

*Full Length Research Paper*

# Comparative study of chemical constituents in sugar cane spirits from Brazil and Cape Verde

Pereira, Regina Furtado Rodrigues<sup>1</sup>, Melo, Diego Quadros<sup>1</sup>, Barros, Allen Lopes<sup>1</sup>,  
Lima, Ari Clecius Alves<sup>2</sup> and Nascimento, Ronaldo Ferreira<sup>1\*</sup>

<sup>1</sup>Department of Analytical Chemistry and Physico-Chemistry – Federal University of Ceará. Rua do Contorno, S/N Campus do Pici, Bl. 940 – CEP: 60451-970 – Fortaleza – CE – Brazil.

<sup>2</sup>Department of Hydraulic and Environmental Engineering – Federal University of Ceará. Rua do Contorno, S/N Campus do Pici, Bl. 713 – CEP: 60451-970 – Fortaleza – CE – Brazil.

Accepted 13 August, 2012

**This work focuses on the development, validation, and use of analytical methods for the identification and quantitation of relevant constituents in sugar cane spirits from Brazil and Cape Verde. The compositions of both artisanal and industrial products were investigated. Repeatability, recovery, linearity of analytical curves, limits of detection, and limits of quantitation were parameters considered for methods validation. Gas chromatography was used for the analyses of acetaldehyde, ethyl acetate, and alcohols. Anions were analyzed by ion chromatography, while metal ions were analyzed by inductively coupled plasma optical emission spectrometry. Good precision and accuracy were achieved, and the concentrations of some constituents were found to be higher than that allowed by law, namely: acetaldehyde, in five samples from Cape Verde and in three samples from Brazil; n-butyl alcohol, in two samples from Cape Verde and in three samples from Brazil; higher alcohols, in three samples from Cape Verde and in two samples from Brazil. On the other hand, mean concentrations of acetaldehyde and higher alcohols were in accordance with legal requirements. Further discussion is presented regarding differences observed between the compositions of the samples studied.**

**Key words:** Sugar cane spirits, cachaça, grogue, quality control, chemical composition, chromatography, methods validation, Brazil, Cape Verde.

## INTRODUCTION

The production and commercialization of sugar cane spirits are important economical activities both in Brazil and Cape Verde. In such countries sugar cane spirits are largely consumed and are also considered a cultural, historical patrimony.

In spite of the economical and cultural relevance of sugar cane derivatives, the lack of legal requirements in Cape Verde regarding production and final quality of sugar cane spirits has only recently been expected to be solved (Expresso das Ilhas, 2012).

Just like in Brazil, in Cape Verde there are a great number of producers of sugar cane spirits, and many production plants are run with no technical knowledge at

all, which in many cases makes the production of sugar cane spirits to be performed as an empirical, rudimentary activity. On the other hand, the search for improvements in this field implies in considering some requirements, for example, the optimization of production processes and the need of proper chemical characterization of the final products.

The quality of sugar cane spirits is closely related to the different stages of its production: preparation of the sugar cane as a raw material (cutting, clean-up, transportation, storing); extraction of the juice; alcoholic fermentation; distillation, the final step in which the beverage is obtained by vaporization/condensation equilibriums of volatile constituents in either a distillation column or in an alembic.

In addition, the product can be aged in wooden barrels, prior to bottling and comercialization (Aquino et al., 2006).

During alcoholic fermentation, sugar is converted

\*Corresponding author. E-mail: [ronaldo@ufc.br](mailto:ronaldo@ufc.br). Tel: +55-85-3366-9958. Fax: +55-85-3366-9038.

**Table 1.** Origin, type, brand and alcoholic grade of the samples analysed.

Brazil				Cape Verde			
Origin/State	Type	Brand	° GL	Origin/Island	Type	Brand	° GL
Ceará	Artisanal	Raparigueta	40	Brava	Artisanal	Biula	38
Ceará	Artisanal	Ibiapaba	40	Santiago	Artisanal	Cana Preta	39
Ceará	Artisanal	Lá do Sítio	45	Santiago	Artisanal	Nha Sonho	39
Ceará	Artisanal	Amansa Sogra	43	Santiago	Artisanal	Fidjo de Pé Doce	39
Ceará	Artisanal	Tonel	48	Santiago	Artisanal	Garafinha	39
Goiás	Artisanal	Atitude	45	Santiago	Artisanal	Terra-Terra	40
Piauí	Artisanal	Piauí	43	Santiago	Artisanal	Ribeira Grande	39
Piauí	Artisanal	Concebida	49	Santiago	Artisanal	Rabelados	39
Rio Grande do Sul	Artisanal	Brazuca	45	Santiago	Artisanal	Rocha Lama	39
Rio Grande do Norte	Artisanal	Serena	50	Santo Antão	Artisanal	Val da Graça	50
Ceará	Industrial	Ypióca	45	Santiago	Industrial	Nôsgrogo (dye)	39
Ceará	Industrial	Sapupara	45	Santiago	Industrial	Serenata	40
Ceará	Industrial	Redenção	43	Santiago	Industrial	Nha Dona	30
Ceará	Industrial	Colonial	50	Santiago	Industrial	Convento	39
Ceará	Industrial	Chave deOuro	48	Santiago	Industrial	NkaPinton	40
Ceará	Industrial	Gole de Ouro	49	Santiago	Industrial	Silva	40
Pernambuco	Industrial	Pitú	45	Santiago	Industrial	Djangago	38
Piauí	Industrial	Mangueira	45	Santiago	Industrial	Nôsgrogo	39
São Paulo	Industrial	51	40	Santo Antão	Industrial	Alcane	38
São Paulo	Industrial	Sagatiba	45	Santo Antão	Industrial	Tropicana	39

mainly into ethyl alcohol and carbon dioxide, but small amounts of other compounds, referred to as *secondary products* of alcoholic fermentation (Crispim, 2000) are also produced and are responsible for the unique sensorial characteristics of sugar cane spirits. Total esters (expressed as ethyl acetate), higher alcohols (that is, n-propyl, isobutyl, and isoamyl alcohols), volatile acidity (expressed as acetic acid), and inorganic species such as sodium, potassium, magnesium, calcium, lead, copper, and iron play an important role in the final aroma and flavour an important role in the final aroma and flavour

(Crispim, 2000; Angelis,1987).

This work focuses on the development and validation of analytical methods for the determination of both organic and inorganic composition of artisanal and industrial sugar cane spirits from Brazil (where such product is named *Cachaça*) and Cape Verde (where it is named *Grogue*). Since in Cape Verde there is no legislation regarding the composition of sugar cane spirits, the results obtained in this work were compared to the limits stated by Brazilian legislation on this subject (MAPA, 2005), as well as to data reported in literature.

## MATERIALS AND METHODS

### Samples

Twenty samples of sugar cane spirits from different islands of Cape Verde – ten artisanal products, ten industrial ones – and twenty samples from different states of Brazil – ten artisanal, ten industrial – were purchased at local markets or obtained directly from the producers (Table 1).

### Chemicals and solutions

Analytical-grade ethanol (99.5%, VETEC, BRAZIL), acetaldehyde (99.9%, VETEC, BRAZIL), ethyl acetate,

**Table 2.** ICP OES analytical conditions.

Parameter	Value
RF power	1,300 W
Plasma gas flow	15 L min <sup>-1</sup>
Auxiliary gas flow	0.5 L min <sup>-1</sup>
Nebulizer gas flow	0.8 L min <sup>-1</sup>
Sample flow	1.4 ml min <sup>-1</sup>
Replicates	3
Signal processing	Peak area – 3 pixels

n-propanol, butanol, isobutanol, and isoamyl alcohols, (99.9%, MERCK, GERMANY), amyl alcohol (99.5%, CARLO ERBA, ITALY), and Milli-Q® (MILLIPORE, USA) water (18.2 MΩ cm<sup>-2</sup> at 25 °C) were used throughout the experiments.

Na<sup>+</sup>, K<sup>+</sup>, Mg<sup>2+</sup>, Ca<sup>2+</sup>, Cu<sup>2+</sup>, Pb<sup>2+</sup>, Zn<sup>2+</sup>, and Fe<sup>3+</sup> solutions were prepared by diluting their respective 1,000 mg/L standard solutions for ICP OES (ACROS ORGANICS, BELGIUM).

Acetate, chloride, sulfate, bicarbonate, and carbonate solutions were prepared with their respective analytical-grade sodium salts (MERCK, GERMANY).

### Gas chromatography (GC)

Volatile compounds were analyzed by gas chromatography with flame ionization detection (GC-17A-FID, SHIMADZU, JAPAN).

Injector and detector temperatures were 200 to 300°C, respectively. The chromatographic column (5% phenyl/95% dimethylpolysiloxane; 30 m x 0.25 mm; 0.25 μm film thickness) (AGILENT, USA), was used with the oven temperature programmed as follows: 40°C (holding time 3 min), 5°C min<sup>-1</sup> up to 65°C, and then 50°C min<sup>-1</sup> up to 200°C.

1-μl aliquots were injected in split mode (1:30), with the carrier gas (hydrogen 4.5 FID) at 60 kPa. The flow rate for each one of the gases in the chromatographic system was: 30 ml min<sup>-1</sup> hydrogen (fuel), 1.0 ml min<sup>-1</sup> hydrogen (carrier gas), and 300 ml min<sup>-1</sup> synthetic air (oxidizing) (WHITE-MARTINS, BRAZIL).

### Ion chromatography (IC)

An ion chromatograph was used in the analyses of anions, with a 25-μL injection loop, KOH eluent generator, and conductivity detector (ICS-3000, DIONEX, USA). AS18 column (25 cm x 4.0 mm) and ASRS-ultra II suppressor were also used.

Temperature, current, and conductance were 40°C, 40 mA, and 0.6 μS, respectively. The gradient program was set as follows: initial concentration 10 mmol L<sup>-1</sup> until 7.2 min; maintained 22 mmol L<sup>-1</sup> up to 13.2 min; maintained 44 mmol L<sup>-1</sup> up to 13.44 min; maintained 44 mmol L<sup>-1</sup> up to 15.0 min.

### Inductively coupled plasma optical emission spectrometry (ICP OES)

Cations were analysed by ICP OES (Optima 4300 DV, PERKIN ELMER, USA), under the analytical conditions presented in Table 2.

### Sample preparation and calibration curves

#### Volatile compounds

Stock solutions (1,000 mg/L) of each analyte (acetaldehyde, ethyl

acetate, n-butanol, n-propanol, isobutanol, and isoamyl alcohol) were prepared in ethanol 40% v/v and used for the determination of their respective retention times by gas chromatography.

Multicomponent solutions were prepared in ethanol 40% v/v and were used in the construction of the calibration curves, with triplicates of eight points. Amyl alcohol was used as internal standard, at the concentration of 1,000 mg/L. Calibration curves were obtained by linear regression (internal standard method).

### Cations

The samples were digested in HNO<sub>3</sub>:H<sub>2</sub>O<sub>2</sub> medium (2:1 v/v) as follows: 2.0 ml of concentrated HNO<sub>3</sub> (SYNTH, BRAZIL) were transferred to a quartz vessel containing 2.0 ml of the sample, and then allowed to rest for 1 h. After that, 1.0 mL of analytical grade H<sub>2</sub>O<sub>2</sub> (VETEC, BRAZIL) was added and the mixture was maintained at 120°C for 2 h in a digestion block (TCM 44, TECNAL, BRAZIL). The resulting solution of each quartz vessel was diluted to 20 ml.

ICP OES calibration curves (external standard method) were obtained by registering the intensity of atomic emissions at specific wavelengths versus the respective concentrations of the analytes. The following concentrations were used in the calibration curves: Na<sup>+</sup>, K<sup>+</sup>, Mg<sup>2+</sup>, and Ca<sup>2+</sup> (5, 10, 15, and 30 mg/L); Cu<sup>2+</sup>, Pb<sup>2+</sup>, Zn<sup>2+</sup>, and Fe<sup>2+</sup> (1, 5, 10, and 15 mg/L).

### Anions

Acetate, carbonate, and chloride peaks were identified by ion chromatography prior to quantitative analyses.

Calibration curves of acetate and carbonate were obtained by ion chromatography according to external standard method, with aqueous solutions of each anion in the range from 10 to 100 mg/L. The curves were constructed by plotting the peak area of the analyte versus its respective concentration. Prior to analysis, the samples were diluted in a 1:10 ratio, 1-ml aliquots being analysed in duplicate.

Due to matrix effects, chloride anion in Cape Verdean samples was analyzed by titration (Mohr's method), as follows (Galinaro et al., 2007; Vogel et al., 2000).

0.5 ml of 0.0166 mol/L K<sub>2</sub>CrO<sub>4</sub> indicator solution was transferred to an erlenmeyer containing 25 ml of the sample. The titration was immediately started by using a 5-ml buret to add 0.0100 mol/L AgNO<sub>3</sub> to the solution in the Erlenmeyer up to the equivalence point, at which the transition from yellow to orange was observed.

In order to evaluate the precision, titrations were performed in triplicate (EURACHEM, 1998).

### Total alkalinity – Carbonate and bicarbonate

The quantification of carbonate and bicarbonate was performed with a Total Alkalinity Titrator (809 Titrando, METROHM-PENSALAB, BRAZIL). 50 mL of the sample were directly titrated with HCl 0.1033 mol/L at 25°C.

### Methods validation

The following parameters were taken into account for the validation of the chromatographic methods: precision, accuracy, recovery, linearity, limit of detection, and limit of quantitation (Eurachem, 1998; ABNT, 2005; Brito et al., 2003).

### Precision

Precision expresses the proximity between the results

**Table 3.** Relative standard deviations (RSD) calculated for analyses performed in triplicate, by different analysts, on different days.

Compound	Analyst #1		Analyst #2		Analyst #3	
	Day #1		Day #3		Day #5	
	C (mg/L)	RSD (%)	C (mg/L)	RSD (%)	C (mg/L)	RSD (%)
Acetaldehyde	103.0	1.5	104.3	0.4	102.3	0.5
n-propanol	485.4	0.2	485.5	0.3	485.5	0.6
Ethyl acetate	131.9	0.8	133.1	0.1	133.6	1.4
Isobutanol	314.5	0.1	312.9	0.2	313.7	0.1
Isoamyl alcohol	1058.9	0.2	1058.0	0.1	1058.2	0.1

of a measurement. Such parameter can be quantified by dividing the standard deviation of repeated measurements obtained with the same sample, under the same conditions, by the respective average result obtained. The resulting value, multiplied by 100%, is called either *coefficient of variation* or *relative standard deviation* (RSD %). In this work, precision was measured regarding intermediate precision and repeatability, with analyses of samples and standard solutions performed in triplicate.

#### Accuracy

Accuracy expresses the closeness of measurements to a reference value considered true. In this case, the measurements refer to the concentration of the analytes. Accuracy can be evaluated by recovery essays (AOAC, 2002), which were carried out with samples from Cape Verde. Recovery of each analyte was calculated by using Equation 1:

$$R = \frac{[\text{ ] in spiked sample} - [\text{ ] in sample}}{[\text{ ] added to the sample}} \times 100\% \quad (1)$$

where [ ] is the analyte concentration.

#### Linearity of the calibration curves

Linearity is related to the proportionality between the concentrations of the analytes and the respective responses given by the method. Linear regression (minimum square methods) can be used for linearity analysis. Homoscedasticity, as well as the absence of abnormal values, must be checked (Eurachem, 1998; US-FDA, 1994).

#### Limit of detection (LOD)

Limit of detection expresses the lowest concentration of a substance that can be detected – not necessarily quantified – by the analytical method. Analyses are performed by lowering the concentrations down to the lowest concentration that results in a signal at least three times more intense than the noise ( $S \geq 3N$ ). Equation 2 can be used to calculate LOD (Eurachem, 1998; Instituto Adolfo Lutz, 1976).

$$LOD = \frac{3,3SD}{b} \quad (2)$$

where SD is the standard deviation and  $b$  is the angular coefficient of the calibration curve.

#### Limit of quantitation (LOQ)

Limit of quantitation is affected by experimental parameters, such as precision and accuracy. It expresses the lowest quantifiable concentration of an analyte and can be calculated with Equation 3

$$LOQ = \frac{10SD}{b} \quad (3)$$

where SD is the standard deviation and  $b$  is the angular coefficient of the calibration curve.

## RESULTS AND DISCUSSION

#### Intralaboratory precision

Relative standard deviations (RSD) were calculated for intralaboratorial analyses (intermediate precision). The values presented in Table 3 were calculated by analyzing one of the real samples studied in this work. The precision of the method was found to be satisfactory, since RSD values were below 8% (ABNT, 2005).

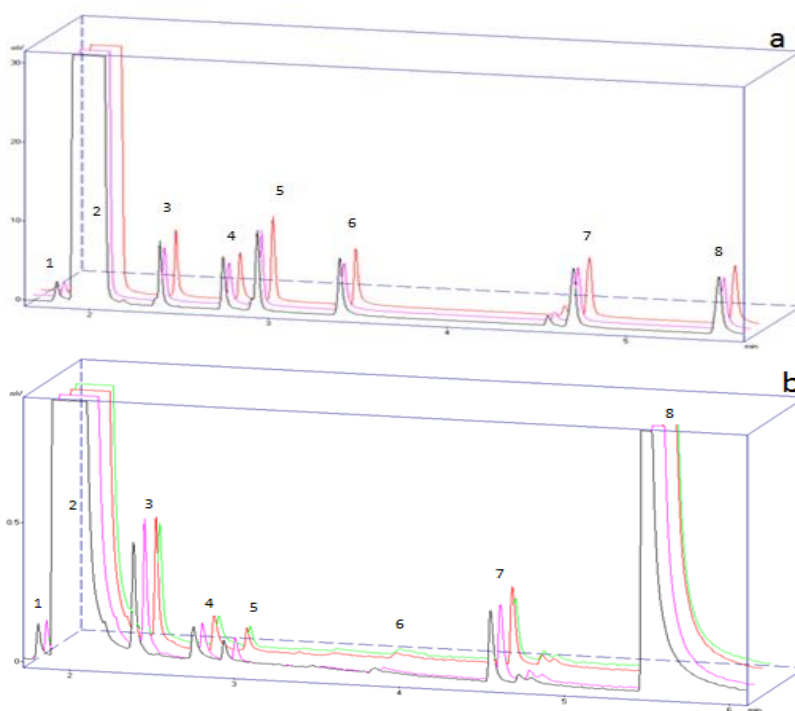
Repeatability was studied by analyzing three solutions of different concentrations within the linear range of the method, in triplicate and on the same day. Results are displayed in Table 4 and show that the higher the concentrations the lower the RSD values, since lower concentrations of organic compounds usually result in greater variations in the response of flame ionization detectors. RSD values were below 5% for all of the compounds studied. Examples of chromatograms obtained in sequential injections are displayed in Figure 1. Their similarities illustrate the precision of the analyses (AOAC, 2002).

#### Accuracy

Six samples of sugar cane spirits from Cape Verde – three artisanal, three industrial – were spiked in two concentrations, 100 and 300 mg/L. Recovery results are

**Table 4.** Relative standard deviations (RSD) calculated for analyses performed with different solutions, by the same analyst, on the same day.

Compound	10 mg/L		500 mg/L		1,300 mg/L	
	C (mg/L)	RSD (%)	C (mg/L)	RSD (%)	C (mg/L)	RSD (%)
Acetaldehyde	10.36	4.72	554.5	0.57	1,207.2	0.27
n-Propanol	16.08	1.06	557.6	0.26	1,122.4	0.52
Ethyl acetate	8.96	0.74	484.8	0.03	1,244.2	0.08
Isobutanol	11.61	2.09	504.0	0.26	1,302.1	0.03
Butanol	< LOD	-	501.8	0.14	1,256.7	0.07
Isoamyl	11.22	0.5	502.9	0.18	1,326.2	0.07

**Figure 1.** Repeatability of the chromatographic method. Chromatograms obtained with (a) multicomponent 500 mg/100 ml anhydrous alcohol (AA) and (b) 10 mg/100 ml AA solutions, injected in triplicate: acetaldehyde (1); ethanol (2); n-propanol (3); ethyl acetate (4); isobutanol (5); n-butanol (6); isoamyl alcohol (7); amyl alcohol (internal standard) (8).

presented in Table 5.

The results show that matrix effects are negligible in the samples studied. The method used is supported by the results, since standard recoveries are higher than 90% (ABNT, 2005).

#### Linearity of the calibration curves

The data obtained from the calibration curves are presented in Tables 6 and 7. Good linearity was observed, with correlation coefficient (R) above 0.99 in most cases

(ABNT, 2005; Brito et al., 2003; AOAC, 2002; US-FDA, 1994).

A t-test at 95% confidence level was performed, obtained for isobutanol, thus indicating that such parameter must be included in the equation.

#### Limits of detection (LOD) and limits of quantitation (LOQ)

The determination of LOD and LOQ was based on parameters of the calibration curves. The data were obtained

**Table 5.** Recovery results for volatile organic compounds in artisanal and industrial samples from Cape Verde, spiked with 100 mg/L and 300 mg/L of the analytes.

Compound	mg/L	G1		G4		G5		mg/L	G2		G3		G6		
		C <sub>1</sub>	R <sub>1</sub>	C <sub>4</sub>	R <sub>4</sub>	C <sub>5</sub>	R <sub>5</sub>		C <sub>2</sub>	R <sub>2</sub>	C <sub>3</sub>	R <sub>3</sub>	C <sub>6</sub>	R <sub>6</sub>	
Artisanal samples	Acetaldehyde	100	6.9	99.5±1	8.4	96.5±4.2	16.7	100.2±2.4	100	8.6	101.7±0.3	16.6	99.3±4	77	100.1±3
		300	32.3	100.1±0.2	33.5	103.8±2	42.5	100.1±1.0	300	33.7	100.0±0.8	42.8	101.5±6	33.6	100.2±0.3
	n-Propanol	100	23.6	101.1±2.0	20.5	102.7±2	40.2	98.8 ± 1.5	100	23.4	98.2±1.7	51.6	99.8±1.5	58.3	102.7±2
		300	42.4	98.4 ± 0.3	37.5	100.0±0.1	58.2	99.9 ± 0.4	300	41.7	99.6±0.1	69.3	97.9±0.3	74.8	99.3±1.3
	Ethyl acetate	100	40.0	102.4±1.3	23.4	102.2±1.0	27.8	99.6±0.85	100	27.6	98.7±1.3	66.8	99.6±0.8	46.6	99.0±1.8
		300	59.1	100.2±2	45.4	100.5±0.3	50.6	100.6±0.8	300	53.4	101.6±0.9	89.6	101.0±0.9	68.5	98.5±0.4
	Isobutanol	100	23.6	100.7±0.7	15.6	102.2±1.0	20.3	97.9 ± 2.1	100	16.7	97.7±2.1	29.8	100.6±2	18.3	98.9±1.6
		300	44.6	99.6 ± 0.4	37.4	100.4±0.7	41.2	99.7 ± 0.4	300	38.6	98.8±0.2	52.5	98.4±1.2	41.3	100.6±0.5
	n-Butanol	100	13.4	103.8±1.5	<LOD	-	<LOD	-	100	8.8	97.4±0.7	<LOD	<LOD	14.5	98.9±1.6
		300	27.4	99.3 ±3.7	-	-	-	-	300	22.8	99.5±1	-	-	27.6	97.8±1.1
	Isoamyl alcohol	100	46.2	101.8±1.2	65.1	99.1±1.2	41.3	102.05±1.3	100	79.2	102.9±0.9	32.1	102.2±2	34.9	99.0±3.2
		300	63.2	99.6 ± 0.4	83.1	100.4±0.2	58.4	99.3 ± 0.8	300	96.4	98.3±1.4	50.1	99.3±0.5	53.7	100.5 ± 0.6

R<sub>n</sub> = recovery (%); C<sub>n</sub> = (mg/100 ml AA).

**Table 6.** Experimental calibration curves parameters of the volatile constituents studied.

Compound	Correlation coefficient (R)	Angular coefficient	Linear coefficient	Concentration range (mg/L)
Acetaldehyde	0.9943	0.0003	0.0066	50-1,500
n-Propanol	0.9953	0.0009	-0.014	25-1,300
Ethyl acetate	0.9918	0.0008	-0.004	25-1,300
Isobutanol	0.9944	0.0014	-0.0148	25-1,300
n-Butanol	0.9966	0.0012	-0.025	25-1,300
Isoamyl alcohol	0.9941	0.0014	-0.0158	25-1,300

by performing 10 injections of 10 mg/L standard solutions.

Most of the analytes were present in the samples in concentrations above their respective LOD (Table 8), with acceptable precision and accuracy (Eurachem, 1998).

#### Quantitation of organic compounds

Typical chromatograms obtained are displayed in

Figure 2a, b, and c. It can be seen that complete separation of the constituents is achieved within 6 min, the greatest peak area being observed for isoamyl alcohol, due to its higher concentration as a mixture of its n-amyl and isoamyl isomers. N-butanol was found to be the least concentrated alcohol in the samples (Nascimento et al. 1999; Giudi et al., 1993; Nykänen and Nykänen, 1991). Table 9 displays the concentrations of the organic compounds analyzed. Further discussion is

presented below.

#### Acetaldehyde

The concentration of acetaldehyde in the final product is an important parameter in the quality control of sugar cane spirits.

Acetaldehyde average concentrations in samples from Brazil [17.2 mg/100 ml anhydrous alcohol (AA)] and from Cape Verde (17.6 mg/100

**Table 7.** Experimental calibration curves parameters of the inorganic constituents studied.

Compound	Correlation coefficient (R)	Angular coefficient	Linear coefficient	Concentration range (mg/L)
Chloride	0.9999	0.4007	1.1958	0.02-20
Acetate	0.9998	0.1384	0.0056	0.02-5
Sulfate	0.9999	0.2768	0.0317	0.04-40
Sodium	0.9988	2,520	-4,942	5-30
Calcium	0.9997	1,289	-476	5-30
Magnesium	0.9997	1,714	-635	5-30
Potassium	0.9992	5,453	-814	5-30
Zinc	0.9996	344	-504	1.0-15
Copper	0.9808	44,340	-13,906.2	1.0-15
Lead	0.9992	367.9	-76.2	1.0-15
Iron	0.9997	3,282	-426.9	1.0-15

**Table 8.** LOD and LOQ values obtained for chemical constituents of sugar cane spirits.

Constituent	LOD (mg/L)	LOQ (mg/L)	Constituent	LOD (mg/L)	LOQ (mg/L)
Acetaldehyde	23.4	77.9	Calcium	0.02	0.07
Ethyl acetate	10.5	35.14	Copper	0.003	0.01
Isoamyl alcohol	7.6	11.5	Iron	0.003	0.01
Isobutanol	5.74	19.15	Lead	0.003	0.01
n-Butanol	8.63	28.78	Magnesium	0.013	0.04
n-Propanol	13.9	46.2	Potassium	0.005	0.015
Acetate	0.02	0.09	Sodium	0.003	0.01
Chloride	0.01	0.05	Zinc	0.007	0.02
Sulfate	0.02	0.07			

ml AA) were found to be similar to each other and higher than most of the average values reported in literature.

In five samples from Cape Verde and in three samples from Brazil the level of acetaldehyde exceeded the upper limit stated by Brazilian legislation, with the highest concentrations being found in artisanal products.

Acetaldehyde concentration was quite different from a sample to another. This may be probably due to different characteristics of the microorganisms used in fermentation, as well as to excessive concentration of metals. According to Nascimento et al. (1998), the presence of copper and other metals may affect the occurrence of sulfurated compounds and also result in increased acetaldehyde concentrations.

### **Ethyl acetate**

The concentrations of ethyl acetate ranged from 4.08 to 240.98 mg/100 ml AA in Cape Verdean samples and from 2.80 to 49.4 mg/100 ml AA in Brazilian samples, with the highest concentrations being found in industrial products.

Although a wide concentration range was observed, the allowed upper limit for ethyl acetate was exceeded only in

one industrial product from Cape Verde.

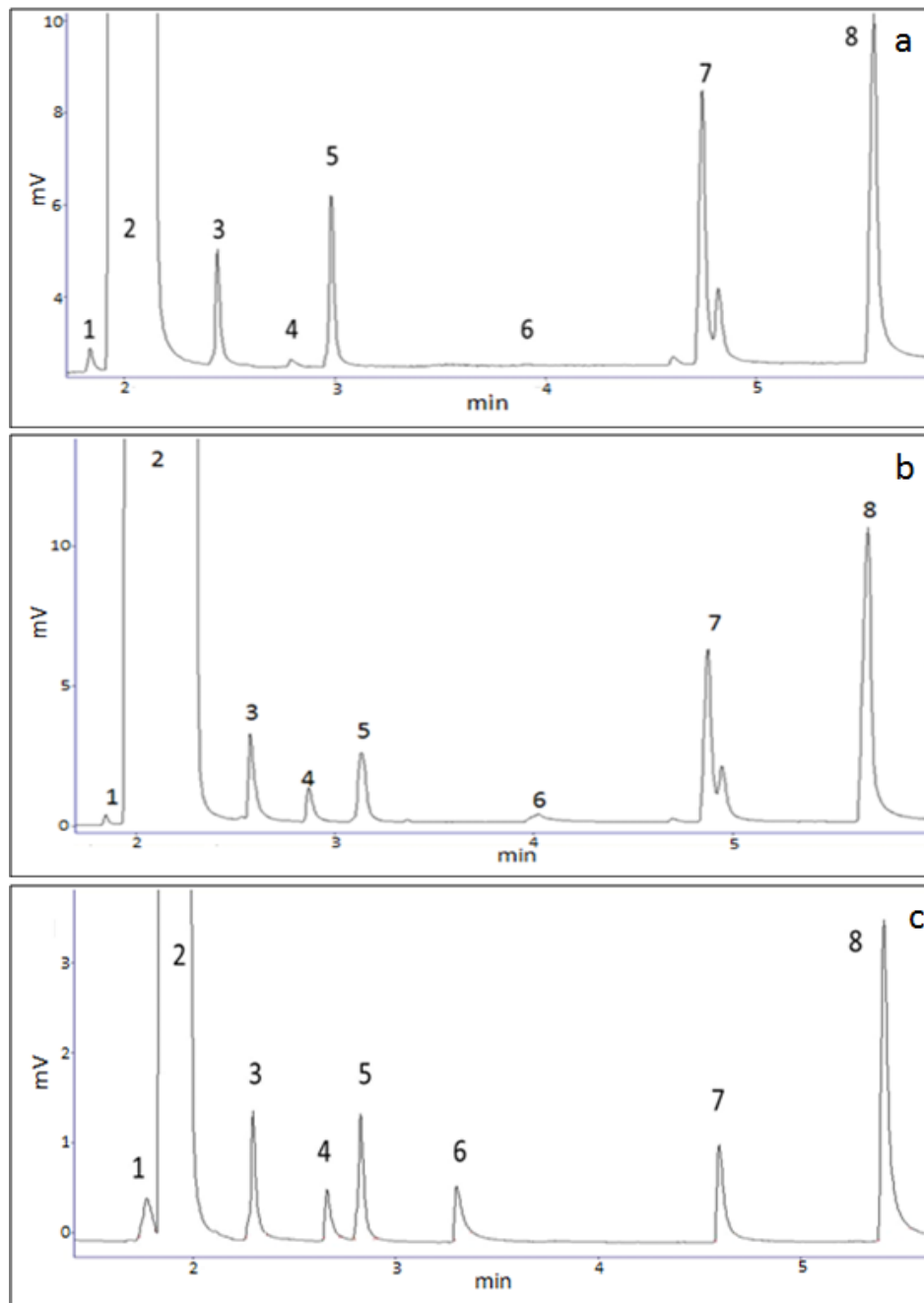
The excessive concentration of ethyl acetate may be related to the type of inoculums used, to improper separation of the distilled fractions, and also to errors when monitoring critical parameters such as time, temperature, and pH (Nascimento et al., 2009).

### **Alcohols**

According to Brazilian legislation, the upper limit of n-butanol concentration in sugar cane spirits is 3.0 mg/100 ml AA, which was exceeded in all of the samples analyzed. The concentrations of n-butanol in sugar cane spirits may be affected by the levels of amino acids or proteins in the fermentation step, as well as by the type of yeast used (Campos et al., 2010).

The concentrations of n-propanol were also found to be over a wide range, with higher values being detected in artisanal products. This may be caused by errors in the control of fermentation and distillation processes, or even by the use of yeasts with low biological activity (Giudi, 1993).

Isobutanol concentration ranged from 7.8 mg/100 ml AA to 63.2 mg/100 ml AA in the samples from Cape Verde,



**Figure 2.** Chromatogram of (a) a sample from Brazil, (b) a sample from Cape Verde, and (c) a multicomponent solution (300 mg/L): acetaldehyde (1); ethanol (2); n-propanol (3); ethyl acetate (4); isobutanol (5); n-butanol (6); isoamyl alcohol (7); amyl alcohol (internal standard) (8).

and from 14.6 mg/100 ml AA to 74.4 mg/100 ml AA in the samples from Brazil. Average concentrations were found to be lower than most of the values reported (Table 10), with the highest concentrations being observed in industrial samples.

Isoamyl alcohol is the most concentrated constituent of the samples studied. Its concentration ranged from 44.6

mg/100 ml AA to 344.0 mg/100 ml AA in the samples from Cape Verde, and from 54.8 mg/100 ml AA to 239.9 mg/100 ml AA in the samples from Brazil. The highest concentrations were found in industrial samples. Average concentrations were lower than most of those cited in literature (Table 10).

Higher alcohols concentration (that is, the sum of the



**Table 9.** Maximum (Max), minimum (Min), and mean (M) concentrations of volatile constituents in samples from Brazil (BR) and Cape Verde (CV) (mg/100 ml AA); upper limits in Brazilian legislation and average values reported in literature.

Compound	Brazilian legislation	BR			CV			Literature mean concentration
		Max	Min	M	Max	Min	M	
Acetaldehyde	30	38.5	4.2	17.2	42.1	4.7	17.6	8.62 (Janzantti et al., 2004); 14.9 (Souza et al., 2009); 8.6 (Parazzi et al., 2008); 52.3 (Silva et al., 2009); 7.85 (Nascimento et al., 1997)
Ethyl acetate	200	49.4	2.8	24.8	241	4.1	70	46.7 (Janzantti et al., 2004); 58.5 (Souza et al., 2009); 76.2; 31.6 (Nascimento et al., 2009); 46.7 (Parazzi et al., 2008); 44.0 (Nascimento et al., 2008); 54.6 (Silva et al., 2009)
n-Butanol	3	40.0	3.3	14.1	60.0	3.1	18.3	30.0 (Janzantti et al., 2004); 1.3 (Souza et al., 2009); 1.1 (Parazzi et al., 2008)
n-Propanol	-	157.3	15.9	71.8	205.1	9.2	71.2	182.5 (Janzantti et al., 2004); 67.6 (Parazzi et al., 2008); 72.8 (Penteado et al., 2009); 34.06 (Silva et al., 2009)
Isobutanol	-	74.4	14.6	42.7	63.2	7.8	37.1	80.8 (Janzantti et al., 2004); 58.4 (Parazzi et al., 2008); 36.9 (Penteado et al., 2009); 85.3 (Silva et al., 2009)
Isoamyl alcohol	-	239.9	54.8	132.9	344	44.6	132.3	281.0 (Janzantti et al., 2004); 198.0 (Parazzi et al., 2008); 112.4 (Penteado et al., 2009); 266.2 (Silva et al., 2009)
Higher alcohols	360	-	-	247.4	-	-	240.6	544.3 (Janzantti et al., 2004); 236; 1324.0 (Souza et al., 2009); 222.1 (Penteado et al., 2009); 385.6 (Silva et al., 2009)

concentrations of isobutanol, n-propanol and isoamyl alcohol) was found to be low in the samples studied, except in two artisanal products from Brazil and in three samples from Cape Verde –one artisanal and two industrial. In these samples, the sum of the concentrations of isobutanol, n-propanol and isoamyl alcohols exceeded the limits allowed by Brazilian legislation.

Average levels of higher alcohols in artisanal samples were found to be higher than those in industrial ones. This may result from the lack of standardization in the production process, since higher alcohols are produced in the metabolism of amino acids and proteins by the yeast, during the

fermentation step.

Depending on the type of yeast used, aldehydes can be reduced to higher alcohols. The concentration of higher alcohols in the final product is also affected by other conditions during fermentation, such as sugar concentration, temperature, aeration, the source of nitrogen and its concentration (Campos et al., 2010; Mayer et al., 2003).

#### Ionic species

The concentration of cations in sugar cane spirits is important to the quality of the final product and

to the health of the consumers (Mayer et al., 2003).

Metal ions greatly influence sensorial characteristics of spirits, by removing bad flavours and and smells caused by sulfur compounds. As micro-nutrients, metals can also favour the fermentation processes (Campos et al., 2010). Table 10 presents maximum, minimum, and mean concentrations of cations in the samples studied, as well as concentration ranges reported in literature.

Results of the analyses of anions in the samples from Brazil and Cape Verde are displayed in Tables 11 and 12, respectively.

Original values that were used to calculate the

**Table 10.** Maximum (Max), minimum (Min), and mean (M) concentrations of cations in samples from Brazil and Cape Verde and concentration ranges reported in literature.

Cation	Literature (mg/L) (Boscolo et al., 2000; Parazzi et al., 2008)	Brazil C (mg/L)			Cape Verde C (mg/L)		
		Max	Min	M	Max	Min	M
Na <sup>+</sup>	29.2 - 0.61	20.0	1.0	7.7	13.3	1.9	7.8
K <sup>+</sup>	31.3 - 34	2.2	-	-	<LOD	-	-
Mg <sup>2+</sup>	63.0 - 0.70	7.9	3.9	5.4	11.2	-	-
Ca <sup>2+</sup>	44.6 - 36	7.7	1.4	6.8	3.7	-	-
Cu <sup>2+</sup>	5.0-0	1,1	0,03	0,34	6	0,5	2,6
Fe <sup>2+</sup>	2.24 - 0.009	2.0	1.40	1.8	<LOD	-	-
Zn <sup>2+</sup>	0.49 - 0.01	2.6	1.30	1.7	<LOD	-	-
Pb <sup>2+</sup>	0.20-0	2.7	0.03	1.4	<LOD	-	-

**Table 11.** Concentrations (mg/L) of anions in samples from Brazil.

Sample	Sulphate	RSD %	Acetate	RSD %	Chloride	RSD %
C1	0.09	0.04	0.009	1.64	0.41	4.4
C2	0.08	2.09	0.52	2.86	0.71	4.5
C3	0.04	3.45	0.86	2.06	0.51	5.4
C4	0.03	3.69	0.92	3.32	0.83	4.5
C5	0.06	1.25	0.31	0.13	0.03	1.1
C6	0.05	3.06	0.76	2.41	0.6	2.1
C7	0.03	1.58	0.39	4.06	<LOD	---
*C8	0.19	6.88	1.72	3.01	0.75	1.81
*C9	<LOD	-	<LOD	-	0.16	1.70
*C10	0.04	3.97	0.99	3.35	0.84	0.27
*C11	0.04	3.16	0.79	4.18	1.04	3.5
C12	0.03	0.99	0.25	6.78	<LOD	-
*C13	0.05	2.95	0.74	2.03	<LOD	-
*C14	0.045	3.05	0.76	0.28	0.07	-
C15	0.08	0.31	0.08	2.22	0.55	-
*C16	0.17	0.70	0.17	1.33	0.33	5.00
C17	0.05	0.22	0.05	4.49	1.12	0.11
*C18	0.155	1.49	0.37	1.96	0.49	4.01
*C19	0.03	0.90	0.22	1.15	<LOD	-
*C20	0.05	1.99	0.49	4.06	<LOD	-

\*Artisanal samples.

data presented in Tables 10, 11, and 12 were subjected to statistical analysis, in order to verify the statistical significance of the concentrations found.

The results of a t-test at 95% confidence level revealed that there were no significant differences between the mean concentrations found in industrial and artisanal samples, in each country. This result may be a consequence of the similarities between the samples studied, in spite of their artisanal or industrial origin.

Since the artisanal or industrial classification of the samples was found to have no statistical significance on the results obtained for each country, the same statistical

test was performed for the mean values found in the whole groups of samples from Brazil and from Cape Verde, no more being considered the classification as artisanal or industrial samples. The results of the t-test are presented in Table 13.

Twenty samples from each country were studied. The critical t-value for 40-2 = 38 degrees of freedom is 1,684 at 95% confidence level.

According to the t-values obtained (Table 13), the differences between the concentrations of chloride, copper, ethyl acetate, and sulphate are statistically significant when Brazilian and Cape Verdean groups of

**Table 12.** Concentrations (mg/L) of anions in samples from Cape Verde.

Sample	Sulphate	RSD%	Acetate	RSD%	Chloride	RSD%	Bicarbonate
*G1	3.91	0.34	2.45	0.01	4.79	-	23.32
G2	11.92	3.42	0.50	0.03	2.16	0.78	105.15
G3	3.46	1.91	2.93	-	2.30	-	<LOD
*G4	4.28	0.81	0.65	0.06	1.00	3.530	<LOD
*G5	6.28	3.12	1.73	0.03	0.48	1.030	7.52
G6	5.36	0.02	1.97	-	0.23	-	<LOD
*G7	12.03	2.05	2.35	0.01	1.56	-	<LOD
G8	5.09	2.89	0.71	0.04	0.69	0.37	<LOD
G9	3.39	0.64	0.65	0.04	4.43	-	4.27
*G10	3.93	0.25	0.74	0.01	1.05	0.35	5.8
G11	3.91	1.60	1.39	0.02	2.13	-	<LOD
G12	3.56	2.97	1.21	0.01	3.13	2.58	4.27
*G13	3.57	3.21	0.88	0.04	0.65	3.76	4.27
G14	5.61	0.71	1.00	0.02	183	-	5.82
G15	4.28	2.03	1.27	0.03	4.91	2.110	4.72
*G16	3.62	4.94	0.83	0.01	1.83	-	4.25
G17	3.25	4.24	0.25	0.03	0.29	4.030	<LOD
*G18	3.09	1.03	2.54	-	4.20	-	4.77
*G19	3.46	2.88	1.31	0.01	1.24	-	5.02
*G20	3.77	3.77	0.92	0.04	1.71	-	5.75

\*Artisanal samples.

**Table 13.** t-Values (95% confidence level) for the values of some constituents of sugar cane spirits from Brazil and Cape Verde.

Compound	t-value
Acetaldehyde	1.25
Ethyl acetate	3.054
Propanol	0.050
Isobutanol	1.45
N-butanol	0.640
Isoamyl alcohol	0.0270
Copper	5.19
Chloride	3.68
Sulfate	8.15

samples are compared to each other.

Figure 3 graphically displays the mean concentrations of volatile compounds, cations, and anions found in the samples studied.

Regarding total alkalinity ( $\text{HCO}_3^-$  and  $\text{CO}_3^{2-}$ ), carbonate was not detected in any of the samples studied. Bicarbonate was found in all of the samples from Cape Verde and in some samples from Brazil, and the results revealed the significance of its concentration when compared to that of other anions.

The presence of acetate affects the quality of sugar cane spirits by causing the production of acids and esters,

for example, ethyl acetate. Relatively low concentrations of acetate were found in all samples.

Acetate is also related to the aroma of sugar cane spirits, and its presence is influenced by the composition of the fermentation medium and the type of yeast used (Ferrer et al., 2005).

The levels of ethyl acetate were significantly higher in the samples from Cape Verde. Its occurrence is probably related to the poor quality of water used throughout the production process.

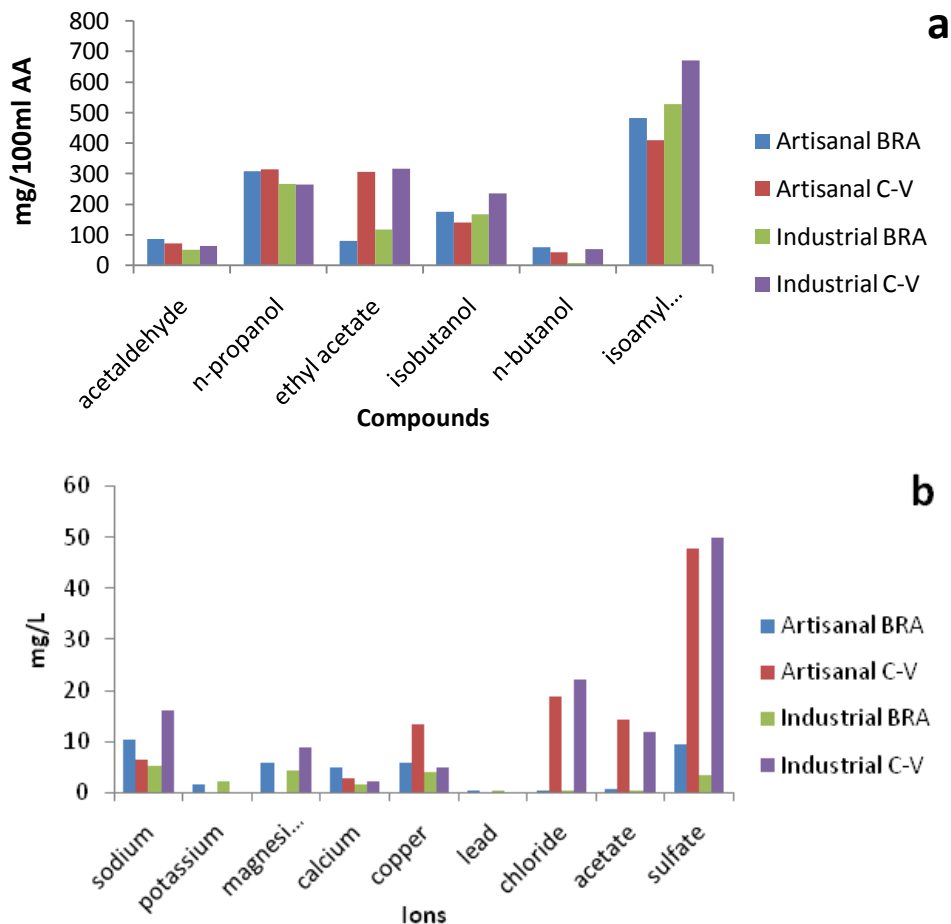
Sulfate was the most concentrated anion in most samples from Cape Verde and its levels in Cape Verdean samples were found to be higher than in Brazilian ones.

An artisanal product from Cape Verde had the highest concentrations of sulphate, acetaldehyde, and ethyl acetate among all samples. It was observed that this sample had a nauseating, irritating aroma (Angelis, 1987).

Chloride was the second and third most concentrated anion in the samples from Cape Verde and Brazil, respectively. The levels of chloride in the samples from Cape Verde were significantly higher than in the samples from Brazil.

The presence of n-butanol, which was detected in samples from both countries, may result from issues during distillation.

Copper concentration was found to be higher than 5.0 mg/L in twelve samples from Cape Verde, and more than twice this value was quantified in six samples from Cape



**Figure 3.** Comparative presentation of mean concentrations of constituents in sugar cane spirits from Brazil and Cape Verde. (a) volatile organic compounds; (b) cations and anions.

Verde and in three samples from Brazil.

These results indicate a potential risk to the health of the consumer, since copper is related to neurological diseases and to the presence of ethyl carbamate, which is a carcinogenic compound (Green et al., 1997). A strategy adopted by producers of sugar cane spirits to lower the levels of copper in the final product is the utilization of stainless steel alembics, thus avoiding the lixiviation of copper during distillation (Weber et al., 2008; Nascimento et al., 1998).

Results presented in this work and elsewhere report that more than 50% of sugar cane spirits from Brazil contain copper levels below the upper limit specified in Brazilian legislation (Mayer et al., 2003; Green et al., 1997; Weber et al., 2008; Nascimento et al., 1998). The difference between the limit concentration for copper allowed in Brazil (5 mg/L) and in the European Union (2 mg/L) may prevent the commercialization of sugar cane spirits from Brazil in a number of countries.

Copper was the most abundant cation in the analyzed samples, as it was found in all of the samples from Cape

Verde and in 70% of the samples from Brazil. Its concentration ranges reported in literature include  $7.6 \times 10^{-3}$  to 5.5 mg/L (Caldas et al., 2009), 0.67 to 6.70 mg/L (Miranda et al., 2010), and 0.55 to 34.0 mg/L (Caldas et al., 2011). In this work, its mean concentration in the samples studied was found to be statistically lower in the samples from Brazil.

The concentrations of lead exceeded the allowed upper limit in four samples from Brazil. Major inequalities between Brazilian and Cape Verdean samples were also observed in mean concentrations acetate ion.

Considering the lack of legal requirements or guidelines for the production of Grogue, these results may be an indication of similarities between the production processes of artisanal and industrial spirits studied.

## Conclusion

Satisfactory precision and accuracy are attained in short times of analysis with the proposed chromatographic

method.

The coefficients of variation calculated for the parameters considered were found to be below the maximum 5% limit required for the method validation. Recoveries were found to be in accordance with the Brazilian legislation.

It was observed that in some samples from Cape Verde the concentrations of copper are much higher than allowed by law, what may result in poor sensorial quality of the product as well as in damages to the consumer's health.

The samples were quite different in composition from one another, especially regarding secondary alcohols and ions, thus resulting in differences in sensorial characteristics.

The fact that significantly lower levels of some undesirable constituents were observed in products from Brazil demonstrates the importance of implementing technical guidelines in production and quality control of sugar cane spirits.

## ACKNOWLEDGEMENTS

The authors are grateful to CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) and CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) for grants and fellowships.

## REFERENCES

- Associação Brasileira de Normas Técnicas (ABNT) (2005). NBR 14029, Agrotóxico e afins – Validação de métodos analíticos. 2nd Ed., Brazil.
- Angelis DF (1987) In: Microbiologia da Fermentação Etanólica. Rio Claro: Unesp, cap. 5, pp. 41-62.
- AOAC (Association of official analytical chemists) (2002). Official methods of analysis. Washington, USA.
- Aquino FWB, Rodrigues S, Nascimento RF, Casimiro ARS (2006). Simultaneous determination of aging markers in sugar cane spirits. *Food Chem.*, 98:569-574.
- Boscolo M, Bezerra CWB, Cardoso DR, Lima Neto BS, Franco DW (2000). Identification and Dosage by HRGC of minor alcohols and ester in Brazilian sugar cane spirit. *J. Braz. Chem. Soc.* 11(1):86-90.
- Brito NM, Junior OP, Polese L, Ribeiro ML (2003). Validação de Métodos Analíticos: Estratégia e Discussão. *R. Ecotoxicol. e Meio Ambiente*, 13:129-146.
- Caldas LFS, Francisco BB, Netto PA, Cassella JR (2011). Multivariate optimization of a spectrophotometric method for copper determination in Brazilian sugar-cane spirits using the Doehlert design. *Microchem. J.* 99:118-124.
- Caldas NM, Oliveira SR, Neto JAG (2009). Feasibility of internal standardization in the direct and simultaneous determination of As, Cu and Pb in sugar-cane spirits by graphite furnace atomic absorption spectrometry. *Anal. Chim. Acta* 636:1-5.
- Campos CR, Silva CF, Dias DR, Basso LC, Amorim HV, Schwan RF (2010). Features of *Saccharomyces cerevisiae* as a culture starter for the production of the distilled sugar cane beverage, cachaça in Brazil. *J. Appl. Microbiol.* 108:1871-1879.
- Crispim JE (2000). Manual de Produção de Aguardente de Qualidade. Livraria e Editora Agropecuária. Guaíba. p. 336.
- EURACHEM (1998). The fitness for purpose of analytical methods; a laboratory guide to method validation and related topics, 1<sup>st</sup> Ed. p. 13-40.
- Expresso das Ilhas (2012). Santo Antão: Legislação sobre a produção do grogue pronta até finais do ano. Webpage <<http://www.expressodasilhas.sapo.cv/pt/noticias/go/santo-antao--legislacao-sobre-a-producao-do-grogue-pronta-ate-finais-do-ano>> – accessed on 2012-05-20.
- Ferrer M, Soliveri J, Plou FJ, López-cortés N, Reyes-duarte D, Christensen, M, Copa-patiño JL, Ballesteros S A (2005). Synthesis of sugar esters in solvent mixtures by lipases from *Thermomyces lanuginosus* and *Candida antarctica* B, and their antimicrobial properties. *Enzyme Microb. Technol.* 36:391-398.
- Galinaro CA, Cardoso D R, Franco DW (2007). Profiles of polycyclic aromatic hydrocarbons in Brazilian sugar cane spirits: discrimination between cachaças produced from non-burned and burned sugar cane crops. *J. Agric. Food Chem.* 55:3141-3147.
- Giudi P, Zambonelli C, Kunkee RE (1993). Increased production of n-propanol in wine by yeast strains having an impaired ability to form hydrogen sulfide. *Am. J. Enol. Viticult.* 44(1):17-21
- Green AM, Clark AC, Scollary GR (1997). Determination of free and total copper and lead in wine by stripping potentiometry. *Fresenius J. Anal. Chem.* 358(6):711-717.
- Janzanti NS (2004). Compostos Voláteis e Qualidade de sabor da Cachaça. Thesis (Doctorate in Food Science) – Faculty of Food Engineering, University of Campinas, Brazil.
- INSTITUTO ADOLFO LUTZ (1976). Normas Analíticas do Instituto Adolfo Lutz. Métodos Químicos e Físicos para a Análise de Alimentos. 2<sup>nd</sup> Ed. v. 1, São Paulo.
- International Standardization Organization (ISO) (1994). Precision of test methods, ISO 5725-2.
- Mayer H, Marconi O, Floridi S, Montanari L, Fantozzi P (2003). Determination of Cu(II) in beer by derivative potentiometric stripping analysis. *J. Inst. Brew.* 109(4):332-336.
- Ministério da Agricultura, Pecuária e Abastecimento (MAPA) (2005). Normative Instruction #13, June 29, Brazil.
- Miranda K, Dionísio AGG, Filho ERP (2010). Copper determination in sugar cane spirits by fast sequential flame atomic absorption spectrometry using internal standardization. *Microchem. J.* 96:99-101.
- Nascimento ESP, Cardoso DR, Franco DW (2008). Quantitative ester analysis in cachaça and distilled spirits by gas chromatography mass spectrometry (GC MS). *J. Agric. Food Chem.* 56:5488-5493.
- Nascimento ESP, Cardoso DR, Franco DW (2009). Comparison of esters determination techniques in cachaça. *Quim. Nova* 32(9):2323-2327.
- Nascimento RF, Cardoso DR, Lima NS, Franco DW (1998). The influence of the material used in building the distiller in the sugar-cane-spirit chemical profile. *Quim. Nova* 21(6):735-743.
- Nascimento RF, Bezerra CWB, Furuya SMB, Schultz MS, Polastro LR, Lima-Neto BS, Franco DW (1999). Mineral profile of Brazilian cachaças and other international spirits. *J. Food Compos. Anal.* 12:17-25.
- Nascimento, RF, Marques, JC, Lima Neto, BS, de Keukeleire, D, Franco, DW (1997). Qualitative and quantitative high-performance liquid chromatographic analysis of aldehydes in Brazilian sugar cane spirits and other distilled alcoholic beverages. *J. Chromatogr. A.* 782:13-23.
- Nykänen L, Nykänen I (1991). Distilled beverages. In: Volatile compounds in food and beverages. pp. 547-580. Maarse H. (ed.), Marcell Dekker Inc., New York.
- Parazzi C, Arthur MC, Lopes CJ, Borges RM (2008). Determination of the main chemical components in Brazilian sugar cane spirit aged in oak (*Quercus* sp.) barrels. *Ciênc. Tecnol. Aliment.* 28(1):193-199.
- Penteado JCP, Masini JC (2009). Heterogeneity of secondary alcohols in Brazilian sugar cane spirits from diverse origins and processes of manufacture. *Quim. Nova* 32(5):1212-1215.
- Silva AP, Santos OJ, Araujo DI, Faria CF, Pereira FA, Oliveira AV, Vicente AM, Brandão LR (2009). Chromatographic evaluation of volatile compounds in Brazilian sugar cane spirits produced with yeasts from different locations. *Ciênc. Tecnol. Aliment* 29(1):100-106.
- Souza LM, Ferreira KS, Passoni LC, Bevitori BA, Melo KV, Viana AR (2009). Organic compounds contents in cachaças produced in the Northern Rio de Janeiro State - RJ. *Quim. Nova* 32(9):2304-2309.
- United States Food and Drugs Administration (US-FDA) (1994). Reviewer Guidance, Validation of Chromatographic Methods.

Rockville. p. 33.  
Vogel AI, Mendham J, Denney RC, Barnes JD, Thomas MJK (2000).  
Vogel's quantitative chemical analysis. 6th Ed. Prentice Hall, New  
Jersey.

Weber JV, Sharypov VI (2008). Ethylcarbamate in foods and  
beverages: a review. Environ. Chem. Lett. 7:233-247.