

Identification and Dosage by HRGC of Minor Alcohols and Esters in Brazilian Sugar-Cane Spirit

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A presença de 51 compostos voláteis, entre álcoois e ésteres, em aguardentes de cana (cachaça) foi investigada por cromatografia gasosa de alta resolução. Os seguintes compostos foram identificados e quantificados: metanol, 1,4-butanodiol, álcool 2-feniletílico, álcool amílico, álcool cetílico, álcool cinâmico, n-decanol, geraniol, álcool isoamílico, isobutanol, mentol, n-butanol, n-dodecanol, n-propanol, n-tetradecanol, propionato de amila, acetato de etila, benzoato de etila, heptanoato de etila, valerato de isoamila, propionato de metila, butirato de propila. O teor médio de álcoois superiores (262 mg/ 100 mL de álcool anidro a.a.) e o teor médio de ésteres (24 mg/ 100 mL a.a.) em cachaças são menores que os encontrados em outros destilados. O teor médio de metanol em cachaças, (6 mg/100 mL a.a.) é o mesmo que o encontrado em rum e menor em relação ao encontrado em destilados de uva. Com relação aos compostos analisados, não foram observadas diferenças significativas no perfil químico qualitativo das cachaças analisadas.

The presence of 51 volatile compounds, among alcohols and esters in Brazilian sugar-cane spirit (*cachaça*), were investigated by high-resolution gas chromatography (HRGC). The following alcohols and esters were identified and quantified: methanol, 1,4-butanodiol, 2-phenylethyl alcohol, amyl alcohol, cetyl alcohol, cynamyl alcohol, n-decanol, geraniol, isoamyl alcohol, isobutanol, menthol, n-butanol, n-dodecanol, n-propanol, n-tetradecanol, amyl propionate, ethyl acetate, ethyl benzoate, ethyl heptanoate, isoamyl valerate, methyl propionate, propyl butyrate. The average higher alcohols content (262 mg/100 mL in anhydrous alcohol a.a.) and total esters content (24 mg/ 100 mL a.a.) in *cachaças*, are smaller than in other spirits. The average methanol content in *cachaças* (6 mg/100 mL a.a.) is the same as in rum, but smaller than in wine spirit. No qualitative differences of chemical profile among *cachaças* have been observed.

Keywords: sugar-cane spirit, cachaça, higher alcohols, esters.

Introduction

Brazilian sugar-cane spirit, which has been popularized in the world as “*cachaça*” (ca-sha-sa), is the most consumed spirit in Brazil¹. The production of *cachaça* is estimated in two billion liters per year².

Cachaça is produced from the distillation of the sugar-cane juice which was fermented by *Saccharomyces cerevisiae* and its ethanol content, according to the Brazilian regulation³, must be in the range of 38 to 54% v/v. The aging process is not yet a common practice among *cachaça* producers, therefore, in general, *cachaça* is consumed as distilled.

Like other spirits, *cachaça* is mainly characterized organoleptically by the presence of other minor components such as higher alcohols, esters, carboxylic acids and carbonilic compounds, which are important for the taste and flavor of spirits^{4,5}. These compounds are formed from carbohydrates and amino acids during amino acid biosynthesis and catabolism, respec-

tively⁶. The comparison of the aldehydes and carboxylic acids fractions in *cachaças* have been previously reported^{7,8}.

The knowledge of the *cachaça* chemical components is a matter of concern, not only to distillers or inspection bureau, but to for analytical chemists due to the chemical complexity of the matrix and the low level of analyte occurrence.

The current official analytical procedures are based on volumetric methodology, therefore precluding any type of speciation. As the sensorial properties and toxicology of compounds change along the homologues series, it is relevant to know more details about each chemical category present in the spirits⁸.

Aiming to contribute to the *cachaça* chemical quality control, this study reports the occurrence and dosage of minor alcohols and esters in *cachaças*, establishing an average of their chemical composition. For this purpose, high-resolution gas chromatography with a flame ionization detector (HRGC-FID) was used.

Experimental

Materials

The analyses were carried out in a Hewlett-Packard 5890-A GC equipped with a split-splitless inlet injector and a flame ionization detector (FID). Two fused silica capillary columns were used: HP-FFAP cross-linked poly(ethylene glycol)-TPA phase; (50m x 0.2mm I.D. x 0.33 μ m film thickness) and HP-20M Carbowax: poly(ethylene glycol); (50m x 0.32mm I.D. x 0.33 μ m film thickness). Chromatographic data were collected in HP chemstation software.

Chemicals of analytical grade from Merck, Aldrich Chemicals and Carlo Erba were used as standards. The presence of the following compounds was investigated: methyl alcohol, n-propyl alcohol, 2-propyl alcohol, n-butyl alcohol, 2-butyl alcohol, isobutyl alcohol, t-butyl alcohol, 1,3-butyl alcohol, 1,4-butyl alcohol, n-pentyl alcohol, isoamyl alcohol (mixture of 2-methyl-butyl and 3-methyl-butyl), 2-pentyl alcohol, 3-pentyl alcohol, neo-pentyl alcohol, n-hexyl alcohol, 2-hexyl alcohol, n-octyl alcohol, 2-octyl alcohol, n-decyl alcohol, n-dodecyl alcohol, n-tetradecyl alcohol, menthol, geraniol, allyl alcohol, cynamyl alcohol, cetyl alcohol, ethylene glycol, propylene glycol, 2-phenylethyl alcohol, 2-amino-2-methyl-1-propyl alcohol, 2-amino-1-butyl alcohol, methyl formate, ethyl formate, methyl acetate, ethyl acetate, n-propyl acetate, isopropyl acetate, n-butyl acetate, isobutyl acetate, sec-butyl acetate, n-pentyl acetate, isopentyl acetate, n-hexyl acetate, ethyl propanoate, isobutyl propanoate, ethyl heptanoate, ethyl butyrate, methyl benzoate, ethyl benzoate, methyl miristate and methyl stearate. These compounds were chosen to be investigated based on their occurrence in other spirits^{9,10}.

Twenty five regular commercial *cachaças* from different regions of Brazil* were analyzed: Caninha da Roça(SP), Caranguejo(CE), Chave de Ouro(CE), Pirassununga 51(SP), Jamel(SP), Oncinha(SP), Pitu(SP), Velho Barreiro(SP), Ypioca Ouro(CE), Ypioca Prata(CE), Germana(MG), Ganyvit(SP), São Francisco(RJ), Marquesi(SP), Massayo(AL), Azuladinha(AL), Trinca 3(CE), Vila Velha(SP), Box 32(SC), Lua Cheia(MG), Cavalinho(SP), Salinas(MG), Baronesa(MG), Bodoco(MG) and Sapupara(CE).

Experimental conditions

Inlet and detector temperatures: 250°C; Injected volume: 1.0 μ L; split ratio: variable, but generally 1:20; carrier gas: hydrogen (1.2 mLmin⁻¹); oven temperature program

(HP-FFAP): 55°C (5 min); 2°C min⁻¹ \rightarrow 100°C (3 min), 5°C min⁻¹ \rightarrow 190°C (30 min); 5°C min⁻¹ \rightarrow 220°C (15 min); oven temperature program (HP-20M) 45°C; 1°C min⁻¹ \rightarrow 100°C (3 min), 5°C min⁻¹ \rightarrow 190°C (30 min); 5°C min⁻¹ \rightarrow 220°C (15 min). The identification was made through standard addition and using both columns, although quantitative determinations of the identified peaks were accomplished using the HP-FFAP column.

Sample preparation

In a typical procedure, 300 mL of the *cachaça* or standard solutions were added into a 500 mL separation funnel and two extractions were performed by liquid-liquid extraction (LLE) with two fractions of 40 mL of pentane:methylene chlorine (2:1 v/v)¹¹. The samples were spiked with internal standards (n-hexanol) which is absent in *cachaças*. The extracted volume was reduced by evaporation to 5 mL. The standard curves always showed correlation coefficients close to unit. Some compounds, like ethyl acetate, methanol, n-propanol, isobutanol and isoamyl alcohol (2-methyl-1-butanol and 3-methyl-1-butanol), were quantified by injection of the samples without any treatment.

Results and Discussion

Figures 1 and 2 show typical chromatographic profiles of a *cachaça* analyzed by direct injection of the sample and from the injection of its extract, respectively.

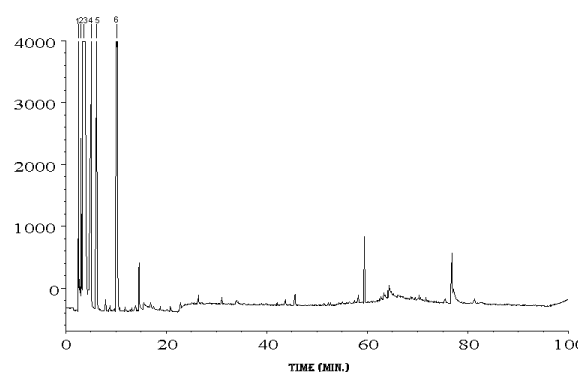


Figure 1. HRGC-FID chromatogram of a *cachaça* sample obtained by direct injection. HP-FFAP chromatographic column (50m x 0.2 μ m I.D. x 0.33 mm film thickness) was used, see experimental conditions. (1) ethyl acetate; (2) methanol; (3) ethanol; (4) propanol; (5) isobutanol; (6) iso-amyl alcohol. Numberless peaks are unknown compounds.

Among 51 analytical standards investigated at the detection level of 10.0 μ g L⁻¹, 23 compounds were positively identified and quantified. The alcohol and ester content in the samples are shown in Tables 1 and 2, respectively.

*Abbreviations for the Brazilian states: (AL) Alagoas; (CE) Ceará; (MG) Minas Gerais; (RJ) Rio de Janeiro; (SC) Santa Catarina; (SP) São Paulo.

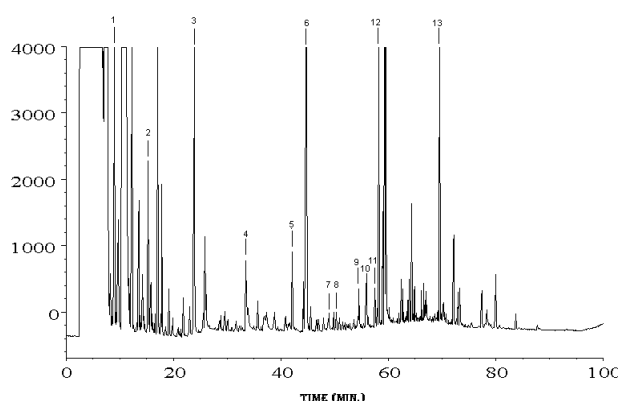


Figure 2. HRCC-FID chromatogram of an extract from the same *cachaça* in Figure 1. HP-FFAP chromatographic column (50m x 0.2µm I.D. x 0.33 mm fiolm thickness) was used, see experimental conditions. (1) propyl butyrate; (2) amyl alcohol; (3) ethyl heptanoate; (4) amyl propionate; (5) mentol; (6) ethyl benzoate; (7) 1,4 butanodiol; (8) 2-phenylethyl alcohol; (9) decanol; (10) dodecanol; (11) tetradecanol; (12) cynamic alcohol; (13) cetyl alcohol. Numberless peaks are unknow compounds.

The Brazilian limit values for total esters and total higher alcohols are 200 mg 100mL⁻¹ of anhydrous alcohol a.a. and 300 mg 100 mL⁻¹ a.a., respectively.³ These limits do not specify which chemical is present in *cachaças*.

As it can be observed in Tables 1 and 2, the mean values presented for higher alcohols and ester content are smaller than the allowed limit values. In general, the higher alcohols content are near to the limit and the ester mean content is about one tenth of the limit.

Many of the compounds found in *cachaças* are reported to occur in spirits. However, in general, no quantitative data are available for these minor compounds. In Table 3, *cachaça* and other spirits are compared regarding some specific common compounds present in their composition.

The average content of n-propanol and n-butanol in *cachaças* is at the same level of other spirits, while isobutanol is less abundant in *cachaças*. Rum exhibits smaller isoamyl alcohol content than *cachaças*, while in whisky and wine spirit samples this alcohol is twice as abundant as in *cachaças*. Amyl alcohol content in *cachaça* is surprisingly smaller than in whisky and rum.

Ethyl acetate is the main ester in alcoholic beverages and its higher concentration in *cachaças* is about half of the one found in rum and whisky. The fact that the total ester content in *cachaça* is smaller in relation to other beverages probably reflects that *cachaças* are, in general, non-aged beverages.

No high methanol content has been found in any *cachaça*. The mean methanol content in regular commercial *cachaças* is smaller than the reported values for wine spirits, but it is at the same level of the rum. There is no mention for the methanol quantification in whisky.

Table 1. Methanol and higher alcohols content in *cachaças*^a.

Alcohols	Sample ^b																										average
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y		
Methanol	9.20	0.87	5.83	6.90	2.51	6.13	5.30	6.59	8.25	4.45	7.89	6.83	4.56	6.22	8.52	9.12	4.15	3.56	2.90	5.65	7.11	2.56	4.36	4.11	8.05	5.66±2.24	
1,4-Butanodiol	0.18	0.18	0.20	0.12	0.09	0.07	0.05	0.08	0.11	0.13	0.15	0.19	0.17	0.17	0.18	0.11	0.16	0.08	0.10	0.16	0.04	0.12	0.16	0.11	0.10	0.13±0.05	
2-Phenylethyl	0.01	-	0.01	-	0.09	0.08	0.02	-	0.06	-	0.06	-	0.02	-	0.05	0.01	-	-	-	0.03	0.05	0.06	-	0.05	0.04	0.02±0.03	
Amyl	0.13	0.11	0.05	0.14	0.14	0.10	0.11	0.10	0.15	0.17	0.19	0.15	0.15	0.15	0.07	0.11	0.17	0.10	0.20	0.11	0.09	0.14	0.15	0.11	0.13	0.13±0.04	
Cetyl	7.20	3.90	8.65	6.80	7.50	3.70	7.20	9.04	6.10	9.40	4.11	5.00	3.70	3.33	10.1	2.50	8.70	8.70	3.00	6.40	7.25	3.80	6.10	2.20	8.90	6.13±2.43	
Cynamic	5.60	7.50	6.30	9.20	5.40	6.80	12.0	9.50	7.20	7.30	5.25	9.00	5.60	6.80	6.80	6.00	11.0	4.80	6.10	7.00	7.70	5.50	5.80	3.50	6.40	6.95±1.94	
Decanol	0.30	0.12	0.51	0.11	0.15	0.02	0.06	0.08	0.13	0.20	-	0.30	0.30	0.11	0.50	0.45	0.11	0.09	0.13	0.40	0.60	0.19	0.18	0.07	0.11	0.21±0.17	
Geraniol	0.54	0.72	0.82	0.25	0.77	0.79	0.31	0.47	0.48	0.35	0.67	0.66	0.54	0.78	0.61	0.59	0.80	0.55	0.73	0.86	0.40	0.56	0.74	0.66	0.77	0.62±0.17	
Isoamyl	145	131	162	168	152	139	125	152	131	143	138	137	67	134	157	13	96	160	108	151	144	112	138	198	111	138±26	
Isobutanol	50	73	68	61	51	49	60	50	40	59	74	69	63	51	78	48	56	77	43	61	44	80	68	96	74	62±14	
Menthol	0.60	0.45	0.25	0.55	0.62	0.65	0.57	0.38	0.45	0.45	0.65	0.55	0.41	0.33	0.71	0.66	0.49	0.36	0.44	0.47	0.68	0.45	0.73	0.39	0.55	0.51±0.13	
n-Butanol	1.11	1.15	0.94	0.98	1.04	1.15	1.20	1.33	1.45	1.53	0.97	1.07	1.15	0.90	1.10	1.03	1.08	1.04	1.50	1.33	1.62	1.02	0.91	1.23	0.90	1.15±0.21	
n-Dodecanol	0.01	-	0.01	0.01	0.02	-	0.01	-	0.02	0.04	-	0.03	-	-	0.01	0.02	-	-	-	-	0.05	-	-	-	-	0.01±0.01	
n-Propanol	40	41	42	49	51	47	44	51	37	38	52	43	60	55	47	41	39	54	46	39	38	41	54	57	49	46±7	
n-Tetradecanol	0.07	0.1	0.05	0.03	-	-	0.01	0.09	0.09	0.11	0.01	0.16	-	0.02	-	-	0.17	-	0.01	0.03	-	-	-	0.03	-	0.04±0.05	
total	251	259	291	296	270	249	254	274	225	260	276	266	202	252	302	243	213	307	209	268	244	245	274	360	252	262±34	

^a Results in mg 100 mL of anhydrous alcohol.

^b The order of the samples is not corresponding to the samples introduction order in the text.

- Not detected

Table 2. Esters content in cachaças^a

Alcohols	Sample ^b																										average
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y		
Amyl Propionate	0.02	0.01	0.01	0.01	0.01	0.01	—	0.01	0.02	0.03	0.03	0.01	0.02	0.02	—	0.02	—	0.01	0.01	—	0.02	0.03	—	0.02	0.02	0.02±0.01	
Ethyl acetate	12.6	24.5	22.3	33.2	18.5	15.4	13.8	14.6	25.9	15.5	17.9	22.3	22.5	24.5	3.0	28.9	34	33.2	24.3	39	16.6	28.5	18.5	30	28.9	23.8±7.3	
Ethyl Benzoate	0.49	0.36	0.57	0.84	0.13	0.25	0.68	0.75	0.33	0.54	0.27	0.48	0.32	0.25	0.68	0.44	0.58	0.36	0.75	0.33	0.77	0.35	0.51	0.31	0.27	0.46±0.2	
Ethyl Heptanoate	0.05	0.04	0.03	0.05	0.05	0.04	0.05	0.05	0.04	0.05	0.09	0.04	0.04	0.05	0.03	0.08	0.06	0.06	0.05	0.04	0.05	0.06	0.06	0.04	0.03	0.05±0.01	
Isoamyl valerate	0.01	0.01	0.01	0.01	0.01	—	—	0.02	0.01	0.01	—	—	—	0.01	0.01	0.02	0.01	0.01	0.02	—	0.01	0.02	0.01	0.01	0.01	0.01±0.01	
Methyl Propionate	0.03	0.02	0.01	0.03	0.02	0.03	0.03	0.04	—	0.03	0.04	0.01	0.01	0.03	0.01	0.02	0.03	0.04	0.05	0.01	0.02	0.01	0.03	0.02	0.01	0.02±0.01	
Propyl Butyrate	0.01	0.01	0.02	0.02	0.02	0.02	0.02	0.03	0.02	0.05	0.02	0.03	0.03	0.03	0.02	0.03	0.01	0.05	0.02	0.03	0.02	0.02	0.02	—	0.04	0.02±0.01	
Total	13.2	24.9	22.9	34.1	18.7	15.7	14.5	15.4	26.3	16.2	18.3	22.8	22.9	24.8	30.7	29.5	34.6	33.7	25.2	39.4	17.4	28.9	19.1	30.4	29.2	24.4±7.3	

^aResults in mg 100 mL of anhydrous alcohol.^bThe order of the samples is not corresponding to the samples introduction order in the text.
— Not detectedTable 3. Comparison of methanol, higher alcohols and ethyl acetate among different spirits^a.

Compound	Cachaça	Rum ^b	Whisky ^c	Wine ^d
Methanol	5.7	6.6		22
Amyl alcohol	0.13	8.7	97	
Isoamylalcohol	136	104	250	230
Isobutanol	63	162	90	136
n-butanol	1.11	1.00	0.40	
n-propanol	48	38	31	44
Total alcohols	263 ^e	314	468	410
Ethyl acetate	23.5	69.7	74.1	

^a Results in mg 100 cm⁻³ a.a.; ^b(Sing et al 1995); ^c (Reazin 1981); ^d (Lurton et al 1995);^e All alcohols studied; blank (not mentioned).

The methanol level in blood for acute intoxication is about 100 mg 100 L⁻¹.¹³ According to the results in Table 1, an adult weighing 70 kg should ingest about 180 L of regular commercial *cachaça* to undergo methanol intoxication.

Recently, some Brazilian newspapers have reported cases of methanol intoxication due to *cachaça* ingestion. This poisoning is directly related to beverages prepared and sold on “moonshine style”, without any type of chemical control. The Brazilian Ministry of Agriculture³ has established a limit of 0.25 mL (200 mg) of methanol in 100 mL a.a.

The origin of the high methanol content in these products is quite controversial, but surely the regular fermentation and distillation process do not account for that. As far as we know, there are no cases of methanol poisoning reported as a consequence of regular commercial *cachaças* ingestion.

The results reported here and in a previous paper⁷ have shown that the average content of aldehydes and higher alcohols in *cachaças* is smaller than in whisky. These two classes of compounds are considered to contribute to the “hangover” syndrome.

By taking into account the above comments and the qualitative chemical profile of *cachaça*, whisky, rum and wine spirits, a moderate Brazilian sugar-cane spirit intake is expected to cause no health problems.

Conclusions

The minor alcohols and esters content in *cachaças* was investigated. This study has clearly shown that the level of methanol content in *cachaça* is the same as in rum, but smaller than in wine spirit. Higher alcohols content in *cachaças* is smaller than in rum, whisky and wine spirit. The same occurs with the total esters content.

The average values for higher alcohols in *cachaças* are slightly smaller than the Brazilian regulation limit and the esters average content is about one tenth of this limit.

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