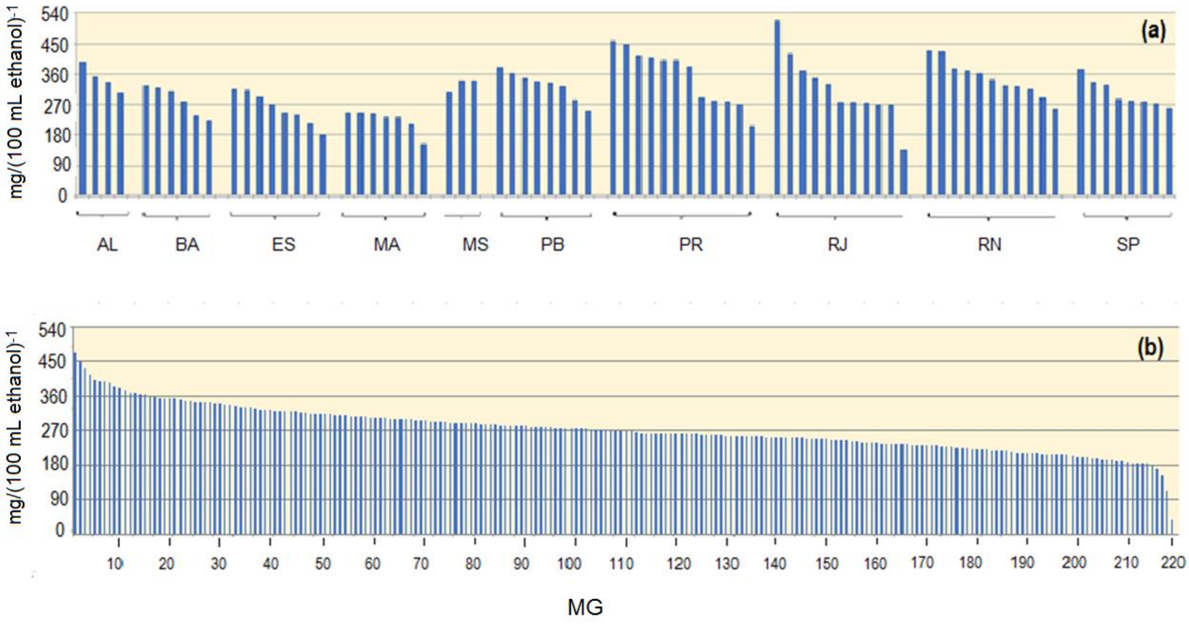
Figure 4. Total higher alcohols (propyl, isobutyl and isoamyl alcohols) (a) in cachaça samples from ten Brazilian states; (b) in 220 samples of cachaça from the state of Minas Gerais.



Source: Authors.

There is a large predominance of values between 180 and 360 mg (100 mL ethanol)$^{-1}$ in Minas Gerais and in the other states. This range included 76% of the cachaça samples from ten Brazilian states (Figure 4a) and 91% of the samples from Minas Gerais (Figure 4b).

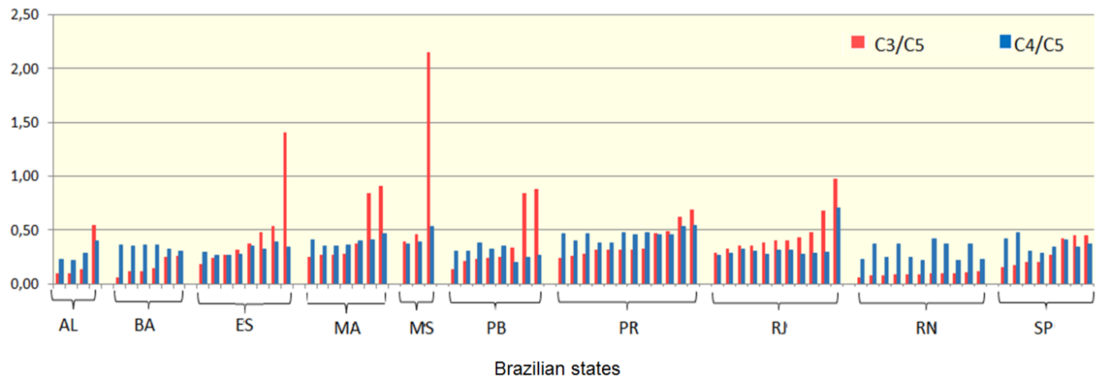
There were samples from several states that contained concentrations of higher alcohol that were greater than the limit established in the legislation [360 mg (100 mL ethanol)$^{-1}$]. However, the averages of the samples from all the states were below than the legal limit (Table 2). The C3/C5 and C4/C5 relationships of the other states are specified for each sample (Figure 5) and by the means for each state (Figure 6).

Table 2. Mean concentrations of higher alcohols by Brazilian state.

State	Mean concentration mg (100 mL ethanol)$^{-1}$
Paraná	353.2
Alagoas	346.5
Rio Grande do Norte	346.5
Mato Grosso do Sul	329.0
Paraíba	327.4
Rio de Janeiro	316.2
São Paulo	301.5
Bahia	281.6
Minas Gerais	272.9
Espirito Santo	257.9
Maranhão	223.5

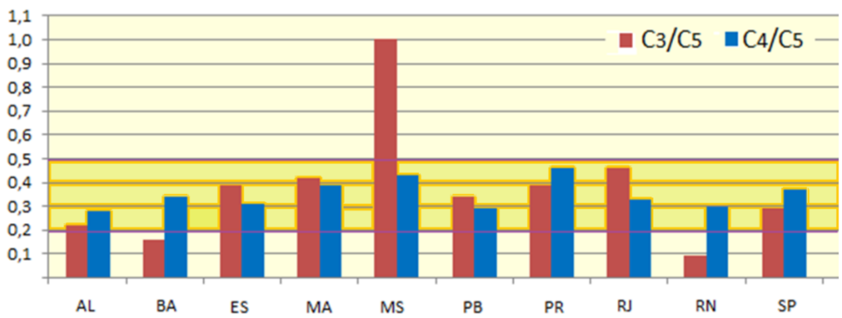
Source: Authors.

Figure 5. C3/C5 and C4/C5 ratios in cachaça samples from ten Brazilian states.



Source: Authors.

Figure 6. Average values of the C3/C5 and C4/C5 ratios in samples from ten Brazilian states



Source: Authors.

Greater homogeneity was observed for the C4/C5 ratios (Figure 5). They almost always fell between 0.20 and 0.50. Only four results (5%) were greater than 0.50, and the highest value was 0.70. The C3/C5 ratio, on the other hand, exhibited marked variations between samples from some states (Espírito Santo, Mato Grosso do Sul, Paraíba, Rio de Janeiro), and they were exceptionally low in samples from Rio Grande do Norte. In Figure 6, the range of ratios representative of the state of Minas Gerais (previously discussed) is shaded in yellow. Thus, it can be seen that the mean C4/C5 ratios of all the states were in the range of 0.20 to 0.50. In the case of the C3/C5 ratio, the mean was less than 0.20 in samples from Bahia and Rio Grande do Norte, and greater than 0.5 in samples from Mato Grosso do Sul.

With the exception of the three samples of cachaça of industrial origin acquired in the Belo Horizonte commerce, all the other samples were sent to the Laboratory directly by the producers, whose names and production scales were not revealed. However, considering that producers of industrial cachaça have their own laboratory to control their products, it can be assumed that most of the samples analyzed were from alembic stills; a smaller portion might have come from standardizing companies that purchase cachaça from third parties (from alembic and industrial stills) for purposes of mixing and standardizing the brand itself. The results of the analysis of industrial cachaça are shown in Tables 4 and 5.

Table 4. Higher alcohols in industrial cachaça [mg (100 mL ethanol)$^{-1}$].

Higher alcohol	Sample		
	1	2	3
Propyl alcohol	57.5	49.7	7.8
Isobutyl alcohol	53.8	68.2	14.1
Isoamyl alcohol	194.6	146.9	27.8
TOTAL	305.9	264.8	49.7

Source: Authors.

Two samples of industrial cachaça contained concentrations of higher alcohol on the same order of magnitude as the alembic cachaças. In one sample (sample 3, Table 4), however, the three alcohols occurred at markedly lower concentrations.

Table 5. C3/C5 and C4/C5 ratios in three samples of industrial cachaça.

Ratio	Sample		
	1	2	3
C3/C5	0.30	0.34	0.28
C4/C5	0.28	0.46	0.51

Source: Authors.

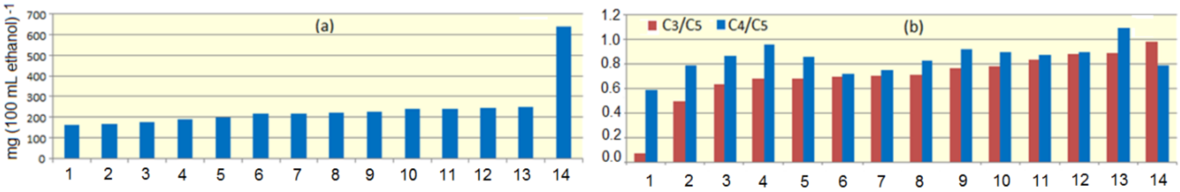
In the three samples of industrial cachaça (Table 5) the C3/C5 and C4/C5 ratios were compatible with the predominant ranges in the various states. Within the scope of all the samples considered, the occurrence of variable proportions between the levels of higher alcohols and, especially, the wide range of values found for the C3/C5 ratio requires some considerations related to the metabolic pathways and productive processes of cachaça.

The C4 and C5 alcohols (isobutyl and isoamyl alcohols) are produced by yeasts mainly from the degradation of the amino acids valine and leucine, when available in quantities greater than that required to meet the physiological needs. In this case, the excess portions are used as nitrogen sources for the biosynthesis of other amino acids, and the yeasts secrete the remaining carbon skeletons after being converted into alcohols. Propyl alcohol can be produced by the catabolism of methionine and threonine, as well as from an alternative route of valine catabolism. However, these are less frequent options, restricted to special conditions such as the lack of valine and leucine. On the other hand, there are alternative pathways for the formation and secretion of propyl alcohol that are directly associated with the catabolism of α-aminobutyric acid (non-genetically encoded amino acid) and, in particular, propyl alcohol can be produced by specific routes associated with contaminating bacteria, such as Clostridium and Propionibacterium: Wood-Werkman pathway, involving the decarboxylation of methylmalonyl-CoA, and ~~the~~ acrylate pathway, involving dehydration of lactic acid. Both routes are followed by the reduction to propanal and then to propyl alcohol (Scully & Orlygsson, 2019). Thus, an atypical increase in the proportion between propyl and isobutyl alcohols can indicate both nutritional deficiencies (affecting leucine and valine levels) and contamination by solvogenic bacteria, which occur naturally in sewage sludge.

Within the scope of the production process, cachaça is manufactured according to two very distinct procedures, according to which they are referred to as "alembic cachaça" (natural must containing bacteria from the environment, with batch distillation) or "industrial cachaça" (must exempt of bacteria, with continuous distillation). In the production of alembic cachaça, corn flour is traditionally used as an adjuvant in the yeast propagation stage, and it remains in the bottom of the vats during successive batches of fermentation. In addition to vitamins and minerals, cornmeal provides several amino acids, including possible precursors to the three higher alcohols, but with a large predominance of leucine (LARKINS, 2019), whose catabolism leads to the formation of isoamyl alcohol. In the case of industrial cachaça, it is assumed that supplementation of the must with sources of inorganic nitrogen prevails, which could justify a sharp decrease in the levels of higher alcohols. However, under typical fermentation conditions for industrial cachaça, the must might contain relevant amounts of amino acids secreted by the enzymatic lysis of dead bacteria and yeasts.

Finally, the results of the analysis of imported commercial whiskeys are presented. Contents of total alcohols are arranged in ascending order (Figure 7a), and C3/C5 and C4/C5 ratios are arranged in ascending order of the C3/C5 ratio (Figure 7b).

Figure 7. Analysis of imported whiskeys (nine scotch whiskeys and one bourbon): (a) content of total higher alcohols; (b) C3/C5 and C4/C5 ratios.



Source: Authors.

The levels of total higher alcohols in the 13 Scotch whiskeys ranged from 163.8 to 247.8 mg (100 mL ethanol)$^{-1}$, with an average of 211.3 ± 42.0 mg (100 mL ethanol)$^{-1}$. The C3/C5 and C4/C5 ratios ranged from 0.49 to 0.98 (mean was 0.75) and 0.79 to 1.10 (mean was 0.86), respectively. The only American whiskey (bourbon, sample 14) analyzed stood out markedly from the other samples. The concentration of higher alcohols was greater than 600 mg (100 mL ethanol)$^{-1}$, the C3/C5 ratio was equal to 0.07 and the C4/C5 ratio was equal to 0.59. These are positive indications for the recognition of the relationships between the contents of higher alcohols in the set of benchnarks for characterization of distillates and fraud investigation, as exemplified in Table 6.

Table 6. Typical ranges of higher alcohol content.

Beverage	C3 + C4 + C5 mg (100 mL ethanol)$^{-1}$	C4/C5
Cachaça	180 - 360	0.20 – 0.25
Scotch whiskey	160 – 270 (?)	> 0.50

Source: Authors.

4. Final Considerations

The range of 180 to 360 mg (100 mL ethanol)$^{-1}$ can be considered as being representative of the sum of the higher alcohol levels in cachaça produced throughout the country. At least for Minas Gerais (the state from which most samples were analyzed), the typical levels of propyl alcohol and isobutyl alcohol are in the range of 20 to 100 mg (100 mL ethanol)$^{-1}$; that of isoamyl alcohol comprises the range of 100 to 250 mg (100 mL ethanol)$^{-1}$. The range of 0.20 to 0.50 is representative of the C4/C5 ratio of cachaças from Minas Gerais. Apparently, this range applies to the C4/C5 ratio of cachaça produced throughout the country, regardless of the production process (industrial scale or alembic cachaça). The C3/C5 ratio exhibits greater fluctuation, a fact that can be associated with the specificities of the metabolic pathways involved in the formation of the three alcohols. The Scotch whiskey samples analyzed contained smaller amounts of higher alcohols, but similar to that of cachaça (160 to 270 mg (100 mL ethanol)$^{-1}$). The difference in the C4/C5 ratio was accentuated and consistent, staying in the range of 0.20 to 0.25 for cachaça and above 0.50 for Scotch whiskeys. The only sample of American whiskey analyzed differed markedly, both from cachaça and from Scotch whiskeys. Because the higher alcohols in cachaça are already routinely quantified by means of gas chromatography in several laboratories in the country, including research and inspection laboratories, the results presented here can be expanded without any burden for the sector. It is believed that this effort can contribute to the improvement of the criteria for the characterization of cachaça and the prevention of fraud, thereby increasing the value of the product and showing respect to consumers.

In Brazil, the official parameters of analysis have a very limited scope in the differentiation between distilled beverages inasmuch as they are based only on the quantification of indices related to functional groups, without distinction of the chemical compounds. To move forward, the introduction of the routine identification and quantification of the structures of the acids, esters and aldehydes, both the aliphatic compounds formed during the production process, and the phenolic compounds introduced during the aging of cachaça, is essential.

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