RECIMA21 - REVISTA CIENTÍFICA MULTIDISCIPLINAR
ISSN 2675-6218

RELEVANCE OF ETHYL LACTATE AMONG THE CACHAÇA ESTERS

RELEVANCIA DEL LACTATO ETÍLICO ENTRE LOS ÉSTERES DE CACHAÇA

RELEVÂNCIA DO LACTATO ETÍLICO ENTRE OS ÉSTERES DA CACHAÇA

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Submetido em: 22/06/2021 e26467
Aprovado em: 12/07/2021

ABSTRACT
The advancement in the technology of cachaça — sugarcane spirit produced in the Brazilian territory — has led to the search for parameters that allow the improvement of its chemical characterization and sensory specificities. Esters are important aroma agents, but their routine monitoring, as endorsed in Brazilian legislation, is restricted to the quantification of ethyl acetate, which is formed through the metabolism of yeast (*Saccharomyces cerevisiae*). However, being produced in a rural environment using fresh cane juice, fermentation of "cachaça de alembic" involves the presence and activity of lactic acid-producing bacteria, making the presence of ethyl lactate among the cachaça esters predictable. In this work, the levels of ethyl lactate and ethyl acetate were compared in 247 samples, corresponding to 56 brands produced in eleven Brazilian states. Ethyl lactate was found in levels that significantly alter the result of the quantitative participation of esters in the composition of cachaça. This fact is especially relevant considering that lactic acid bacteria are GRAS and widely recognized as a resource for improving the sensory quality of wines and other alcoholic beverages. Thus, studies on the contribution of lactic acid bacteria to the sensory quality of cachaça should be encouraged. Ethyl lactate, in addition to signaling a chemical specificity naturally occurring in cachaça, is a marker of other possible chemical and sensory peculiarities whose research should be stimulated.

KEYWORDS: Lactic bacteria. Distilled beverages. Aroma components. Cachaça composition.

RESUMEN
Los avances en la tecnología de la cachaça - aguardiente de caña de azúcar producida en el territorio brasileño - ha dado lugar a la búsqueda de parámetros que permitan mejorar su caracterización química y especificidades sensoriales. Los ésteres son agentes aromáticos importantes, pero su control rutinario, aprobado por la legislación brasileña, se limita a la cuantificación del contenido de acetato de etilo, que proviene del metabolismo de las levaduras (*Saccharomyces cerevisiae*). Sin embargo, al producirse en un medio rural a partir de jugo fresco de caña de azúcar, la fermentación de la cachaça de alembique conlleva la presencia y actividad de bacterias productoras de ácido láctico, lo que hace predecible la participación de lactato de etilo entre los ésteres de esta bebida. En este trabajo se compararon los contenidos de lactato de etilo y acetato de etilo en 247 muestras correspondientes a 56 marcas producidas en once estados brasileños. Los resultados mostraron la presencia de lactato de etilo en niveles que cambian significativamente el resultado de la participación cuantitativa de los ésteres en la composición de la cachaza. Este hecho es especialmente relevante si se tiene en cuenta que las bacterias del ácido láctico son GRAS y se han utilizado como recurso para mejorar la calidad sensorial de los vinos y otras bebidas alcohólicas.

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ISSN 2675-6218

Por ello, es importante impulsar estudios sobre la contribución de las bacterias del ácido láctico a la calidad sensorial de la cachaza. El lactato de etilo, además de señalar una especificidad química natural en la cachaza, es un marcador de otras posibles peculiaridades químicas y sensoriales cuya investigación debe fomentarse.

PALABRAS CLAVE: Bacterias del ácido láctico. Bebidas destiladas. Componentes aromáticos. Composición de la cachaza.

RESUMO

O avanço na tecnologia da cachaça — aguardente de cana produzida no território brasileiro — tem ensejado a busca por parâmetros que permitam aprimorar sua caracterização química e especificidades sensoriais. Os ésteres são importantes agentes de aroma, mas seu monitoramento rotineiro, conforme referendado na legislação brasileira, restringe-se à quantificação do teor de acetato de etila, que advém do metabolismo das leveduras (*Saccharomyces cerevisiae*). No entanto, sendo produzida em ambiente rural a partir do caldo de cana fresco, a fermentação da "cachaça de alambique" enseja a presença e atividade de bactérias produtoras do ácido lático, tornando previsível a participação do lactato de etila entre os ésteres da cachaça. Nesse trabalho, foram comparados os teores de lactato de etila e acetato de etila em 247 amostras correspondentes a 56 marcas produzidas em onze estados brasileiros. Os resultados evidenciaram a presença do lactato de etila em teores que alteram significativamente o resultado da participação quantitativa dos ésteres na composição da cachaça. Tal fato é especialmente relevante tendo em vista que bactérias láticas são GRAS e têm sido empregadas como recurso para aprimoramento da qualidade sensorial de vinhos e outras bebidas alcoólicas. Assim, é importante estimular estudos sobre a contribuição das bactérias láticas para a qualidade sensorial da cachaça. O lactato de etila, além de sinalizar uma especificidade química de ocorrência natural na cachaça, é um marcador de outras possíveis peculiaridades químicas e sensoriais cuja pesquisa deve ser estimulada.

PALAVRAS-CHAVES: Bactérias láticas. Bebidas destiladas. Componentes do aroma. Composição da cachaça.

INTRODUCTION

In addition to ethanol, distilled beverages contain a large number of secondary components, as acetaldehyde, acetic acid, propanol, isobutanol, isoamyl alcohol, ethyl acetate and other aldeydes, acids, higher alcohols and esters (Scanavini, 2010). In cachaça (the beverage produced in Brazilian territory by the distillation of fermented must from sugarcane juice) current legislation determines the quantification of these compounds by functional category, being expressed as total aldehydes, total volatile acids, total higher alcohols and total esters (Brasil, 2005).

The ester class contains the most relevant compounds for the sensorial quality of alcoholic beverages (Pigott & Patterson, 1989; Qian & Shellhammer, 2012). Among them, the predominance of ethyl acetate is traditionally recognized in all distilled alcoholic beverages (Martin & Caress, 1971; Kostik et al., 2014; Coldea et al., 2017).

In the last decades, however, lactic acid bacteria were recognized because of their important role in the sensory improvement of alcoholic beverages, and their intentional inoculation is a common practice (Edwards & Beelman, 1989; Revel et al., 1999; Gámbaro et al., 2002; Bauer & Dicks, 2004; Kurdys et al., 2018). Researchers highlight the effect of lactic acid bacteria on sensory enrichment,

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which, at least in part, results from the formation of ethyl lactate by the reaction between lactic acid secreted by bacteria and ethanol secreted by yeasts (Sumby et al., 2010).

There are marked sensory differences between ethyl acetate and ethyl lactate. In alcoholic beverages, the presence of ethyl acetate can be noticed under 20 mg/L, providing a fruity aroma (Salo, 1970; Verstrepen et al., 2003). However, negative associations with glue, varnishes and solvents appear at concentrations greater than 100 mg/L. For ethyl lactate, the perception threshold is around 110 mg/L, the sensory effect is positive and it results in associations with nuts, vanilla and butter even at concentrations greater than 300 mg/L (Knoll et al., 2011; Kurdys et al., 2018).

There are no reports of intentional inoculation of lactic acid bacteria to cachaça fermentation must and this practice is not foreseen in its production process. However, ethyl lactate has already been identified in samples of cachaça (Nascimento et al., 2008; Fernandes et al., 2017; Karp et al., 2019). So far, however, the occurrence of this ester has been interpreted only as an indicator of failures in the cleaning of sugarcane juice, which should be remedied with the technological advancement of the sector.

The legislation regarding cachaça is currently under review by government agencies, with a view to advances in the scope of product characterization and inspection and operational procedures. The present study sought to quantify the extent of occurrence of ethyl lactate in Brazilian cane spirit with a view to better discerning its role in the identity of the beverage.

MATERIAL AND METHODS

Samples: Samples of cachaça were sent to the Laboratório LABM (Belo Horizonte) by the producers from eleven Brazilian states (Alagoas, Bahia, Espírito Santo, Maranhão, Mato Grosso do Sul, Minas Gerais, Paraíba, Paraná, Rio de Janeiro, Rio Grande do Norte, and São Paulo) during the period from January to December 2019. Records of 247 analyses, corresponding to 56 cachaça brands, were examined. Of this total number of samples, the producer of one of the brands sent 24 samples during the 2019 harvest, eight of which were extracted from the same tank; these samples were evaluated separately.

Alcohol content: The ethanol concentration was determined using Incoterm brand alcoholometers (Porto Alegre, RS) equipped with a decimal scale and previously calibrated (ranges from 30.0 to 40.0 and 40.0 to 50.0% by volume). Incoterm thermometers were also previously calibrated, with a range of -10.0 °C to 50.0 °C with divisions of 0.05 Cº.

Ethyl lactate and ethyl acetate

Analytical standards: Absolute ethyl alcohol (99.9%, Merck), 99.0% ethyl lactate, 99.5% ethyl acetate and 99.0% 1-pentanol (Sigma-Aldrich) were all of chromatographic grade.
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).

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Analytical conditions: The oven temperature was maintained at 35°C for 3 min; the oven was heated from 35 °C to 80°C at a rate of 5.0°C min^{-1}; the temperature was maintained at 80°C for 3 min, and then heated from 80°C to 165°C at 6.1°C min^{-1}. The injector temperature was 140 °C; the detector temperature was 180°C; injection volume, 2 µL in split mode (1:1); nitrogen carrier gas (6.0 mL min^{-1}). Peak quantification was obtained using calibration curves previously prepared with standard solutions for each component.

Expression of results

The means and standard deviations for each brand and from the variable number of samples were calculated for the concentrations of ethyl acetate and ethyl lactate, always expressed in mg per 100 ml of anhydrous ethanol. In the presentation of the results, the sequence of the brands was based on specific criteria, with no correspondence between the numbers and respective brands in the different figures.

Statistical analysis

Student's t-test was applied: (a) to assess whether the quantification of esters by the ethyl acetate content was significantly altered by the increase in ethyl lactate content; (b) to compare the ranges of variation in the levels of ethyl acetate and ethyl lactate within each brand.

RESULTS AND DISCUSSION

The number of samples per brand varied between 2 and 24 (Table 1). Of the 56 brands, one was selected for separate evaluation because it involved monitoring two crops, totaling 24 samples. The remaining 55 samples were evaluated together.

Table 1. Cachaça samples analyzed

Number of samples analyzed per brand	Number of brands	Total number of samples
2	16	32
3	15	45
4	8	32
5	4	20
6	5	30
7	2	14
9	3	27
11	1	11
12	1	12
24	1	24
TOTAL	56	247

The data collected is presented in Table 2. The numbering of the brands corresponds to the alphabetical order of the respective names. As can be seen in Table 2, the concentration of ethyl lactate was even higher than that of ethyl acetate in four brands (brands 13, 26, 41, 53). Only seven brands contained less than 5 mg (100 mL ethanol)$^{-1}$ of ethyl lactate. The average levels of ethyl acetate and ethyl lactate in the 56 brands analyzed were 41.8 and 17.7 mg (100 mL ethanol)$^{-1}$.

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Considering an alcohol content of 40%, these averages correspond to 167.2 and 70.8 mg L^{-1}, respectively. The interpretation of these values on the basis of the literature regarding the composition of wines allows us to suppose that, even if it does not reach the threshold of sensory perception, the ethyl lactate content can play an important role as a balancing factor and for the attenuation of the unpleasant effect of high concentrations of ethyl acetate (Kurdys et al., 2018). According to the Student t test, the ester concentration of the analyzed brands at the 99.9% confidence level is altered by the inclusion ethyl lactate.

To facilitate visualization, results of minimum and maximum levels of ethyl acetate and ethyl lactate are shown in Figure 1 for 55 brands in decreasing order of the ranges of variation (within each brand). One of the brands (brand 56) was reserved for specific evaluations (Figures 2 and 3). Large variations in the concentrations of ethyl acetate and ethyl lactate between brands and, even more importantly, within the same brand can be observed. Variations in total ester concentrations would be expected if some of the samples had been aged, and this variation would depend on the duration of the aging process. However, the proportions of individual esters would not be expected to vary significantly with aging. Thus, there is a necessity for standardization of the raw material and the conditions of fermentation and distillation so that products with a more uniform composition can be obtained.

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Table 2. Means and standard deviations of the concentrations of ethyl acetate and ethyl lactate in 56 brands of cachaça from eleven Brazilian states

BRAND	ETHYL ACETATE		ETHYL LACTATE		BRAND	ETHYL ACETATE		ETHYL LACTATE	
	Mean	Standard Deviation	Mean	Standard Deviation		Mean	Standard Deviation	Mean	Standard Deviation
1	57.7	21.4	39.4	8.9	29	44.2	46.8	11.6	5.8
2	135.3	49.8	23.6	4.5	30	37.4	16.7	19.9	8.7
3	56.8	14.8	8.2	3.2	31	28.9	8.5	14.8	2.3
4	58.3	32.7	19.8	8.2	32	36.2	14.4	24.4	4.5
5	39.8	9.9	19.8	1.5	33	43.5	9.9	15.6	0.1
6	21.7	9.9	20.1	19.9	34	62.6	48.3	20.9	19.6
7	28.3	1.8	4.1	1.1	35	28.9	14.5	11.5	2.0
8	20.5	4.4	5.3	3.9	36	23.1	0.0	13.1	0.0
9	80.0	13.1	20.7	9.9	37	63.5	57.1	6.3	4.9
10	75.5	16.8	16.1	6.4	38	71.5	26.5	21.8	4.4
11	23.3	2.5	14.3	1.1	39	31.8	14.5	11.7	4.1
12	34.3	23.9	6.3	3.5	40	25.8	6.6	10.4	2.3
13	28.1	8.5	53.2	16.1	41	19.1	2.8	23.1	4.1
14	65.2	24.8	29.7	13.6	42	65.3	46.5	12.7	3.3
15	14.2	1.0	4.5	2.1	43	32.8	9.6	4.8	1.8
16	35.7	4.8	25.5	11.3	44	96.7	21.3	16.7	5.4
17	46.2	12.7	16.7	2.5	45	25.0	9.3	6.6	2.6
18	65.1	35.4	34.1	13.8	46	43.8	1.5	3.3	0.3
19	18.3	4.6	9.6	4.3	47	43.6	10.8	15.8	15.2
20	36.2	17.2	6.2	9.3	48	22.6	7.8	11.0	10.0
21	32.2	17.1	22.2	7.2	49	26.8	9.0	12.4	3.4
22	27.0	8.8	0.6	0.1	50	21.4	11.5	22.3	19.9
23	18.7	8.9	3.0	1.8	51	20.0	0.3	17.6	0.6
24	19.5	5.0	8.3	3.3	52	124.2	46.8	23.2	14.9
25	39.4	28.0	27.2	12.2	53	23.7	4.2	90.4	61.6
26	22.1	1.0	42.9	29.3	54	17.8	0.1	0.4	0.0
27	47.0	29.3	27.4	25.1	55	31.6	7.6	7.3	6.6
28	33.3	18.0	6.0	3.5	56	48.9	20.8	37.1	9.8

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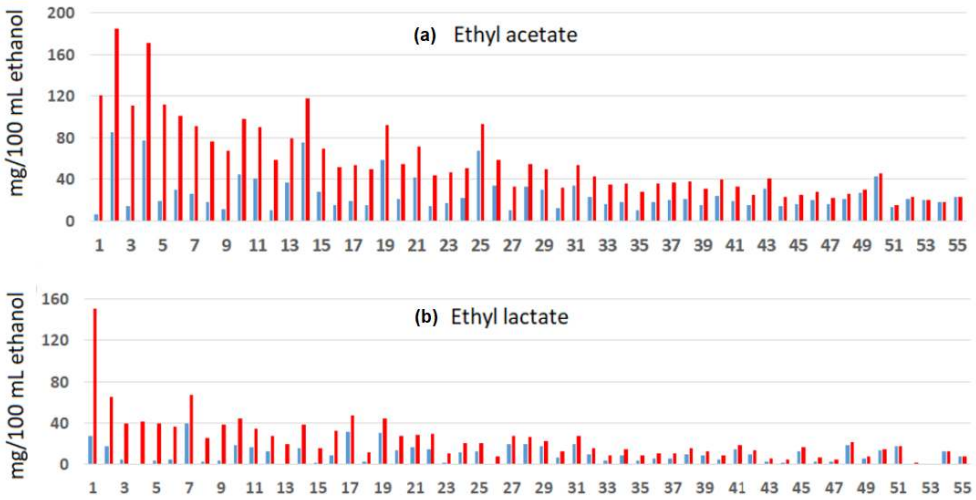

Figure 1. Minimum and maximum concentrations found in 55 brands of cachaça: (a) ethyl acetate; (b) ethyl lactate

The occurrence of ethyl lactate in cachaça has been attributed to operational failures in fermentation and distillation (Serafim et al., 2013). It is assumed that, because they come exclusively from natural sources, lactic acid bacteria are contaminants to be combated, which can cause detrimental effects on the final quality of the beverage and on standardization efforts. The median percentage values of standard deviations in relation to the average levels of ethyl acetate and ethyl lactate were 35.4 and 43.6 mg (100 mL ethanol)$^{-1}$, respectively. According to the Student t test, these values are not significantly different at the 95% confidence level. Therefore, although devoid of specific controls and monitoring, it appears that lactic acid bacteria do not occur so randomly, and, in fact, they can contribute to flavor development in a way similar to that of yeasts, at least within the scope of aliphatic esters. Although coming exclusively from the natural environment of each factory, the ethyl lactate content does not allow the discrimination of origins, unless the producers adopt effective efforts to standardize their content within narrower ranges in the different batches produced. This statement is supported by Figure 2, in which the analysis of 24 samples from a single producer during a harvest (northern Minas Gerais, May to November 2019) are presented. At the beginning of the harvest, there was a clear predominance of ethyl acetate over ethyl lactate, which demonstrates the predominance of alcoholic yeasts in the wort. However, some formation of ethyl lactate always occurs. Throughout the harvest, progressive changes occur in the populations of yeast and bacteria within the must. In the case under study, one of the changes was demonstrated by the prevalence of ethyl acetate at the beginning of the harvest, whereas the levels of the two esters were similar in the middle of the harvest (samples 13 to 15), and ethyl lactate began to predominate in the final stages (warmer months) (samples 16 to 24). Compared to the first sample, the last sample indicates the

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complete reversal of the trend, with a large predominance of ethyl lactate over ethyl acetate. These data prove that the standardization procedures for annual harvests and between successive harvests are really essential, both for sensory adjustment and for chemical characterization of each brand.

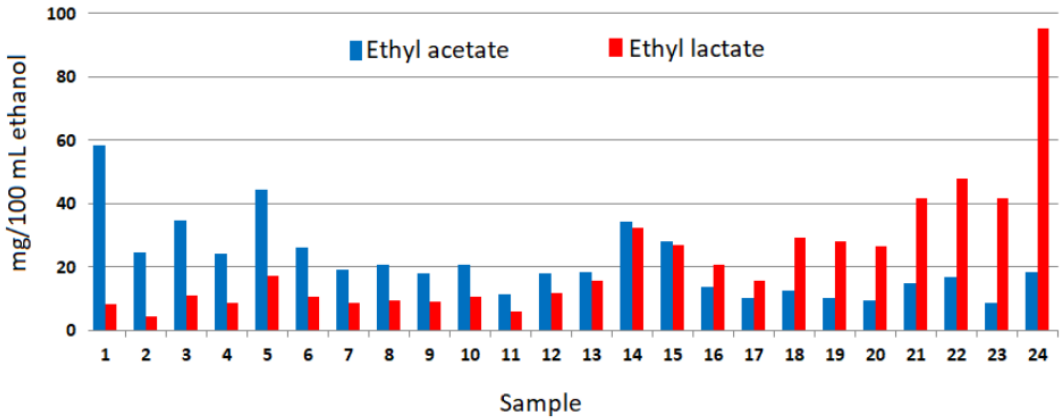

Figure 2. Evolution of the ethyl acetate and ethyl lactate concentrations in 24 cachaça samples from the same producer throughout the 2019 harvest.

An additional evaluation can be extracted from Figure 3, in which the results of samples from the same fermentation tank throughout the entire harvest period are presented. As before, there is a marked predominance of ethyl acetate in the first sample (new yeast, colder weather), followed by the progressive increase in the levels and proportions of ethyl lactate. After the analysis of the sixth sample, the producer made interventions in the fermentation vat to revitalize the yeasts. These procedures led to the decrease in the ethyl lactate concentration in the following fermentation processes, although the predominance of this ester was still maintained.

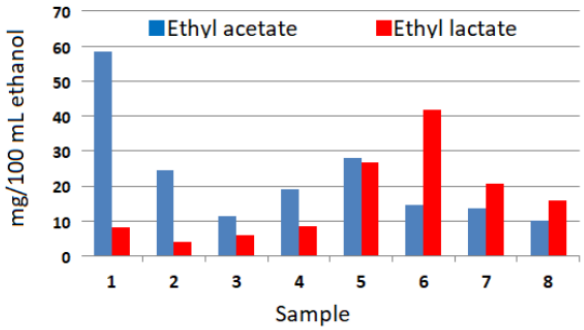

Figure 3 - Evolution of the levels of ethyl acetate and ethyl lactate in eight samples of cachaça from the same fermentation tank during the 2019 harvest.

CONCLUSION

By measuring the ethyl lactate content in cachaça, one can gauge the participation of lactic acid in the production process. The levels of ethyl lactate can vary between different producers because of operational peculiarities (especially those associated with the preparation of the broth to be

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fermented), as well as between different batches of the same producer, due to natural factors. Therefore, monitoring ethyl lactate content is relevant and can contribute to the scope of the characterization chemical identity of the cachaça. It can also be used as a tool for standardization criteria of lots and vintages and as a reference for stimulating new advances in the scope of the appreciation of cachaça on the world stage. In addition, the analysis of ethyl lactate in cachaça does not introduce any difficulty in the analytical procedure; it is only necessary to identify and quantify an additional peak (ethyl lactate), which occurs under the same chromatographic conditions required for the analysis of acetate ethyl and other aroma components routinely analyzed.

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