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Avanço na caracterização da cachaça de alambique: lactato de etila

Advance in the characterization of alembic cachaça: ethyl lactate

Avance en la caracterización de la cachaça de alambique: lactato de etilo

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Resumo

Na metodologia atual de análise de cachaça, o limite de 200 mg/100 mL etanol estabelecido na legislação brasileira para a totalidade dos ésteres presentes na cachaça é aferido exclusivamente pela dosagem do acetato de etila, éster majoritário nas cachaças produzidas em escala industrial (destilação contínua), cujos procedimentos de assepsia aplicados ao caldo têm foco no combate à atividade das bactérias. No entanto, sabe-se que os produtores da cachaça de alambique (destilação por bateladas) fermentam o caldo fresco, que incorpora naturalmente bactérias láticas do ambiente. Foi feito um levantamento dos registros de análise de 300 amostras, correspondentes a 110 marcas de cachaça. As análises foram realizadas por métodos laboratoriais recomendados e por cromatografia de gas. Constatou-se a ocorrência extensiva do lactato de etila em proporções variáveis, porém com a mesma ordem de grandeza do acetato de etila. Concluiu-se que, além das peculiaridades olfativas afetas aos teores peculiares em cada marca, a simples presença do lactato de etila (acima de um nível que possa ser referido como "traços") permite inferir se a cachaça é originária de alambique ou de produção industrial.

Palavras-chave: Componentes secundários; Ésteres; Aroma; Cachaça de alambique.

Abstract

In the current methodology for the analysis of cachaça, the limit of 200 mg/100 mL ethanol established in Brazilian legislation for the totality of esters present in cachaça is measured exclusively by the dosage of ethyl acetate, the principal ester in cachaça produced on an industrial scale (continuous distillation), whose asepsis procedures applied to the broth are focused on combating the activity of bacteria. However, it is known that producers of alembic cachaça (batch distillation) ferment the fresh juice, which naturally incorporates lactic bacteria from the environment. A survey of the analysis records of 300 samples, corresponding to 110 cachaça brands, was performed. The analyses were performed using the recommended laboratory methods and gas chromatography. The extensive occurrence of ethyl lactate was found in varying proportions, but with the same order of magnitude as ethyl acetate. It was concluded that, in addition to the olfactory peculiarities related to the particular contents in each brand, the simple presence of ethyl lactate (above a level that can be referred to as traces) permits one to infer whether the cachaça originates from alembic or from industrial production.

Keywords: Secondary components; Esters; Aroma; Alembic cachaça.

Resumen

En la metodología actual para el análisis de la cachaza, el límite de 200 mg/100 mL de etanol establecido en la legislación brasileña para la totalidad de ésteres presentes en la cachaça se mide exclusivamente por la dosificación de acetato de etilo, el éster principal en las cachazas producidas a escala industrial (destilación continua).), cuyos procedimientos de asepsia aplicados al caldo están enfocados a combatir la actividad de las bacterias. Sin embargo, se sabe que los productores de cachaça alambique (destilación por lotes) fermentan el jugo fresco, que naturalmente incorpora bacterias lácticas del medio ambiente. Se realizó un relevamiento de los registros de análisis de 300 muestras, correspondientes a 110 marcas de cachaza. Los análisis se realizaron mediante los métodos de laboratorio rccomendados y la cromatografía de gases. La presencia extensa de lactato de etilo se encontró en proporciones variables, pero con el mismo orden de magnitud que el acetato de etilo. Se concluyó que, además de las peculiaridades olfativas relacionadas con los contenidos peculiares de cada marca, la simple presencia de lactato de etilo (por encima de un nivel que se puede denominar pequeñísima cantidad permite inferir si la cachaza se origina en alambique o en producción industrial.

Palabras clave: Componentes secundários; Ésteres; Aroma; Cachaça de alambique.

1. Introduction

Humanity has known fermented alcoholic beverages for thousands of years. Distilled alcoholic beverages appeared along with the medieval practices of Alchemy. Only from the 20th century, however, by the combination of scientific advances in several areas, was it possible to clarify the complexity of the phenomena involved both in the production and in the sensory quality of alcoholic beverages (Nykanen & Suomalainen, 1983; Charalambous, 1994).

Currently, for each olfactory experience, it is possible to track and identify the combined and interactive effect of hundreds of volatile components, whose structures and proportions are determined by the specificities of the raw materials, fermentation agents and procedures related to each stage of production (Piggott & Patterson, 1989; Qian & Shellhammer, 2013). These advances gave rise to criteria for "certification of origin" for alcoholic beverages, resulting in an increased appreciation of certified beverages in the international market.

In Brazil, we have an exclusive right over the production of "Cachaça", defined as "brandy produced in Brazilian territory from the distillation of fermented must from sugarcane juice, with an alcohol content between 38 and 48 percent in volume, at 20 ºC" (Brasil, 2005a,b). However, there are still no official parameters that allow one to relate the sensorial diversity of the beverage to the immense variety of producing regions and the procedures adopted in production.

There have been important advances in the identification, monitoring, and prevention of possible contaminants. But the same advances did not occur with the parameters related to the sensorial quality of Cachaça: except for secondary alcohols, which have three identified and quantified structures (n-propyl, isobutyl and isoamyl alcohols), the components of the other classes (acids, aldehydes, and esters) are expressed as a group, without highlighting the structural peculiarities within each functional category (Table 1).

Table 1. Concentrations of cachaça congeners (mg/100 mL ethanol).

Parameter	Minimum	Maximum
Total volatile acidity, in acetic acid	-	150
Total esters, in ethyl acetate	-	200
Total aldehydes, in acetaldehyde	-	30
Furfural + Hydroxymethylfurfural	-	5
Total higher alcohols (Sum of propyl, isobutyl and isoamyl alcohols)	-	360
TOTAL	200	650

Source: Brasil (2005a).

However, most Brazilian laboratories currently employ instrumental resources (such as gas chromatography) for the analysis of cachaça. With such resources, it is possible to routinely identify and quantify, with great analytical precision and reproducibility, several specific components within each functional class. In this work, results, and considerations regarding the specific quantification of ethyl acetate and ethyl lactate, principal components of the total esters group in cachaça are presented.

2. Material and Methods

Samples: The analysis records of 300 cachaça samples were consulted, as received by the laboratory (LABM - Belo Horizonte) during the period between 2018 and 2019. In the survey, the mean values corresponding to 56 brands (for which more than one sample was analyzed) and 54 isolated values (corresponding to brands that had a single sample analyzed) were extracted, totaling 110 results. All the samples were analyzed using previously validated procedures (internal control and interlaboratory analyses) and their records are traceable. For reasons of ethical secrecy, they are not identified. The results are expressed in sequential numbering, adopting the increasing order of ethyl acetate content as a criterion.

Alcohol concentration: The concentration of ethanol was determined using previously calibrated INCOTERM brand alcoholometers (Porto Alegre, RS) equipped with a decimal scale (ranges from 30.0 to 40.0 and 40.0 to 50.0% in volume); and previously calibrated INCOTERM thermometers with a range of -10.0ºC to 50.0ºC and divisions of 0.05

centigrade degrees. The samples were previously distilled, according to a standardized procedure (ABNT - Associação Brasileira de Normas Técnicas, 1997).

Ethyl acetate and ethyl lactate

Analytical standards: absolute ethyl alcohol (99.9%) Merck (Darmstadt, Germany), ethyl acetate (99.5%), ethyl lactate (99.0%), and 1-pentanol (99.0%) Sigma- Aldrich Co. (Saint Louis, MO, USA) were all of chromatographic grades.

Equipment: GCROM Generation 8000 gas chromatograph, flame ionization detector (GC-FID); Cwax 20M chromatographic column (30 m x 0.53 mm x 1.0 µm) 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 per min); 3 min at 80°C; ramp from 80ºC to 165°C at 6.1°C per min. Injector temperature, 140 °C, detector temperature, 180°C, injection volume, 2 µL in split mode (1:1); nitrogen carrier gas (6.0 mL/min). Peak quantification from calibration curves previously prepared with standard solutions for each component. Ten duplicate tests were performed.

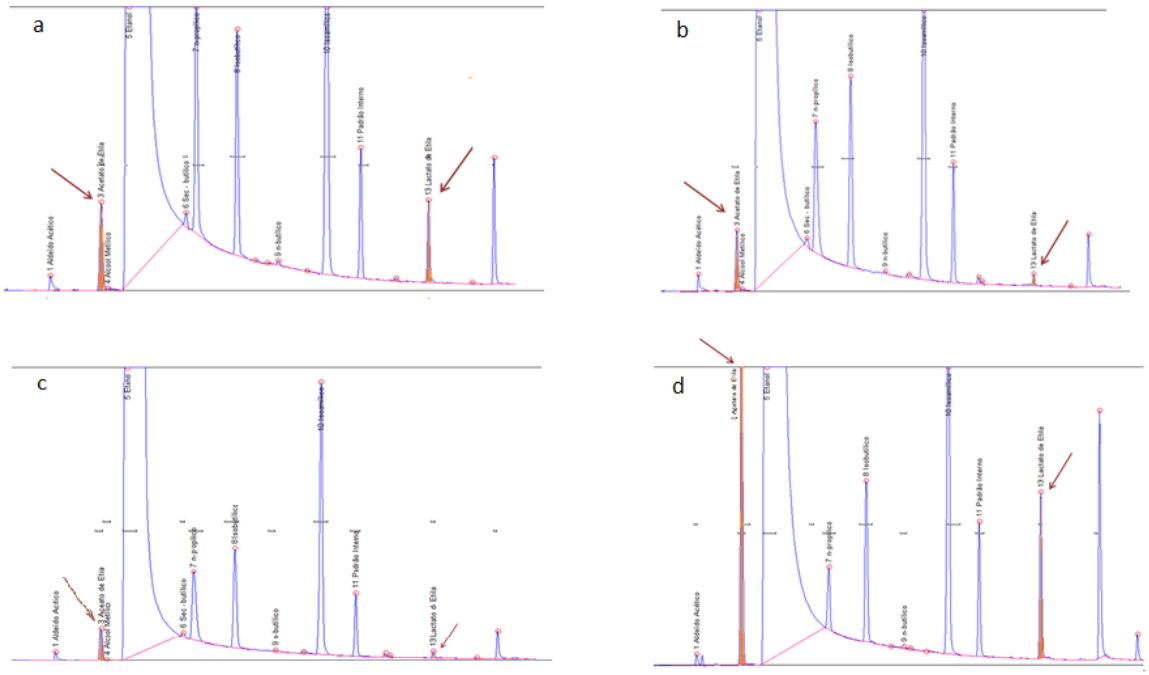
Calculations: The concentrations of ethyl acetate and ethyl lactate in mg/100 mL of ethanol were calculated from the results of the chromatographic analysis (in mg/100 mL of the sample), multiplied by 100 and divided by the alcohol concentration (in mL ethanol/100 mL of the sample at 20ºC) of the respective samples.

3. Results and Discussion

Four chromatograms that illustrate the position of the peaks corresponding to ethyl acetate and ethyl lactate and their relative proportions are presented in Figure 1. Ethyl acetate is eluted early and its peak lies between the peaks corresponding to acetaldehyde and methanol. The peak corresponding to ethyl lactate appears in the final phase after the peaks of ethanol and other alcohols routinely monitored in the analysis of cachaça. In the chromatograms, which correspond to four out of the 300 samples analyzed, the variation between the peak sizes of the two esters can be seen, namely: (a) Median and similar peaks; (b) Median peak for ethyl acetate and very small peak for ethyl lactate; (c) Very small peaks for ethyl acetate and ethyl lactate; (d) Sharp peak for ethyl acetate and large peak for ethyl lactate. The results deduced from the chromatograms of the 110 brands analyzed are

summarized in Figure 2. Each column shows the concentration of ethyl acetate (in blue) and ethyl lactate (in orange), as well as the sum of these two esters (total height of each column).

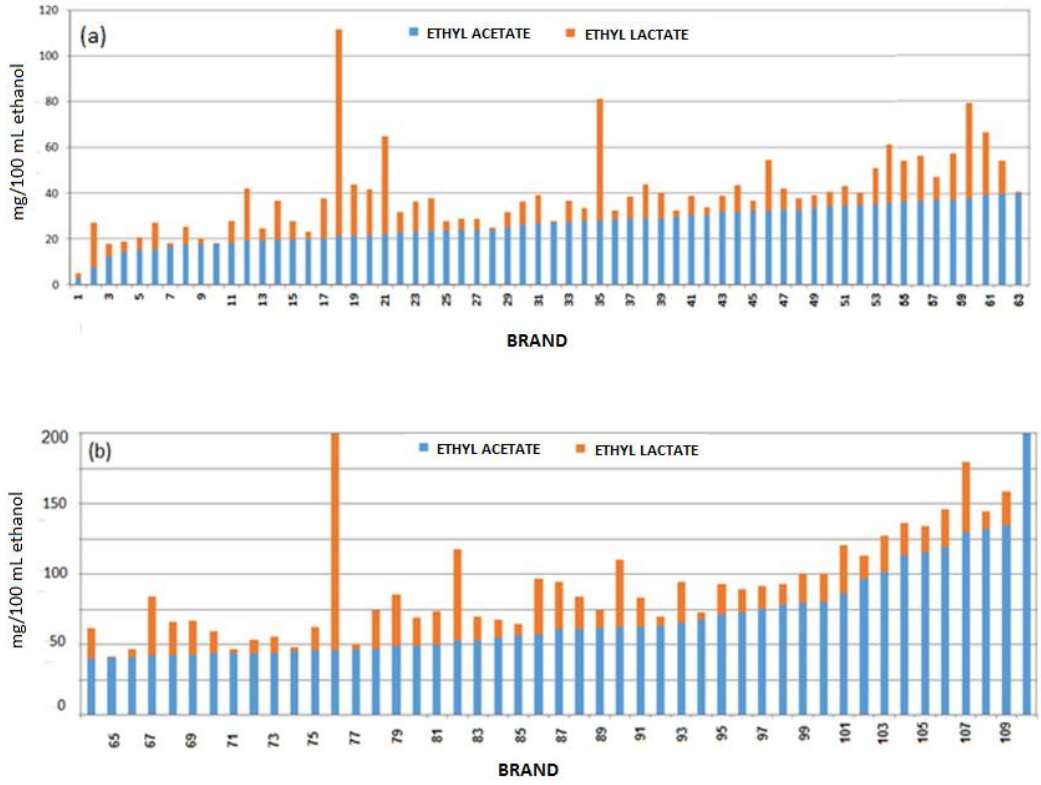
Figure 1. Chromatographic profiles of four cachaça samples.



Source: Authors.

The 200 mL/100 mL ethanol limit established in the legislation for the total esters content (Table 1) was exceeded by only two brands (samples 76 and 110; Table 2). Eliminating the two brands (with extreme levels of ethyl acetate and ethyl lactate, respectively) the means and standard deviations of the other 108 brands are shown in Table 3.

Figure 2. Content of ethyl acetate and ethyl lactate in 110 brands of Cachaça
(a)with ethyl acetate <40 mg/100 mL ethanol;
(b) with ethyl acetate ≥ 40 mg/100 mL ethanol.



Source: Authors.

Table 2. Samples of cachaça with ester concentrations greater than the legal limit.

Sample number	Ethyl acetate	Ethyl lactate	Ethyl acetate and Ethyl lactate
	mg/100 mL ethanol		
76	209.1	4.9	214.0
110	46.2	197.0	243.2

Source: Authors.

Ethyl acetate predominates in the class of esters, given its direct relationship with the metabolism of the yeasts themselves (*Saccharomyces cerevisiae*), which secrete both ethanol, the main product, and acetic acid, a component of the set of secondary metabolites from anaerobic fermentation of sugars (Maia, 1992). Furthermore, it is well known that, because of the interaction with secondary alcohols produced during fermentation, acetic acid is a

precursor to several other esters, although in much smaller proportions (Maarse, 1991; Maia, 1992).

Table 3. Means and standard deviations of ethyl acetate and ethyl lactate concentrations in 108 cachaça brands (mg/100 mL ethanol).

Parameter	Variable range		Mean	Standard deviation
	Mínimum	Maximum		
Ethyl acetate	2.9	135.3	43,5	27.6
Ethyl lactate	0.2	90.4	16,3	14.4
Total	5.0	179.7	59.8	34.4

Source: Authors.

Yeasts do not produce lactic acid, which is essential for the synthesis of ethyl lactate. But it is known that this acid is secreted by an immense number of bacteria, composed of twelve genera and hundreds of species. All of these bacteria are generically referred to as lactic acid bacteria, precisely because they share the property of secreting lactic acid as the end product of anaerobic fermentation of carbohydrates (Batt & Tortorelli, 2014). Lactic acid bacteria are considered omnipresent because they are disseminated in the most varied natural habitats (Oliveira et al. 2015). In fact, the presence of lactic acid bacteria in the sugar cane since the moment of harvest is widely recognized, as well as the fact that its populations can increase sharply, depending on the subsequent procedures for handling, transporting, storing, and crushing the cane. Therefore, it is a fact that the fermentation wort incorporates lactic acid bacteria from the natural environment unless the production process incorporates specific procedures to combat and prevent it from happening (Ventura et al, 2007; Kurboke, 2017). This fact explains the possibility of the presence of ethyl lactate in cachaça, already pointed out by several authors, such as Nascimento (2008).

In the production of food and beverages, lactic acid bacteria are recognized as the main secretors of compounds endowed with desirable aromas (Smid & Kleerebezem, 2014). Hence, the presence of ethyl lactate in cachaça can be interpreted as an indicator of diversity and sensory richness. However, cachaça manufacturers do not intentionally inoculate lactic acid bacteria into the alcoholic fermentation wort, as has been done in other beverages (Sanchez, 2019). Thus, the presence of ethyl lactate in cachaça also indicates that the product comes

from the fermentation of fresh juice, which has not been subjected to asepsis procedures to prevent the growth of bacteria.

Another important inference refers to the specific concentrations of these esters, as well as their relative proportion in each cachaça. As is shown in Figure 1, the levels of ethyl acetate and ethyl lactate varied markedly between different brands. On the average, the ethyl lactate content was 16.3 mg/100 mL ethanol and corresponded to 37% of the mean ethyl acetate content (43.5 mg/100 mL ethanol). Individually, however, the levels of ethyl lactate varied from traces (0.2 mg/100 mL ethanol) to 90.4 mg/100 mL ethanol. In the case of ethyl acetate, the individual values varied between 2.9 mg/100 mL ethanol and 192.7 g/100 mL ethanol. Therefore, the possibility of associating the relative levels and proportions of ethyl acetate and ethyl lactate with sensory criteria and specificities of the production process and the producing region can be inferred.

Concerning the sensory characteristics, ethyl acetate is one of the few aliphatic esters whose olfactory association (such as shoemaker's glue, nail polish, etc.) is identified as negative. Almost all other aliphatic esters are associated with desirable and even noble aromas. Among them, ethyl lactate evokes associations with butter, almonds, and coconut, which are positively evaluated (Sanchez, 2019; Maia, 2019).

Regarding food safety, lactic acid bacteria are considered safe and free from restrictions (Oliveira et al, 2015). They are identified as active in the fight against pathogenic bacteria (Ganzle, 1999; Duarte, 2013). Among numerous other applications, they make up the main focus of probiotic foods (Sobczak et al, 2014; Batt & Tortorelli, 2014) because they are indispensable in the gastrointestinal tract of mammals (Hove et al, 1999). Therefore, although there are procedures designed to combat the presence of lactic acid bacteria in alcoholic fermentation must (Ventura, 2007), their application would not appear to increase the safety of the product or its sensory quality.

Finally, both ethyl acetate and ethyl lactate are aliphatic esters and are produced exclusively because of the characteristics of the broth and the stages of fermentation and distillation. They are distinctly different from the phenolic esters that are incorporated into the cachaça after distillation due to the contact with the wood of the barrels and kegs and that require a specific methodology for analysis, which is not yet routine. Thus, the quantification of these two esters can indicate differentiations between cachaças of various origins.

4. Conclusion

The recognition of the contribution of lactic acid bacteria in an alcoholic fermentation for the production of cachaça signals the possibility of numerous advances in the area of sensory quality, as well as the certification of the identity of the producing regions. The specific dosage of the contents of ethyl acetate and ethyl lactate in cachaças permits a distinction between "alembic cachaça", produced by thousands of producers in all states of the country, and "column cachaças", produced by a small number of manufacturers on an industrial scale, which is about a thousand times greater than that of alembic cachaça. The distinction stems from the fact that manufacturers resort to broth asepsis procedures that combat the intervention of lactic bacteria to make the reproducibility of large-scale production feasible. There are standardized procedures in the production of alembic cachaça, from the cane harvest stage to the beginning of fermentation, which preserves the presence of lactic bacteria, expressing peculiarities of the soil and climate of each region of origin (Maia & Campelo, 2006) Scientific and technological advances in this sector, especially through the identification of parameters that attest to the specificities of the different brands in each scale of production, are indispensable for the strengthening of the cachaça productive sector, as well as for the valorization of this typically Brazilian beverage.

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