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Qualitative and quantitative high-performance liquid chromatographic analysis of aldehydes in Brazilian sugar cane spirits and other distilled alcoholic beverages

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Abstract

A study is presented on the high-performance liquid chromatographic analysis of eighteen aldehydes in Brazilian sugar cane spirits and other international brandies. The aldehydes were separated by reversed-phase high-performance liquid chromatography as 2,4-dinitrophenylhydrazones (DNPHs). A very good chromatographic separation was achieved for eighteen different aldehyde-DNPHs. The proposed methodology is quite simple and not very time-consuming. Ten aldehydes were identified in 75 beverages and quantified using the external standard method with UV detection at 365 nm. A detailed knowledge of the aldehyde content should significantly contribute to improving the quality control of distilled spirits. © 1997 Elsevier Science B.V.

Keywords: Sugar cane spirits; Alcoholic beverages; Aldehydes; 2,4-Dinitrophenylhydrazone

1. Introduction

The annual production of the Brazilian sugar cane spirit, caninha, also called cachaça or pinga, is around $2 \cdot 10^9$ l/year, which classifies it as one of the world's most prominent brands [1]. However, less than 1% of the volume produced is consumed outside South America. Efforts are being made to introduce caninha as an international category brand aimed at increasing export. Detailed knowledge of the chemical composition is very important for quality control and for evaluating the effects on consumers' health. As part of our attempts to learn more about the constituents of caninha, we are developing in our laboratory dedicated methodologies to study the chemical composition and to correlate the analytical results with organoleptic properties.

The toxicity associated with aldehydes is well known and their presence in alcoholic beverages is quite often related to nausea, vomiting, uneasiness, sweating, confusion, decrease in blood pressure, rapid heartbeat and hangover headaches [2]. Acetal-

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dehyde in the presence of alcohols reacts with the amino groups in nucleosides to yield mixed acetals, which are claimed to increase the risk of breast cancer in women [3,4]. Considerable interest is expressed by the World Health Organization (WHO), particularly the International Agency for Research on Cancer (IARC), to identify and list all congeners present in alcoholic beverages that may be carcinogenic, mutagenic or toxic [3]. Several aldehydes, such as formaldehyde, acetaldehyde, acrolein and benzaldehyde, are reputed to be carcinogens [3–7].

The high-performance liquid chromatographic (HPLC) determination of the aldehydes as their 2,4-dinitrophenylhydrazones (aldehyde-DNPHs) was preferred over gas chromatographic [8–10] and spectrophotometric methods because of the superior separation efficiency [11–13]. Most of the work on DNPHs dealt with carbonyl compounds in environmental analyses by reversed-phase HPLC [14–20], but studies on the analysis of aldehydes in alcoholic beverages are also presented in the literature [21–27].

We describe here a HPLC method for the qualitative analysis of aldehydes in alcoholic beverages, as well as quantitative analysis of the ten most relevant aldehydes in distilled spirits.

2. Experimental

2.1. Reagents

The standard aldehydes (eighteen in total; for specification, see Fig. 1) were of analytical grade (Merck, Aldrich). Methanol and acetonitrile were of HPLC grade (Merck, Mallinckrodt). Water was distilled using a Milli-Q system (Millipore). 2,4-Dinitrophenylhydrazine (Aldrich) was purified by three successive recrystallizations from methanol.

2.2. Preparation of 2,4-dinitrophenylhydrazones of the standard aldehydes

The aldehyde-DNPHs were obtained as described previously [28]: 2,4-dinitrophenylhydrazine (0.4 g; ca. 2 mmol) was dissolved in concentrated sulfuric acid (2 ml) and distilled water (3 ml). To this solution, the standard aldehydes (0.1 g), dissolved in ethanol (15 ml), were added. The corresponding aldehyde-DNPHs were isolated via filtration and purified (twice) by recrystallization from absolute ethanol. The purity was confirmed by melting point determination, elemental analysis (C, H, N) and HPLC analysis.

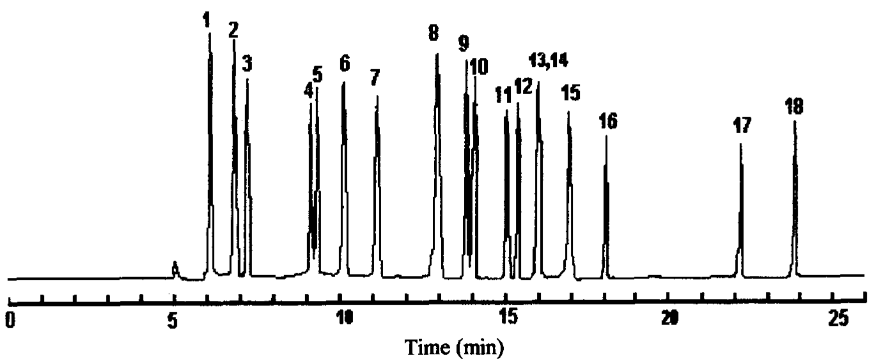

Fig. 1. HPLC chromatogram of the 2,4-dintrophenylhydrazones of standard aldehydes on a Shimadzu C_{18} column (15 cm×6.0 mm I.D.; 5 μm) with a flow-rate of 1 ml/min. Gradient profile: methanol–water (65:35, v/v) for 6 min, then to methanol–water (85:15, v/v) in 10 min, methanol–water (80:20, v/v) in 20 min and methanol–water (65:35, v/v) in 25 min. UV detection was at 365 nm. Identification of peaks: 1=formaldehyde; 2=5-hydroxymethylfurfural; 3=acetaldehyde; 4=acrolein; 5=furfural; 6=propionaldehyde; 7=*p*-anisaldehyde; 8=butyraldehyde; 9=benzaldehyde; 10=crotonaldehyde; 11=isovaleraldehyde; 12=*n*-valeraldehyde; 13=cinnamaldehyde; 14=2-methylbenzaldehyde; 15=*n*-hexanaldehyde; 16=*n*-heptanaldehyde; 17=*n*-nonaldehyde and 18=*n*-decanaldehyde.

For quantitative analysis, standard solutions of the ten most relevant aldehyde-DNPHs were obtained by suitable dilution of the stock solutions (1000 mg/l in acetonitrile) in ethanol–water (45:55, v/v). Four points were used to obtain the calibration curves, in the following concentration ranges: formaldehyde-DNPH, 0–5 ppm; 5-hydroxymethylfurfural-DNPH, 0–10 ppm; acetaldehyde-DNPH, 0–200 ppm; acrolein-DNPH, 0–10 ppm; furfural-DNPH, 0–15 ppm; propionaldehyde-DNPH, 0–3 ppm; butyraldehyde-DNPH, 0–10 ppm; benzaldehyde-DNPH, 0–10 ppm; isovaleraldehyde-DNPH, 0–5 ppm and *n*-valeraldehyde-DNPH, 0–5 ppm.

2.3. Samples

Brazilian caninhas (artisanal samples, characterized by an asterisk, and commercial samples, 56 in total) were collected from several regions of Brazil. International category brands (nineteen samples) were obtained from local supermarkets (aguardente, brandy, cognac, grappa, tequila, various whiskies, rums and vodkas) for comparison purposes.

Cachaça-Adabo* (SP), Armazém Vieira (SC), Azuladinha (AL), Barci (SP), Boazinha (MG), Box 32 (SC), Bento Gonçalves* (RS), Cambará* (PR), Camilo* (SP), Caninha da Roça (SP), Carangueijo (CE), Cavalinho (SP), Chave de Ouro (CE), Correinha (MG), Córrego Azul (SP), 51 (SP), Curvelo (MG), Delicate (SP), D. Mário* (SP), ESALQ* (SP), Executivo (SP), Fazenda Isabela* (SP), Fazenda Porto Alegre* (SP), Fim de Século (RJ), Germana (MG), Herr Blumenau (SC), Jamel (SP), Januária (MG), Lua Cheia (MG), Luiz Alves* (SC), Mangueira (PI), Maranhão* (MA), Marília (SP), Marquesi (SP), Massayó (AL), Murim Mirim (RN), Nêga Fulô (RJ), Oncinha (SP), Pedregulho* (SP), Periquita (PI), Piraquara (MG), Pitú (PE), Praianinha (RJ), Ribeirão Bonito* (SP), São Francisco (RJ), Sapupara (CE), Sertaneja* (SP), Sertãozinho* (SP), Trinca 3 (CE), Velho Barreiro (SP), 29 (SP), 21 (SP), Vila Velha (SP), Ypioca Ouro (CE), Ypioca Prata (CE), Zuliane* (SP). States in Brazil: SP, São Paulo; SC, Santa Catarina; AL, Alagoas; MG, Minas Gerais; RS, Rio Grande do Sul; PR, Parana; CE, Ceará; RJ, Rio de Janeiro; PI, Piauí; MA, Maranhão and RN, Rio Grande do Norte.

International brands, Aguardente, Bagaceira (Portugal); Brandy, Fundador (Spain); Cognac, Courvoisier (France); Grappa (Italy); Tequila (Mexico); Whiskies, Evan Williams (USA), Heaven Hill (USA), Lougan (USA), Jim Bean (USA), Johnnie Walker (USA), Buchanan's (Scotland), Glenfiddich (Scotland), Pass Port (Scotland), White Mackay (Scotland), William Grant's (Scotland); Rums, Appleton (Jamaica), Havana Club (Cuba); Vodkas, Stolichnaya (Russia), Wyboroya (Russia).

2.4. Sample derivatization

A 0.4% solution of 2,4-dinitrophenylhydrazine was prepared by dissolving 2,4-dinitrophenylhydrazine (0.4 g; ca. 2 mmol) in acetonitrile (100 ml). In a volumetric flask, 1.0 ml of the 2,4-dinitrophenylhydrazine solution, 4.0 ml of the sample (without previous concentration) and 50 μl of 1 *M* $Hclo_4$ were introduced, consecutively. The resulting solution was stirred at room temperature for at least 45 min, followed by syringe injection of 25 μl into the HPLC apparatus.

2.5. Quantitative analysis

Quantitative conversion of the aldehydes in the distilled spirits to their 2,4-DNPHs is guaranteed by using a large excess of 2,4-dinitrophenylhydrazine [16,22,23]. The most relevant aldehyde-DNPHs (ten in total) were quantified in the alcoholic matrices using the external standard method, with detection at 365 nm. The calibration curves for each aldehyde-DNPH were obtained by linear regression, plotting peak area versus concentration. The correlation coefficients were very close to unity (Table 1). The standard solutions were prepared in ethanol–water (45%, v/v).

2.6. Chromatographic conditions

Four C_{18} columns were tested: Supelcosil C_{18} (25 cm×4.6 mm I.D.; 5 μm), Shimadzu Shim-park C_{18} (15 cm×6.0 mm I.D.; 5 μm), Shimadzu Shim-park C_{18} (25 cm×4.6 mm I.D.; 5 μm) and Micro-pack C_{18} (30 cm×4.0 mm I.D.; 10 μm). The analyses were carried out on a HPLC (Shimadzu), Model-10

Table 1
Calibration curves ($y=a+bx$) for HPLC determinations of aldehyde 2,4-dinitrophenylhydrazones (2,4-DNPHS)

2,4-DNPHs	*A*	*B*	r^2
Formaldehyde	0.3499	2.565	0.9994
5-Hydroxymethylfurfural	0.0428	1.455	0.9996
Acetaldehyde	−2.708	1.860	0.9993
Acrolein	0.3789	3.492	0.9991
Furfural	0.2033	1.257	0.9993
Propionaldehyde	0.2830	2.622	0.9932
Butyraldehyde	0.3592	2.854	0.9990
Benzaldehyde	0.2461	1.108	0.9961
Isovaleraldehyde	−0.2361	2.702	1.0
n-Valeraldehyde	−0.1610	2.166	0.9998

Y=peak area; x=concentration; A=intercept; B=slope; r^2=correlation coefficient.

AD, equipped with an injector (Shimadzu) (20 μl loop) and a UV–Vis photodiode array spectrophotometric detector (SPD-M6A, Shimadzu). Several gradient programs were examined using methanol–water and acetonitrile–water as mobile phases. The specific gradient profiles are detailed on the corresponding chromatograms (see Figs. 1 and 2).

3. Results and discussion

The aldehyde-DNPHs, corresponding to acrolein, furfural, propionaldehyde, isovaleraldehyde and *n*-valeraldehyde, could not be fully separated using acetonitrile–water as the mobile phase. In fact, only the C_3 compounds were separated, not the C_5 species [14]. Although it was suggested that a ternary mixture of acetonitrile–methanol–water could be used [17], a binary gradient is most frequently employed, as in this study. As expected, the retention times of saturated aliphatic aldehydes increased according to the molecular mass, while the unsaturated aliphatic aldehydes eluted prior to the saturated ones [19]. The most efficient separation of the pertinent aldehyde-DNPHs was obtained on the Supelcosil C-18 and on the Shimadzu C-18 columns using a gradient of methanol and water. However, all of the analytical results shown in Tables 1–4 and in Figs. 1–3 were obtained by analyses carried out using a Shimadzu C-18 (15 cm×6.0 mm; 5 μm) column. We selected the Shimadzu C-18 column as it performed better than the Supelcosil C-18 column.

A typical chromatogram, shown on Fig. 1, high-

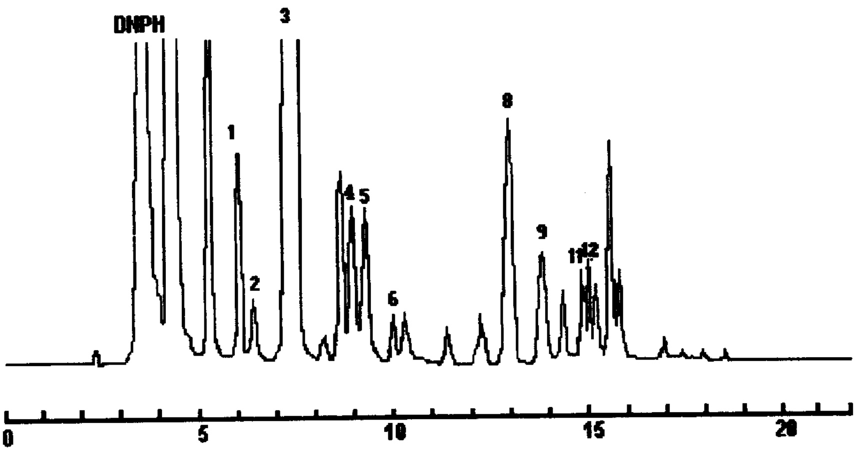

Fig. 2. HPLC chromatogram of the 2,4-dintrophenylhydrazones of aldehydes in cachaça sample No. 06 on a Shimadzu C_{18} column (15 cm×6.0 mm I.D.; 5 μm) at a flow-rate of 1 ml/min. The gradient profile was the same as that given in Fig. 1. UV detection was at 365 nm. Identification of peaks: 1=formaldehyde; 2=5-hydroxymethylfurfural; 3=acetaldehyde; 4=acrolein; 5=furfural; 6=propionaldehyde; 8=butyraldehyde; 9=benzaldehyde; 11=isovaleraldehyde and 12=*n*-valeraldehyde.

Table 2
Recoveries of aldehydes added to caninha sample no. 6 (concentration in mg/l)[a]

Aldehydes	Concentration present	Concentration added	Concentration found (mean±SD)	Recovery (%) (mean±SD)
Formaldehyde	0.440	0.400	0.86±0.08	102±4
5-Hydroxymethylfurural	1.54	1.25	1.77±0.04	99±2
Acetaldehyde	43.0	20.0	62.1±1.3	98±3
Acrolein	1.25	1.20	1.44±0.08	99±1
Furfural	5.12	5.20	10.1±0.32	97±3
Propionaldehyde	0.200	0.100	0.28±0.02	93±3
Butyraldehyde	0.800	0.450	3.42±0.06	93±3
Benzaldehyde	0.300	0.28	0.617±0.03	106±3
Isovaleraldehyde	0.350	0.100	0.28±0.02	93±3
n-Valeraldehyde	0.200	0.300	0.63±0.07	97±2

[a] $n=5$, injections were performed in duplicate.

lights the excellent separation of the standard aldehyde-DNPHs. Fig. 2 illustrates the utility of the method to determine aldehydes in representative samples of both caninhas and whisky.

The ten most relevant aldehydes were quantified in the alcoholic beverages, namely formaldehyde, 5-hydroxymethylfurfural, acetaldehyde, acrolein, furfural, propionaldehyde, butyraldehyde, benzaldehyde, isovaleraldehyde and *n*-valeraldehyde. Aldehydes not detected at the nanogram level are *p*-anisaldehyde, crotonaldehyde, cinnamaldehyde, 2-methylbenzaldehyde, *n*-hexanaldehyde, *n*-heptanaldehyde, *n*-nonaldehyde and *n*-decanaldehyde.

In five identical caninha samples, a known amount of the ten above-mentioned aldehydes was added. The samples were derivatized with 2,4-dinitrophenylhydrazine and the analyses were carried out in duplicate. The experimentally found recoveries are given in Table 2. As can be seen, the values are in the range of 93–106%. The reproducibility of the method were ascertained by carrying out five assays on the same sample over two days; each solution was injected twice. The values of the standard deviations were low and the coefficients of variation were between 3.2 and 7.0% (Table 3). An estimated detection limit for the aldehydes was obtained by successive dilution of a solution containing 500 μg/l of each aldehyde-DNPH in acetonitrile. Considering the signal-to-noise ratio (3:1), the detection limit was in the range of 10–50 μg/l for detection at 365 nm.

Brazilian legislation permits a maximum content of 5 mg of furfural/100 ml of absolute ethanol and 30 mg of total aldehydes, expressed as acetaldehyde/100 ml of absolute ethanol. As can be seen from Table 4, the content of the ten relevant aldehydes in the Brazilian caninhas does not exceed these limits.

Table 3
Reproducibility of the HPLC analysis of aldehyde 2,4-dinitrophenylhydrazones (2,4-DNPHs) in caninha sample no. 6 (concentration in mg/l)[a]

2,4-DNPHs	Mean±SD	Coefficient of variation (%)
Formaldehyde	0.44±0.02	4.5
5-Hydroxymethylfurfural	1.54±0.05	3.9
Acetaldehyde	43.0±1.4	3.2
Acrolein	1.25±0.05	4.0
Furfural	5.12±0.36	7.0
Propionaldehyde	0.20±0.01	5.0
Butyraldehyde	0.80±0.05	6.3
Benzaldehyde	0.30±0.01	3.0
Isovaleraldehyde	0.35±0.01	6.0
n-Valeraldehyde	0.20±0.01	3.0

[a] $n=5$, injections were performed in duplicate.

Table 4
Quantitative HPLC analysis of aldehyde 2,4-dinitrophenylhydrazones in Brazilian sugar cane spirits or caninhas (concentration in mg/100 ml of absolute ethanol)

Caninhas	Formal	HMF	Acetal	Acrolein	Furfural	Propional	Butyral	Benzal	Isovaleral	Valeral	ΣAldehydes
1[a]	0.270	0.237	8.80	0.110	0.230	<LD	0.060	0.150	0.076	0.039	9.97
2	0.140	1.33	14.0	0.390	1.80	0.017	0.380	0.150	0.056	0.140	18.4
3	0.190	0.595	10.0	0.190	0.140	0.023	0.026	0.060	0.048	0.073	11.3
4	0.380	0.297	12.0	0.010	0.240	<LD	0.063	0.410	0.036	0.140	13.6
5	0.360	1.86	17.0	0.430	0.031	0.028	0.130	0.180	<LD	0.091	20.1
6	0.210	0.501	11.0	0.460	2.60	0.022	0.340	0.060	0.130	0.062	15.4
7[a]	0.320	0.735	20.0	0.160	0.250	0.060	0.190	0.260	<LD	<LD	21.9
8[a]	0.880	0.269	14.0	0.300	0.410	<LD	0.703	0.027	0.028	0.130	16.7
9[a]	0.060	0.381	10.0	<LD	<LD	0.031	0.405	0.170	0.024	0.150	11.2
10	0.040	0.065	11.0	0.044	0.310	0.028	0.320	0.020	0.140	0.051	12.0
11	0.060	0.075	16.0	0.015	0.088	0.033	0.360	0.028	0.053	0.084	16.8
12	0.130	<LD	8.50	<LD	<LD	<LD	0.008	0.009	0.206	0.120	8.94
13	0.310	0.325	14.0	0.120	0.180	0.038	1.90	0.109	0.054	0.130	17.1
14	0.090	0.747	15.0	0.051	<LD	<LD	0.014	0.170	0.082	0.210	16.3
15[a]	0.190	0.040	14.0	<LD	<LD	0.037	0.077	0.040	0.064	0.190	14.6
16	0.010	0.373	7.80	<LD	0.170	0.003	0.097	0.230	0.019	0.220	8.91
17[a]	0.240	1.460	12.0	0.100	<LD	<LD	0.043	0.430	0.025	0.090	14.3
18[a]	0.002	0.039	7.40	<LD	<LD	<LD	0.140	0.190	0.026	0.180	7.97
19[a]	0.160	1.330	12.0	0.100	1.01	0.009	0.039	0.140	0.022	0.060	14.8
20[a]	0.220	0.543	3.30	<LD	0.008	<LD	0.032	0.250	0.063	0.240	4.25
21	0.500	0.328	17.0	0.042	0.140	0.008	0.170	0.150	0.160	0.170	18.7
22[a]	0.050	0.507	11.0	0.130	0.210	0.009	0.037	0.410	0.017	0.110	12.5
23[a]	0.270	0.244	12.0	<LD	<LD	<LD	0.820	0.210	0.023	0.062	13.6
24	1.20	0.400	10.3	<LD	0.410	0.014	0.105	0.130	<LD	0.020	12.6
25	0.220	0.187	12.0	0.095	0.380	0.054	0.200	0.063	0.023	0.059	13.2
26	0.130	0.404	13.0	<LD	<LD	0.018	0.380	0.077	0.030	0.150	14.1
27	0.140	0.545	14.0	0.180	0.240	<LD	0.220	0.024	0.170	0.120	15.6
28	0.104	0.705	17.0	<LD	<LD	0.004	0.032	0.048	0.049	0.160	18.1
29	0.140	0.258	4.90	<LD	<LD	<LD	0.018	0.042	0.055	<LD	5.41

Table 4

30[a]	0.018	0.582	10.0	0.058	<LD	<LD	0.054	<LD	0.036	0.052	10.8
31	0.460	0.741	13.0	0.077	0.120	0.022	0.203	0.460	0.140	0.065	15.3
32[a]	0.360	0.508	9.40	0.078	0.360	0.004	0.210	0.250	0.034	0.050	11.2
33	0.400	0.064	11.0	0.380	0.260	0.015	0.170	0.024	0.051	0.310	12.7
34[a]	0.055	0.647	14.0	<LD	<LD	0.001	0.059	0.087	0.084	0.104	15.0
35	0.068	1.25	18.0	0.001	<LD	0.017	0.035	0.025	0.027	0.039	19.4
36	0.051	0.458	0.050	0.660	1.50	0.023	0.100	0.018	0.042	0.087	7.11
37	0.310	0.131	14.0	0.056	0.210	0.035	1.06	0.120	0.016	0.065	16.0
38	0.210	0.147	14.0	<LD	<LD	<LD	0.043	0.028	0.082	0.055	14.6
39[a]	0.062	0.743	15.0	<LD	0.180	0.007	0.031	0.068	0.046	0.093	16.2
40	0.250	0.665	3.80	0.028	<LD	0.039	0.006	<LD	0.020	0.028	4.83
41	0.120	0.140	17.0	0.094	0.405	0.017	0.150	0.019	0.031	0.045	18.0
42	0.022	0.332	12.0	0.130	<LD	0.028	0.220	0.044	0.088	0.170	13.0
43	0.075	0.490	9.10	0.013	<LD	0.013	0.033	<LD	0.047	0.110	9.88
44[a]	0.023	0.368	9.20	0.240	<LD	<LD	0.061	0.071	0.053	0.140	10.1
45	0.070	0.059	4.10	0.066	0.220	<LD	0.040	0.064	0.055	0.083	4.75
46	0.098	0.130	9.20	<LD	0.050	0.013	0.035	0.055	0.029	0.057	9.66
47[a]	0.120	0.149	6.80	<LD	<LD	<LD	0.078	0.540	0.031	0.096	7.81
48	0.035	0.722	11.0	<LD	<LD	0.006	0.015	0.076	<LD	0.066	11.9
49	0.067	0.054	7.40	0.033	0.120	0.004	0.130	0.073	0.047	0.093	8.02
50	0.047	0.266	8.74	0.118	0.273	0.022	0.186	0.250	0.140	0.153	10.2
51	0.094	0.541	7.70	0.044	<LD	<LD	<LD	<LD	0.055	0.120	8.52
52	0.035	0.389	9.80	0.062	<LD	0.017	0.205	0.100	0.140	0.120	11.5
53	0.038	0.415	8.60	0.008	<LD	0.031	0.260	0.078	0.180	0.190	9.79
54	0.150	0.168	12.0	0.130	0.230	0.021	0.084	0.013	0.039	0.046	12.9
55	0.108	0.651	9.80	0.004	0.037	0.003	0.031	0.022	0.049	0.130	10.8
56[a]	0.048	1.37	13.0	0.120	0.400	<LD	0.017	0.037	0.034	0.027	15.0
Mean	0.186	0.490	11.2	0.137	0.400	0.020	0.197	0.130	0.063	0.108	12.7
SD	±0.208	±0.400	±3.91	±0.147	±0.552	±0.014	±0.310	±0.128	±0.048	±0.060	±4.06

[a]Artisanal caninhas.

Formal=formaldehyde; HMF=5-hydroxymethylfurfural; acetal=acetaldehyde; propional=propionaldehyde; butyral=butyraldehyde; benzal=benzaldehyde; isovaleral=isovaleraldehyde; valeral=*n*-valeraldehyde.

LD=detection limit.

Table 5
Quantitative HPLC analysis of aldehyde 2,4-dinitrophenylhydrazones in imported distilled spirits (concentration in mg/100 ml of absolute ethanol)

Imports	Formal	HMF	Acetal	Acrolein	Furfural	Propional	Butyral	Benzal	Isovaleral	Valeral	ΣAldehydes
1[a]	0.861	0.450	17.4	0.780	<LD	<LD	0.055	0.123	0.081	0.668	19.8
2[a]	0.682	0.460	16.1	0.760	1.44	<LD	0.077	0.085	0.077	0.070	19.8
3[a]	0.450	0.418	11.4	0.645	2.591	<LD	0.144	0.031	0.049	0.062	15.8
4[a]	0.175	0.312	7.63	0.200	0.772	0.010	0.100	0.085	0.084	0.041	9.41
5[a]	0.219	0.160	14.1	0.060	0.780	<LD	0.422	0.118	<LD	0.127	16.0
6[a]	0.364	0.681	16.6	0.100	0.745	<LD	0.210	0.190	<LD	0.075	19.0
7[a]	0.198	1.078	9.02	<LD	1.05	<LD	0.169	0.111	<LD	0.062	11.6
8[a]	0.184	0.448	7.33	0.160	0.590	<LD	0.120	0.023	<LD	0.044	8.90
9[a]	0.211	0.950	9.40	0.210	0.810	<LD	0.195	0.196	0.048	<LD	12.0
10[a]	0.195	1.230	6.19	<LD	0.950	<LD	0.155	0.24Z	<LD	<LD	8.96
11[b]	0.106	0.720	19.1	0.060	<LD	0.018	0.024	0.099	0.727	0.180	21.0
12[c]	0.144	1.690	22.4	0.050	0.240	0.006	0.006	1.38	0.052	0.039	26.0
13[d]	0.043	0.489	23.2	0.060	0.073	0.033	0.158	2.79	6.84	1.31	35.0
14[e]	1.120	0.318	14.4	<LD	0.337	0.016	0.088	15.8	0.047	0.041	32.6
15[e]	0.354	1.530	10.4	0.240	<LD	0.035	0.103	0.074	0.227	0.154	13.1
16[f]	0.084	0.580	11.3	0.154	0.540	0.321	0.093	0.462	0.098	0.199	13.8
17[g]	0.011	<LD	0.391	<LD	<LD	<LD	<LD	<LD	<LD	<LD	0.40
18[g]	0.014	<LD	0.700	<LD	<LD	<LD	<LD	<LD	<LD	<LD	0.71
19[h]	0.430	3.520	10.2	0.220	1.960	0.044	1.130	0.062	0.160	<LD	17.7
Mean	0.307	0.884	12.0	0.264	0.919	0.060	0.191	1.287	0.707	0.176	16.0
SD	±0.297	±0.808	±6.33	±0.261	±0.680	±0.106	±260	±3.80	±1.94	±0.330	±9.04

[a] =Imported whiskies (b=bagaceira, c=brandy, d=grappa, e=rum, f=tequila, g=vodka and h=cognac).

Formal=formaldehyde; HMF=5-hydroxymethylfurfural; acetal=acetaldehyde; propional=propionaldehyde; butyral=butyraldehyde; benzal=benzaldehyde; isovaleral=isovaleraldehyde and valeral=*n*-valeraldehyde.

LD=detection limit.

The major aldehyde in all caninhas is acetaldehyde, the highest amount being found in sample 7. The concentration ranges, expressed in mg/100 ml of absolute ethanol, of formaldehyde, 5-hydroxymethylfurfural, acetaldehyde, acrolein, furfural, propionaldehyde, butyraldehyde, benzaldehyde, isovaleraldehyde and *n*-valeraldehyde are 0.01–1.20, not detectable (ND)–1.86, 3.3–20, ND–0.70, ND–2.6, ND–0.06, ND–2.0, ND–0.60, ND–0.20 and ND–0.20, respectively.

Table 5 shows the quantitative results for aldehydes in international distilled spirits. Varying concentrations of the aldehydes listed above were found depending on the type and brand of these alcoholic beverages. The concentration ranges, expressed in mg/100 ml of absolute ethanol, of aldehydes in the samples of whiskies are 0.175–0.861 for formaldehyde, 0.160–1.23 for 5-hydroxymethylfurfural, 6.19–17.4 for acetaldehyde, ND–0.780 for acrolein, ND–2.59 for furfural, ND–0.01 for propionaldehyde, 0.055–0.422 for butyraldehyde, 0.023–0.242 for benzaldehyde, ND–0.084 for isovaleraldehyde and ND–0.127 for *n*-valeraldehyde. Only imported samples 13 and 14 exceed the Brazilian legislative limits for total aldehydes.

Table 6 compares the mean concentrations of aldehydes, expressed in mg/100 ml of absolute ethanol, in the different series of beverages studied. The amount of aldehydes in the international distillates is significantly higher compared to that in the Brazilian caninhas. In fact, most whiskies, rums, brandies and other international alcoholic beverages are usually aged over a longer period than the Brazilian caninhas. It is known that the content of aldehydes in distilled spirits increases due to oxidation processes [24,29]. Fig. 3 displays a histogram comparing the mean amount of the five principal aldehydes; formaldehyde, 5-hydroxymethylfurfural, acetaldehyde, furfural and butyraldehyde.

The occurrence and quantification of furanic aldehydes (furfural, 5-hydroxymethylfurfural) in distilled spirits have been investigated previously [22–26]. These aldehydes receive special attention because they can control the preservation of organoleptic properties during the distribution and storage of distilled spirits [21,22]. Furanic aldehydes are preferably formed at elevated temperatures [25,26,30], but the mechanism has not been clarified to date. It is believed that the furanic aldehydes appear during distillation and aging [22,25]. Mir et al. [26] have shown that the presence of these furanic compounds in spirits aged in oak barrels depends on the actual condition of the barrel as well as on related factors. The presence of the furanic aldehydes, confirmed by us in non-aged caninhas, reinforces the claims that they can indeed be formed during distillation.

The mean amounts of furfural and 5-hydroxymethylfurfural (Table 6) in international distilled spirits was higher than in caninhas, probably due to the charring process of the wood and the aging of these beverages. The respective mean contents of furfural and 5-hydroxymethylfurfural in the Brazilian caninhas (Table 4) are: 0.40 and 0.49 mg/100 ml of absolute ethanol. The respective mean contents in whiskies (Table 6) are 1.08 and 0.618 mg/100 ml of absolute ethanol. According to Jeuring and Kuppers [25], quantification of furanic aldehydes can be used to recognize possible adulterations.

It is estimated that in 50 ml of whisky there are about 0.15 mg of formaldehyde and 4.2 mg of

Table 6
Mean concentration of aldehyde 2,4-dinitrophenylhydrazones in a series of distilled spirits (concentration in mg/100 ml of absolute ethanol)

Series	Formal	HMF	Acetal	Acrolein	Furfural	Propional	Butyral	Benzal	Isovaleral	Valeral
Caninhas (commercial)[a]	0.108	0.321	7.85	0.067	0.148	0.017	0.171	0.083	0.093	0.121
Caninhas (artisanal)	0.186	0.564	11.2	0.139	0.339	0.019	0.169	0.169	0.040	0.107
Other brands[b]	0.256	1.26	12.4	0.13	0.630	0.067	0.228	2.95	1.16	0.320
Whiskies	0.354	0.618	11.5	0.364	1.08	0.01	0.164	0.120	0.068	0.143

Formal=formaldehyde; HMF=5-hydroxymethylfurfural; acetal=acetaldehyde; propional=propionaldehyde; butyral=butyraldehyde; benzal=benzaldehyde; isovaleral=isovaleraldehyde and valeral=*n*-valeraldehyde.

LD=detection limit.

[a] Principal Brazilian commercial caninhas, $n=12$.

[b] Other brands (grappa, tequila, rums, cognac, vodkas). $n=9$; whiskies, $n=10$.

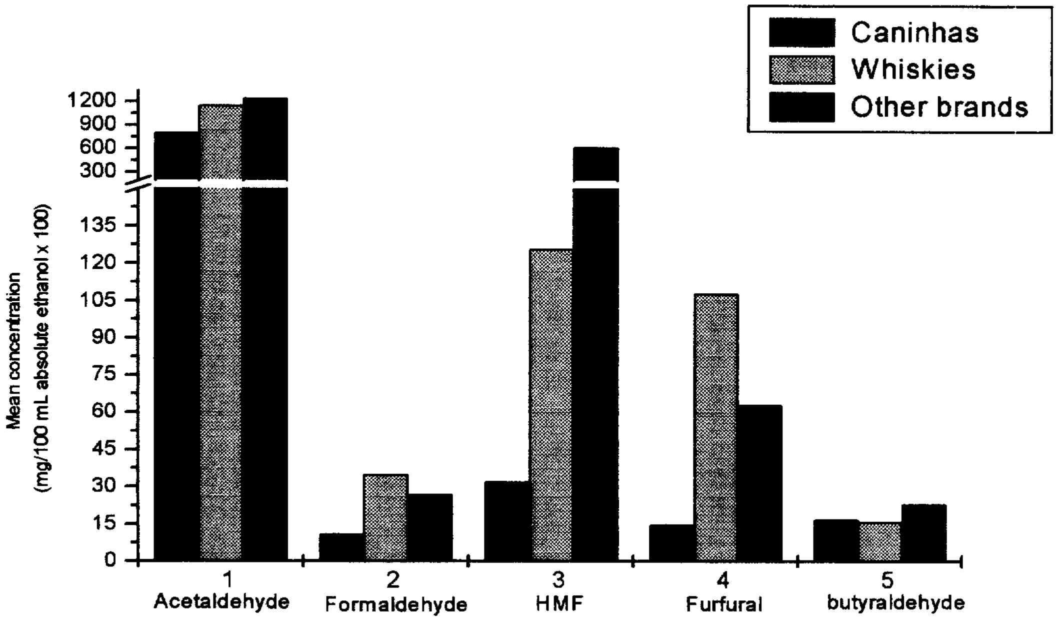

Fig. 3. Histogram indicating the mean concentrations of relevant aldehydes in principal commercial caninhas, imported whiskies and other brands of alcohol. (HMF=5-hydroxymethylfurfural). Principal commercial caninhas (n=12): SD=0.21, 0.38, 4.05, 0.62 and 0.34 for formaldehyde, HMF, acetaldehyde, furfural and butyraldehyde, respectively. Other brands: brandy, grappa, cognac, tequila, rums and vodkas (n=9): SD=0.36, 1.12, 8.34, 0.76 and 0.90 for formaldehyde, HMF, acetaldehyde, furfural and butyraldehyde, respectively. Whiskies (n=10): SD=0.24, 0.35, 4.21, 0.616 and 0.10 for formaldehyde, HMF, acetaldehyde, furfural and butyraldehyde, respectively.

acetaldehyde [27]. If we consider the consumption of distilled spirits, for example 6.9 l per capita in the USA, and extend the above-mentioned values to the distillates we investigated, the intake would be equivalent to 20.7 mg of formaldehyde and 352.8 mg of acetaldehyde. Moreover, taking into account the average content of total aldehydes (maximum of 70 mg/l) in the distilled spirits, as determined by us, the values would increase to 483 mg as the total content of aldehydes. Clearly, these quantities should be of concern to consumers of distilled spirits for health reasons. Although the health effects of aldehydes have mainly been attributed to formaldehyde, acetaldehyde and acrolein [7], the control of other members of the group is needed until their toxicity has been proven. Such evaluation could be easily achieved using the HPLC method described here.

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