

Quantitative Ester Analysis in Cachaça and Distilled Spirits by Gas Chromatography–Mass Spectrometry (GC–MS)

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An analytical procedure for the separation and quantification of ethyl acetate, ethyl butyrate, ethyl hexanoate, ethyl lactate, ethyl octanoate, ethyl nonanoate, ethyl decanoate, isoamyl octanoate, and ethyl laurate in cachaça, rum, and whisky by direct injection gas chromatography–mass spectrometry was developed. The analytical method is simple, selective, and appropriated for the determination of esters in distilled spirits. The limit of detection ranged from 29 (ethyl hexanoate) to 530 (ethyl acetate) $\mu\text{g L}^{-1}$, whereas the standard deviation for repeatability was between 0.774% (ethyl hexanoate) and 5.05% (isoamyl octanoate). Relative standard deviation values for accuracy vary from 90.3 to 98.5% for ethyl butyrate and ethyl acetate, respectively. Ethyl acetate was shown to be the major ester in cachaça (median content of 22.6 mg 100 mL⁻¹ anhydrous alcohol), followed by ethyl lactate (median content of 8.32 mg 100 mL⁻¹ anhydrous alcohol). Cachaça produced in copper and hybrid alembic present a higher content of ethyl acetate and ethyl lactate than those produced in a stainless-steel column, whereas cachaça produced by distillation in a stainless-steel column present a higher content of ethyl octanoate, ethyl decanoate, and ethyl laurate. As expected, ethyl acetate is the major ester in whiskey and rum, followed by ethyl lactate for samples of rum. Nevertheless, whiskey samples exhibit ethyl lactate at contents lower or at the same order of magnitude of the fatty esters.

KEYWORDS: Cachaça; GC–MS; ester; spirits; ethyl acetate; ethyl lactate

INTRODUCTION

The Brazilian sugar-cane spirit, cachaça, is produced from the distillation of the fermented sugar-cane juice and has an ethanol content between 38 and 48% (v/v) (1). The annual production of cachaça is around 2 billion liters, of which less than 1% is exported (2). Recent efforts have been made to improve the quality of cachaça in view to expand its dissemination in the international trade market. As part of our research efforts, a better knowledge of the chemical composition of cachaça has been gained (2–9). Although on some extension the qualitative composition of cachaça has been previously subject of discussion, as far as we know, the quantitative profile of the fatty esters in cachaça has not yet been reported.

Similar to other distilled spirits, cachaça is organoleptically characterized by the content of secondary metabolites, also known as congeners, such as higher alcohols, esters, carboxylic acids, and carbonyl compounds, which contribute to the peculiar flavor of the spirit (9–11). Esters are well-known aroma compounds occurring at relatively high content and exhibiting a low odor threshold and therefore are quite relevant for the beverage sensory properties (9–13).

With the aim of improving the chemical characterization of cachaça, this paper reports a selective and sensitive method for the quantitative determination of eight ethyl esters (ethyl acetate, ethyl butyrate, ethyl hexanoate, ethyl lactate, ethyl octanoate, ethyl nonanoate, ethyl decanoate, and ethyl laurate) and isoamyl octanoate in distilled spirits by direct injection gas chromatography–mass spectrometry (GC–MS) analysis. These esters are known to be the most abundant esters in spirits, and their presence is commonly related to the pleasant fruity bouquet of alcoholic beverages (14–16).

EXPERIMENTAL SECTION

Materials. Ester standards (ethyl acetate, ethyl butyrate, ethyl hexanoate, ethyl lactate, ethyl octanoate, ethyl nonanoate, ethyl decanoate, isoamyl octanoate, and ethyl laurate) and the internal standard 4-methyl-2-pentanol were purchased from Sigma-Aldrich (Steinheim, Germany) and used as received. The high-performance liquid chromatography (HPLC)-grade ethanol was obtained from Fischer Scientific (Fair Lawn, NJ) and used without further purification. Water was previously bidistilled and further deionized using a Milli-Q system Millipore (Millipore, Bedford, MA).

GC–MS Analysis. Sample analyses were carried out in a gas chromatograph model GC17A (Shimadzu, Tokyo, Japan) hyphenated to a mass selective detector model QP5050A (Shimadzu, Tokyo, Japan) using electron impact (70 eV) as the ionization source. The target analytes were separated through a capillary column coated with an

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Table 1. Linear Regression Data and Acquisition Ion Window for the Target Compounds

compounds	acquisition window (min)	ion (<i>m/z</i>)	calibration range ^a	(<i>r</i> ²)	linear equation expression
ethyl acetate	4.00–6.50	70	5.40–97.2	0.9999	$y = (-0.053 \pm 0.007) + (0.038 \pm 0.0001)x$
ethyl butyrate	6.50–8.00	88	0.878–15.8	0.9999	$y = (-0.025 \pm 0.004) + (0.116 \pm 0.0001)x$
4-methyl 2-pentanol	8.00–11.4	69			
ethyl hexanoate	11.4–15.0	88	0.759–13.7	1	$y = (-0.022 \pm 0.002) + (0.229 \pm 0.0003)x$
ethyl lactate	15.0–16.2	75	5.06–91.1	1	$y = (-0.011 \pm 0.0006) + (0.036 \pm 0.00001)x$
ethyl octanoate			1.38–24.9	0.9999	$y = (-0.063 \pm 0.010) + (0.311 \pm 0.0008)x$
ethyl nonanoate	16.2–22.7	88	0.504–9.07	1	$y = (-0.019 \pm 0.004) + (0.338 \pm 0.0008)x$
ethyl decanoate			1.38–24.8	0.9999	$y = (-0.070 \pm 0.012) + (0.362 \pm 0.0009)x$
isoamyl octanoate	22.7–25.0	70	0.758–13.6	0.9999	$y = (-0.053 \pm 0.012) + (0.555 \pm 0.0018)x$
ethyl laurate	25.0–29.0	88	1.04–18.6	0.9999	$y = (-0.015 \pm 0.011) + (0.421 \pm 0.0012)x$

^a Values in mg L⁻¹.

esterified polyethylene glycol phase (HP-FFAP; 50 m × 0.20 mm × 0.33 μm film) (Hewlett-Packard, Palo Alto, CA). The oven temperature was programmed from 35 to 180 °C at a programming rate of 5 °C min⁻¹ and then raised at 20 °C min⁻¹ increments from 180 to 220 °C (5 min hold). The temperature of the injector and detector interface was 220 °C. Helium was used as a carrier gas at a flow rate of 1 mL min⁻¹, and the analyses were performed in the split mode (1:15). Direct sample injection was used with a sampling volume of 1 μL, and the solvent delay time was set for 4 min. The calibration curve was constructed following the EURACHEM and ICH guides (17, 18). Samples with analyte content out of the calibration range were appropriately diluted with an ethanol/water 40% (v/v) solution. The chromatographic data were collected using the CLASS-5000 software (Shimadzu, Tokyo, Japan). The analyses of the target esters were performed in the selective ion monitoring (SIM) mode (Table 1).

Samples. A total of 136 samples of cachaça have been collected *in loco* in the São Paulo state, Brazil. From these samples, 68 cachaças were produced by copper alembic distillation (batch process) and 27 were produced by stainless-steel column distillation (continuum process). The remaining samples of cachaça (41) were produced in mixed alembic, i.e., alembics build up with parts of copper and parts of stainless steel (batch process). The manufacturers kindly provided all information regarding the distiller apparatus used for the spirit production.

In addition, 10 samples of whiskey and 10 samples of rum were analyzed for comparison purposes. Whiskey samples were William Grant's [Scotland, 40% (v/v)], Johnnie Walker Black Label [Scotland, 43% (v/v)], Johnnie Walker Gold Label [Scotland, 43% (v/v)], Johnnie Walker Blue Label [Scotland, 43% (v/v)], Johnnie Walker Green Label [Scotland, 43% (v/v)], The Famous Grouse [Scotland, 40% (v/v)], Ballantine's Finest [Scotland, 43% (v/v)], Passport [Scotland, 40% (v/v)], Maker's Mark [U.S., 45% (v/v)], and Jameson [Ireland, 40% (v/v)]. Rum samples were Havana Club 7 años [Cuba, 40% (v/v)], Havana Club Silver Dry [Cuba, 40% (v/v)], Bacardi Oro [Brazil, 38% (v/v)], Bacardi Blanco [Brazil, 38% (v/v)], Montilla Cristal [Brazil, 38% (v/v)], Montilla Ouro [Brazil, 38% (v/v)], Vigia [Cuba, 40% (v/v)], XK Solera [Mexico, 38% (v/v)], Varadero 7 años [Cuba, 38% (v/v)], and Santiago de Cuba [Cuba, 38% (v/v)].

RESULTS AND DISCUSSION

A characteristic chromatogram observed for cachaça samples in the (A) SCAN mode (scan range of *m/z* 40–400), (B) SIM mode, and (C) standard mixture of esters is depicted in Figure 1. The highest peak in Figure 1A corresponds to isoamyl alcohol (3-methyl-1-butanol), which is present in all analyzed samples at a relative high amount (average = 152 mg 100 mL⁻¹ anhydrous ethanol, minimum = 2.92 mg 100 mL⁻¹ anhydrous ethanol, and maximum = 441 mg 100 mL⁻¹ anhydrous ethanol; see the Supporting Information). When the analyses were run in the SIM mode (Figure 1B), the interferences because of the presence of isoamyl alcohol and other compounds were prevented.

Table 1 reports the linear regression data and the SIM ion window for the esters studied. The constructed calibration plots show good linearity, with correlation coefficients (*r*²) close to the unit for all target compounds. The method linearity was studied,

attending to the expected natural range of occurrence of these compounds in distilled beverages, and it was ascertained by plotting the peak area of each compound against the corresponding content. Linearity (Table 1) remained in the studied concentration range and can be considered satisfactory, from 0.878 to 15.8 mg L⁻¹ for ethyl butyrate and from 5.40 to 97.2 mg L⁻¹ for ethyl acetate.

The analytical methodology was validated (17, 18) by its repeatability, accuracy, linearity, and limits of detection (LODs) and quantitation (LOQs). The repeatability of the method was evaluated through the relative standard deviation (RSD) within a day sequence analyses at three different concentrations (repeatability, *n* = 9). The RSDs for repeatability and accuracy are given in Table 2. The repeatability and accuracy of the method ranged from 0.77 to 5.05% and 1.42 to 12.2%, respectively, and are considered satisfactory for trace analyses (19).

From the calibration curves, the LOQ and LOD were estimated using the ICH guideline (17); LOQ = 10σ/*S* and LOD = 3.3σ/*S*, where σ is the standard deviation of *y* intercepts of the regression line and *S* is the slope of the calibration curve. The calculated value of LOD and LOQ for each analyte is reported in Table 2. The method LOD is below 100 μg L⁻¹ for all studied esters with the exception for ethyl acetate (LOD = 530 μg L⁻¹). These LOD values are found to be around 2 orders of magnitude smaller than those reported in the literature concerning headspace solid-phase microextraction (SPME)–GC–MS analyses of distilled spirits (20, 21). Few methods report the determination of ethyl esters in wine and beer by SPME–GC–MS with detection limits below 100 μg L⁻¹ (21–23). However, as observed by us, these methods could not be extended to the analyses of distilled spirits with high ethanol and isoamyl alcohol content with the maintenance of this low detection limits. This limitation is mainly accounted as a result of the SPME fiber saturation by ethanol and isoamyl alcohol at contents usually found in cachaça (see the Supporting Information).

The summary of the results for the quantitative analyses of 9 esters in samples of 136 cachaças are presented in Table 3. As observed, ethyl acetate is the major ester present in cachaça (median content of 18.0 mg 100 mL⁻¹ anhydrous alcohol) followed by the ethyl lactate (median content of 7.78 mg 100 mL⁻¹ anhydrous alcohol). The occurrence of ethyl lactate in cachaça is related to bacterial contamination of the sugar cane juice by strains of *Lactobacillus* spp. responsible for the concurrent lactic fermentation. A possible source of these bacteria is associated to contaminated raw materials (sugar cane, yeast, and water) or the distillation apparatus itself. This secondary fermentation, which is predominant at pH lower than 4 and temperatures higher than 30 °C, does not adversely affect the ethanol yield. Even though, the extension of this contribution is not well-understood and it is believed to contribute positively to the flavor quality of the spirit (24). It is worthwhile mentioning that the high amount of ethyl lactate concomitant with its low odor threshold in cachaça may hinder

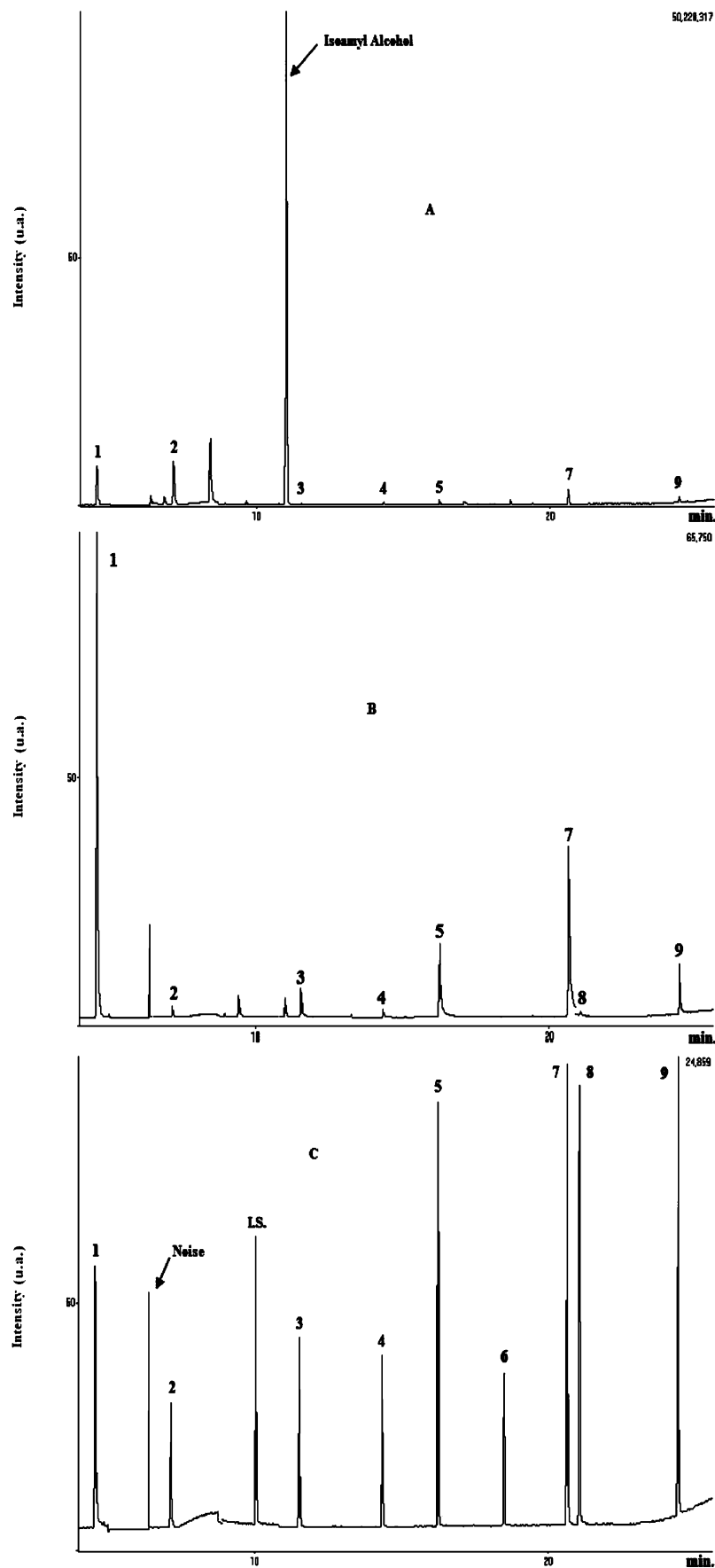


Figure 1. Characteristic chromatograms obtained for cachaça in the (A) SCAN mode (scan range of m/z 40–400), (B) SIM mode, and (C) standard mixture of esters. Peaks (1) ethyl acetate, (2) ethyl butyrate, (3) ethyl hexanoate, (4) ethyl lactate, (5) ethyl octanoate, (6) ethyl nonanoate, (7) ethyl decanoate, (8) isoamyl octanoate, and (9) ethyl laurate.

Table 2. Repeatability, Accuracy, Linearity, and LODs and LOQs for the Nine Esters Analyzed in Cachaça

esters	repeatability (%)	accuracy (%)	LOD ^a	LOQ ^a	LOD ^b	LOQ ^b
ethyl acetate	3.41	98.5	0.53	1.78	0.13	0.44
ethyl butyrate	2.52	90.3	0.097	0.32	0.024	0.080
ethyl hexanoate	0.774	91.8	0.029	0.096	0.0072	0.024
ethyl lactate	2.01	96.4	0.053	0.18	0.013	0.045
ethyl octanoate	1.90	93.6	0.097	0.32	0.024	0.080
ethyl nonanoate	2.60	95.2	0.031	0.10	0.0078	0.025
ethyl decanoate	3.04	93.1	0.098	0.33	0.024	0.082
isoamyl octanoate	5.05	92.8	0.067	0.22	0.018	0.055
ethyl laurate	4.11	93.1	0.082	0.27	0.020	0.068

^a Values in mg L⁻¹. ^b Values in mg 100 mL⁻¹ anhydrous ethanol. The ethanol strength of the solution was 40% (v/v).

Table 3. Average, Median, Maximum, and Minimum Values (mg 100 mL⁻¹ Anhydrous Ethanol) Collected for the Analysis of 136 Samples of Cachaça

compounds	cachaças			
	average	median	maximum	minimum
ethyl acetate	44.0	18.0	433	1.56
ethyl butyrate	0.631	0.138	19.7	0.0365
ethyl hexanoate	0.200	0.138	1.20	0.0342
ethyl lactate	25.5	7.78	244	0.0766
ethyl octanoate	0.661	0.456	0.0991	3.60
ethyl nonanoate	0.0722	0.0577	0.138	0.0377
ethyl decanoate	1.37	0.872	7.33	0.132
isoamyl octanoate	0.114	0.0998	0.234	0.0666
ethyl laurate	0.633	0.418	4.03	0.0831

Table 4. Total Ester, Ethyl Acetate, and Lactate Content (mg 100 mL⁻¹ Anhydrous Ethanol) for Cachaças, with Total Ester Amount Exceeding the Brazilian Legislation (200 mg 100 mL⁻¹ Anhydrous Ethanol)

samples	total ester content	ethyl acetate	ethyl lactate
sample 21	231	65.0	166
sample 27	255	226	28.5
sample 30	374	267	106
sample 37	340	94.5	244
sample 38	200	31.1	169
sample 42	417	385	31.1
sample 68	263	36.0	226
sample 94	383	344	38.2
sample 98	470	433	36.0

the desired aroma of the fatty esters, leading to a spirit with a poor fruity bouquet.

According to the Brazilian legislation (1), the total content of esters in cachaça expressed in equivalents of ethyl acetate must not exceed 200 mg 100 mL⁻¹ anhydrous ethanol. For rums, this limit is in the range of 200–500 mg 100 mL⁻¹ anhydrous ethanol (25). Among the 136 analyzed samples of cachaça, nine samples exceeded the limit established by the Brazilian authorities (Table 4). Notably, four of the above-mentioned samples (numbers 21, 37, 38, and 68) exceed the legislation limit as a consequence of their high content of ethyl lactate.

Currently, the official method adopted by the Ministério da Agricultura, Pecuária e Abastecimento (MAPA, Brazil) is the volumetric analysis based on the back-titration of the acid used to neutralize the alkali excess used for ester hydrolyses (26).

Besides the volumetric method, the Brazilian authorities (MAPA) also recommend a gas chromatographic coupled to a flame ionization detector (GC–FID) as an alternative to volumetric analysis (26). However, the volumetric method quantifies all esters

that could be hydrolyzed, while the suggested chromatographic method is solely based on the ethyl acetate determination. Thus, the method based on ethyl acetate analyses by GC–FID is expected to underestimate the total amount of esters. Ethyl lactate limits remain an issue for the official methods because the concentration of ethyl lactate is around 40% of the total ester content, hence, being the second most abundant ester in that spirit (Table 3). Further analyses of the quantitative data in Table 3 revealed that when applying the official method the total amount of esters in cachaça is underestimated by a maximum of up to 1137%, excluding the ethyl lactate contribution from the analyses.

The fermentation of sugar-cane juice and the distillation step may be considered the most important steps in the production of a cachaça with superior sensorial quality (27, 28). The resultant cachaças are distinguished as “artesian” or “industrial” when they are products distilled in copper alembic or stainless-steel columns, respectively (28). The distillation in copper alembic is known as a batch process corresponding to one theoretical plate, and the distillation in the column is a continuous process involving many assembled theoretical plates (29). Therefore, chemical differences are expected in the spirits produced by these two distillation apparatuses especially because of the influence of the material used in building the distiller (27, 30).

The cachaça samples dealt in this study were separated in three groups depending upon the distillation setup (see the Experimental Section). Figure 2 depicts a histogram of the median value for each analyte according to the type of distillation apparatus. It is clear that cachaças produced by distillation in copper and hybrid alembic exhibit a higher content of ethyl acetate and ethyl lactate than those produced in a stainless-steel column. On the other hand, cachaças produced by distillation in stainless-steel columns tend to exhibit a high content of ethyl octanoate, ethyl decanoate, and ethyl laurate than those distilled in alembics.

Indeed, cachaças distilled in hybrid copper–stainless-steel alembic show a total ester content (73.2 mg 100 mL⁻¹ anhydrous ethanol) greater than cachaças produced by distillation in copper alembic (54.4 mg 100 mL⁻¹ anhydrous ethanol) and stainless-steel column (20.0 mg 100 mL⁻¹ anhydrous ethanol) in agreement with that previously reported by Nascimento et al. (27). There are two key factors that may contribute strongly to the above distinction. One is certainly the catalytic activity of the metal surface of the distiller that is in contact with the distilling vapors promoting chemical reactions as previously reported (27, 30). The other is the distillation temperatures and number of theoretical plates for distillation in alembic or column.

For cachaças distilled in alembic, the distillation is controlled by measuring the alcoholic content of the spirit during the process, and three fractions, namely, head, heart, and tail, are separated on the basis of their alcoholic strengths. The heart [ethanol strength of 38–50% (v/v)] distills in the range of 75–85 °C and makes up 80% of the total volume of distilled cachaça (28, 30). The remaining 20% is a mixture of the head [ethanol strength of 50–70% (v/v)] that distills in the temperature range of 70–75 °C and the tail [ethanol strength of 10–38% (v/v)], which distills in the temperature range of 85–100 °C (28, 30). When a column is used in the distillation process, the three fractions are not separated and the distilled portion shows an ethanol strength of 35–65% (v/v) in the temperature ranging from 94 to 105 °C (28, 30). This corroborates with data in Figure 2, which shows that the spirits produced in alembic exhibit a higher content of esters with short chain length. On the other hand, the spirit distilled in a stainless-steel column shows a higher content of longer chain esters. This

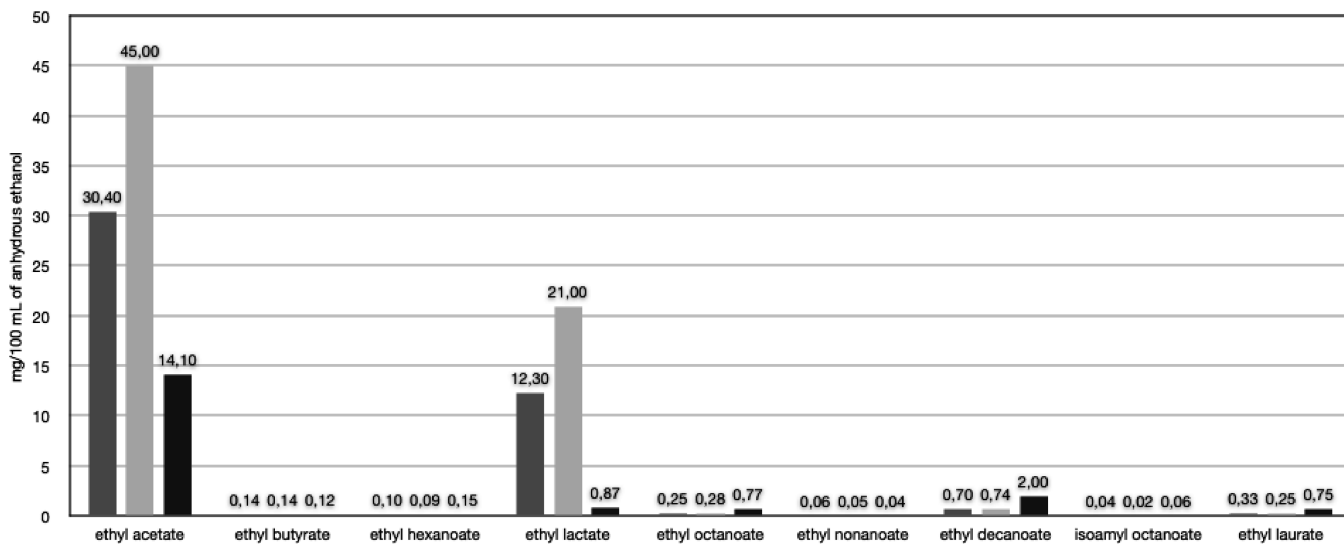


Figure 2. Individual content for the analyzed esters in cachaca produced by copper alembic, stainless-steel column, and hybrid alembic distillation.

Table 5. Average, Median, Minimum, and Maximum Values (mg 100 mL⁻¹ Anhydrous Ethanol) for the Quantitative Profile of 9 Esters in 10 Samples of Whisky and 10 Samples of Rum

ester	whisky				rum			
	average	median	maximum	minimum	average	median	maximum	minimum
ethyl acetate	31.9	26.6	86.9	14.1	41.3	25.4	124	2.57
ethyl butyrate	0.144	0.125	0.240	0.067	0.374	0.396	0.412	0.313
ethyl hexanoate	0.340	0.250	0.805	0.114	0.240	0.240	0.262	0.218
ethyl lactate	2.02	1.94	3.00	1.04	6.39	5.11	11.2	2.86
ethyl octanoate	1.85	1.07	4.30	0.393	0.642	0.642	1.30	0.370
ethyl nonanoate	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
ethyl decanoate	3.42	2.60	7.49	0.729	0.828	0.671	1.86	0.370
isoamyl octanoate	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
ethyl laurate	1.98	1.84	4.63	0.199	0.476	0.476	0.476	0.476

might be due to the increasing boiling point and ester activity coefficient as a function of the ethanol strength and ester chain length (31).

The singular qualitative profile of esters in cachaca produced from alembic and column will affect the fruit aroma of the product. This effect will be sharper for cachaca produced from a stainless-steel column because the aroma threshold for esters at the bottled strength of cachaca [38% ethanol (v/v)] is higher for longer chain esters than for short-chain esters. Conversely, the product from alembic has a higher content of low-molecular-weight esters that presents higher partition coefficients, increasing the fruity aroma note of the beverage.

To compare the qualitative and quantitative profile of esters in cachaca, 10 selected samples of rum and whisky were analyzed. Cachaca is the denomination of the typical Brazilian spirit produced from the distillation of fermented sugar-cane juice, whereas rum is a sugar-cane spirit obtained by the distillation of fermented cooked sugar-cane juice and molasses (32). **Table 5** summarizes the average, median, minimum, and maximum values of the studied esters for the analyzed samples of whisky and rum. Conversely to that observed for cachacas, the fruity/floral odorants ethyl nonanoate and isoamyl octanoate (LODs are 0.0457 and 0.0258 mg L⁻¹, respectively) were not detected at all in the whisky and rum samples analyzed.

As expected, ethyl acetate is the major ester present in whisky and rum followed by ethyl lactate for rum samples. However, this is not observed in the whisky samples that present ethyl lactate at concentrations lower or at the same order of longer chain esters. In fact, the low content of ethyl lactate in whisky is indicative of the absence of *Lactobacillus* spp. during the whisky fermentation

process. Probably, the lack of *Lactobacillus* contamination is because whiskey producers usually use selected yeast and have a strict microbiological control of the yeast, while almost all cachaca and rum producers use wild yeasts. Furthermore, the sugar-cane juice for cachaca production is fermented at higher pH values than whisky, and the temperature during the fermentation is not so well-controlled and may reach up to 45 °C.

The Brazilian rums (Montilla and Bacardi) exhibit a "poor" ester profile once only ethyl acetate and ethyl lactate were detected. A reasonable cause to the low concentration of long- and medium-chain ethyl esters in these rum samples is the use of activated coal filters, a common practice among rum producers, for the removal of off-flavors and spirit discoloration (33).

All samples of whisky and some of rum were aged in wood barrels; however, none of them shown an expressive content of the analyzed ethyl esters. This is in agreement with the origin of ethyl esters as secondary metabolites of yeast and not as extractive compounds from the wood and esterification reactions during the aging process (34).

In conclusion, ethyl acetate and ethyl lactate are the major esters in cachaca and rum derived from the secondary metabolism of the yeast and *Lactobacillus* spp., respectively. Whisky has shown a lower content of fruity odorant ethyl lactate perhaps caused by lower bacterial contamination of the fermentation process. The obtained data strongly suggest as previously described (27, 30) that ester chemical profiles in cachacas are substantially determined by the type of distillation apparatus (copper/hybrid alembic or stainless-steel column) and experimented fermentation conditions control probably would affect the final fruity aroma of the spirit. Furthermore, Brazilian authorities should be aware that the recommended

GC–FID method that lacks the analytical data for ethyl lactate in cachaça and rum will certainly underestimate the total content of esters expressed in equivalents of ethyl acetate in the alcoholic beverage.

Supporting Information Available: Sample list, ethanol content, and complete analytical data for the analyzed samples. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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