



Analytical Methods

Brazilian cachaça: “Single shot” typification of fresh alembic and industrial samples via electrospray ionization mass spectrometry fingerprinting

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ABSTRACT

Copper pot stills (alembics) and industrial stainless steel columns are the most common distillation devices used in Brazil to produce sugarcane spirit (cachaça). The use of both apparatus originates two distinctive products, the so-called (a) alembic (the most valuable) and (b) industrial cachaças, respectively, with subtle but characteristic chemical compositions and sensorial properties. Herein, we demonstrate that the use of direct infusion electrospray ionization mass spectrometry in the negative mode, ESI(–)-MS, is able to provide fast “single shot” fingerprinting discrimination between these two types of spirits. Representative samples of both spirits show ESI(–)-MS with regular sets of diagnostic ions of m/z 279, 255, 199, 171, 143 (alembic) and of m/z 377, 341 (industrial). Principal component analysis (PCA) of the ESI(–)-MS data consistently split the 34 alembic and the 14 industrial samples into two well-defined groups. Sugar addition to the alembic samples was also easily detected.

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1. Introduction

Cachaça, the famous Brazilian sugarcane spirit, has received growing attention owing to worldwide acceptance (Nascimento et al., 1999). This alcoholic beverage is obtained from the distillation of the sugarcane fermented must (wine) by employing basically two types of apparatus: homemade copper pot stills (alembics) or industrial stainless steel columns.

When the distillation is conducted in alembics, three fractions (head, heart, and tail) are separated on the basis of their alcoholic contents. The heart fraction, which comprises an alcoholic degree of 38–50 °GL and represents approximately 80–85% of the total volume of the distilled, is commonly called alembic or homemade cachaça (Maia & Campelo, 2006; Piggott & Lea, 2003). After an ageing period in wood casks of at least 1.5 years, such fraction becomes the finest artisan cachaça (Pinheiro, Leal, & de Araujo, 2003). The head and the tail fractions, which make up 15–20% of the total volume of the distilled fraction, are collected when the alcoholic degree achieves 50–70 °GL and 10–38 °GL, respectively (Garcia-Llobodanin, Achaerandio, Ferrando, Guell, & Lopez, 2007). When industrial stainless steel columns are employed, however,

a continuous distillation process yields a unique fraction with an alcoholic degree of 35–65 °GL. Water and commonly sugar is added afterward to this fraction to generate the final product, the so-called column or industrial cachaça, with an alcoholic degree of 38–48 °GL (Reche et al., 2007).

The Minas Gerais State is by far the largest producer of the finest and most valuable artisan cachaça in Brazil and the exportation of such product is now an important item for its economy. The production of artisan cachaça is also directly related to other important economic activities, such as the bovine culture for milk, meat, and organic fertilizer production. Hence, the sugarcane residues (leaves and tips), as well as the tail distillation fraction (known as vinhoto), can be used to feed the cattle in the dry season, when the pastures becomes scarce. The large number of workers required for the alembic cachaça fabrication is also recognized as a factor minimizing the deleterious effects caused by massive migration from rural areas to urban centers in Brazil (Ribeiro, 1997).

For several marketing and economical reasons, therefore, the establishment of a reliable methodology able to quickly discriminate between alembic and industrial cachaças is important and constitute an old claim of the Brazilian producers of artisan cachaça, especially those from the Minas Gerais State. Recently, Reche et al., 2007 have proposed the use of a “multiple shot” approach

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to differentiate between both types of spirits based mainly on a combination of GC–MS, ICP–OES, and HPLC techniques and principal component analysis (PCA).

Direct infusion electrospray ionization mass spectrometry, ESI–MS, (Fenn, Mann, Meng, Wong, & Whitehouse, 1989; Simon, 1997) has been shown to allow fast typification and quality control of several alcoholic beverages and related chemical mixtures (Araujo et al., 2005; Cooper & Marshall, 2001; de Souza et al., 2007a; de Souza et al., 2007b; Mauri & Pietta, 2000; Moller, Catharino, & Eberlin, 2005; Rodgers, Schaub, & Marshall, 2005; Sawaya et al., 2004; Wu, Rodgers, & Marshall, 2004). In the present investigation, we show that direct infusion ESI–MS in the negative ion mode provides fast (less than a minute) “single shot” typification of alembic and industrial cachaças. The clear visual differentiation has also been substantiated by chemometry via principal component (PCA) and hierarchical clustering (HCA) analysis (Arozarena, Casp, Marin, & Navarro, 2000; da Costa et al., 2004; Herranz, Delaserna, Barro, Martín-Álvarez, & Cabezudo, 1989).

2. Materials and methods

2.1. Samples

The 35 alembic cachaça samples were supplied directly by small homemade producers from the following Brazilian States: Minas Gerais (33 samples), Pernambuco (1 sample), and Paraná (1 sample). Such States are located at the Southeastern, Northeastern, and Southern regions of the Brazilian territory, respectively. The samples from the Minas Gerais State were collected by the authors (PPS and HGLS) at production sites located at five different cities. Analyses on the samples produced at the Paraná and Pernambuco States were conducted to evaluate whether the ESI–MS fingerprintings would be considerably affected by quite distinct regional origin. All the alembic cachaça samples were submitted to no ageing processes.

Samples of industrial cachaças (14) were produced in huge distilleries from the São Paulo State (located at the Southeastern region of Brazil). Among them, 13 samples of six representative commercial brands (that encompass more than 70% of the Brazilian annual production) were purchased at local stores. A unique sample was produced via a novel multidistillation process (for more details see: www.sagatiba.com/shell.php) and was supplied directly by the producer. No one of the industrial samples was submitted to ageing process.

2.2. Mass spectrometry

ESI–MS were acquired by using a Q–TOF mass spectrometer (Micromass, Manchester, UK). General conditions were as follows: source temperature of 80 °C, capillary voltage of 2.1 kV and cone voltage of 40 V. Prior to the ESI–MS analysis, 250 µL of an aqueous solution of ammonium hydroxide 0.1% (v/v) was added to 1 mL of each sample and the mixture vigorously stirred for 15 s. Sample introduction was performed by using a micro syringe at a flow rate of 10 µL min⁻¹, and pumped through an uncoated fused-silica capillary. Each analysis required about 60 s and the mass spectra were scanned in the *m/z* 50–500 range.

To verify the consistency of the ESI–MS records, analyses were additionally performed by using a mass spectrometer with a distinct configuration (Agilent Ion Trap XCT, Santa Clara, CA). The samples were submitted to an identical treatment as previously stated and then directly infused (by a micro syringe) into the ESI source of the XCT instrument (adjusted to operate in the negative ion mode) at a flow rate of 10 µL min⁻¹. ESI source conditions were as follows: heated capillary temperature 200 °C; sheath gas (N₂)

flow rate 6 L min⁻¹; spray voltage 3.5 kV; tube lens offset voltage 25 V. The *m/z* range used in all experiments was also 50–500.

2.3. Chemometric data handling

Multivariate analyses by PCA were performed by running the software MATLAB, version 6.1. The ESI–MS experimental data were compiled to generate a final matrix of 49 objects (samples) and 106 variables (*m/z* values with their respective relative intensities). To remove the noise signals, only ions with a relative abundance higher than 10% were considered for the final data matrix. These data were previously mean-centered and autoscaled to variance 1, aimed at ensuring that all variables contributed equally to the model, independent on the scale in which they were measured.

3. Results and discussion

3.1. General remarks

Although ESI(+)-MS (positive ion mode) were also acquired, only ESI(-)-MS (negative ion mode) yielded fingerprints able to distinguish between both types of spirits. Therefore, only the ESI(-)-MS data is presented and discussed. Additionally, both the QTOF and XCT mass spectrometers furnished quite similar ESI(-)-MS fingerprints; hence only data obtained via the Q-TOF will be shown.

3.2. ESI(-)-MS fingerprinting

Fig. 1 shows the ESI(-)-MS of three typical (fresh) alembic cachaças. As a common feature, the spectra of all the 35 samples exhibit abundant ions of *m/z* 143, 171, 199, 255, 279, and a minor ion of *m/z* 341. We have characterized those of *m/z* 143, 171, 199 as RCO₂⁻ anions, that is, the deprotonated forms of linear and saturated C₈, C₁₀ and C₁₂ carboxylic acids, respectively. The anions of *m/z* 255 and 341 are probably the deprotonated forms of the liquoritigenin flavonoid and sucrose, respectively (de Souza et al., 2007b). Hence, despite the quite distinct geographic regions where the samples were collected and the less-controlled and non-standardized conditions employed in their fabrication, the ESI(-)-MS of the every 35 alembic cachaças were found to be rather similar and thus characteristic.

Fig. 2 shows three examples of ESI(-)-MS of typical industrial cachaças. For these and for all the spectra of the 14 samples collected, a common aspect is clearly recognized: the great predominance of the ions of *m/z* 341, [sucrose – H]⁻, and *m/z* 377 and 379, ascribed to [sucrose + Cl]⁻. This chloride adducts is promptly recognized by the typical 3:1 abundance ratio of the isotopologue anions of *m/z* 377 and 379. The ESI formation of chloride adducts of disaccharides is common and have been proposed by Cole and coworkers (Zhu and Cole (2001)) to result from non-covalent interactions between chloride and the hydroxyl moieties of the disaccharides and other analogous molecules. Among the samples of the industrial cachaças evaluated herein, one was known to be unsweetened (no sugar addition) since it was collected by one of us directly at the distillery as a freshly-distillate spirit. Sugar is frequently added to the industrial cachaças to improve their sensorial properties, a practice admitted by the Brazilian regulatory agencies (Maia et al., 2006; Pinheiro et al., 2003; Ribeiro, 1997). The other samples, purchased at liquor stores, are sweetened industrial cachaças as indicated by their labels, and had been spiked at the production sites with sugar at concentrations near to 6 g L⁻¹ (the maximum level allowed by the current Brazilian legislation). Fig. 2a and b/c show the ESI(-)-MS of the unsweetened and of two typical commercial sweetened industrial cachaça samples,

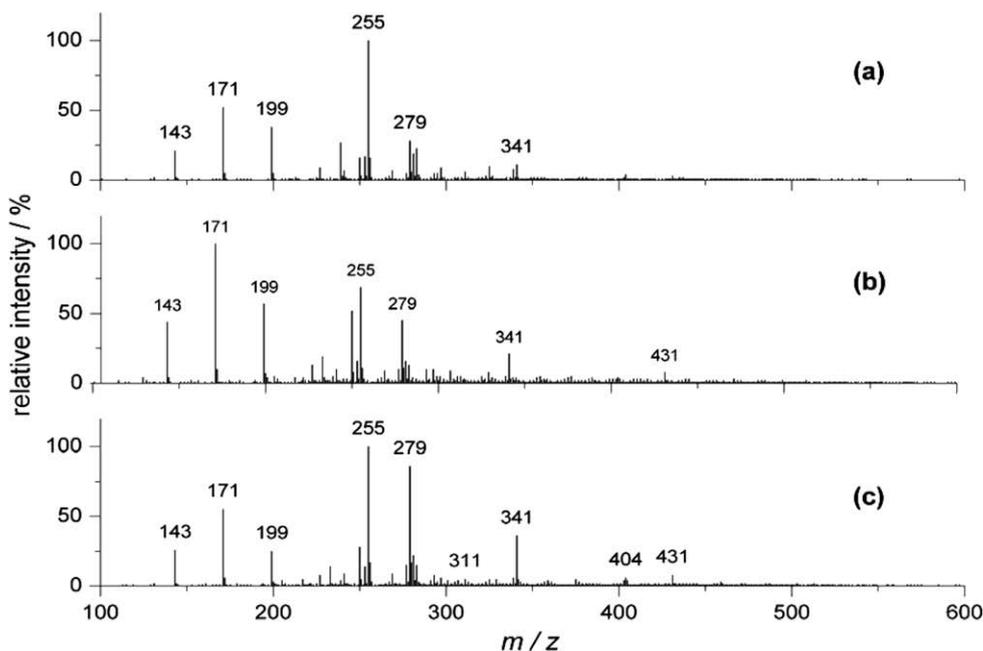


Fig. 1. ESI(–)-MS of three fresh unsweetened typical alembic cachaças obtained upon distillation in alembics (copper pot stills).

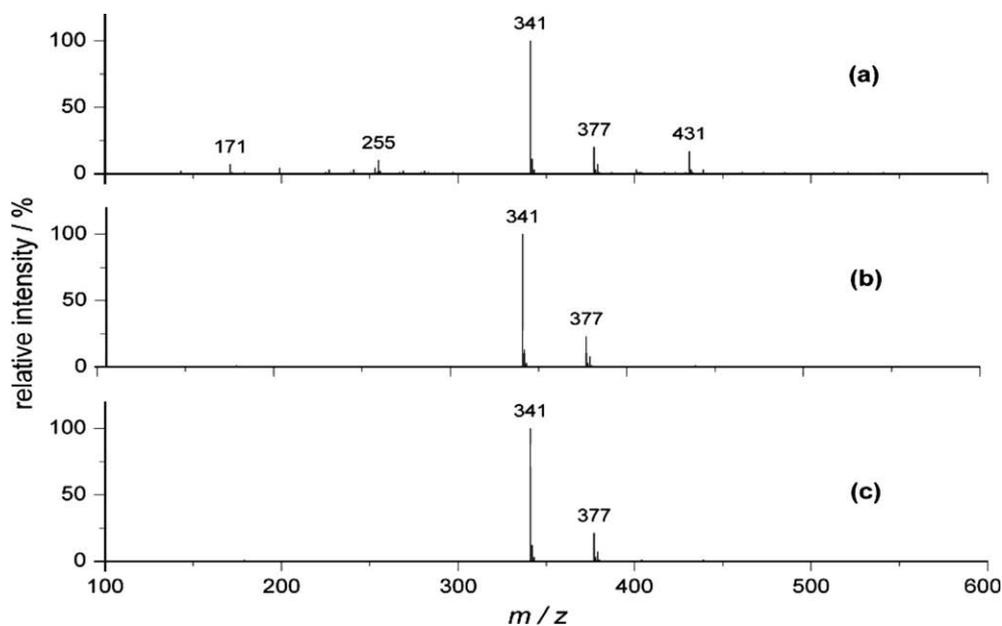


Fig. 2. ESI(–)-MS of three industrial cachaça samples obtained upon distillation in stainless steel columns. Sample (a) is unsweetened and was collected directly at the distillery. Samples (b) and (c) are sweetened samples acquired at local liquor stores.

respectively. As expected, the ESI(–)-MS of the sweetened samples show predominance of the diagnostic anions for sucrose, i.e. [sucrose – H][–] of m/z 341 and [sucrose + Cl][–] of m/z 377 and 379 (3:1). Note that in the ESI(–)-MS of the unsweetened sample (Fig. 2a), the anions of m/z 341 and 377/379, diagnostic for sucrose, are surprisingly still dominant. The trace amounts of sucrose in this spirit, probably derived from an incomplete must fermentation and not entirely removed by the distillation process, could be detected by the highly-sensitive ESI(–)-MS. In addition, a careful comparison between the mass spectra of the typical alembic spirits (Fig. 1), where the diagnostic anions of sucrose are surely no dominants, and the unsweetened industrial sample (Fig. 2a), allows one to assume that stainless steel distillers are not as efficient as cop-

per alembics in promoting the degradation of sucrose during distillation. Note also that other anions of m/z 171 and 255, besides the anion of m/z 431 (see a brief discussion on this anion below), are minor but noticeable in Fig. 2a. In summary, both types of unsweetened and sweetened industrial cachaças display quite similar, nearly undistinguishable ESI(–)-MS fingerprints.

Figs. 1 and 2 demonstrate, therefore, that ESI(–)-MS fingerprints of the alembic and industrial cachaças are quite reproducible and distinct, each type displaying characteristically a series of diagnostic anions. The greater number of ions observed in the ESI(–)-MS of the alembic cachaças seems to be related to the presence of a greater quantity of polar components. This greater number of ions could result from selective reactions induced by

the copper surface during distillation. Such reactions, therefore, should not occur when stainless steel columns are employed. Higher distillation efficiency of the stainless steel columns as compared to the copper alembics may also contribute (Boza & Horii, 2000; Bruno, Vaitsman, Kunigami, & Brasil, 2007; Cardoso, Lima-Neto, Franco, & do Nascimento, 2003). Hence, the methodology described herein is able to quickly discriminate between alembic samples from sweetened/unsweetened industrial spirits via a simple visual inspection of the respective ESI–MS.

To verify whether the addition of sugar to alembic cachaças (a procedure that alembic producers claim not to adopt at all) could be noticed, a typical alembic cachaça was spiked with sugar at the maximum level allowed by the regulatory agencies (6 g L^{-1}) (Piggott & Lea, 2003). The resulting ESI(–)–MS (Fig. 3) clearly shows that the diagnostic ions that characterize alembic samples (m/z 143, 171, 199, 255, and 279) are now completely suppressed by sugar addition whereas intense ions of m/z 341 (deprotonated sucrose), 404, and 431 shows up. The ions of m/z 404 and 431 are interesting and diagnostic since they correspond to be nitrate (NO_3^-) and oxalate ($\text{C}_2\text{O}_4^{2-}$) adducts of sucrose, that is: $[\text{sucrose} + \text{NO}_3]^-$ of m/z 404 and $[\text{sucrose} + \text{H} + \text{C}_2\text{O}_4]^-$ of m/z 431. These ions are also detectable, but with much minor relative abundances, in the genuine alembic samples (Fig. 1). Probably, nitrate and oxalate anions are less efficiently removed from the spirit upon alembic distillation. The diagnostic absence of the $[\text{sucrose} + \text{Cl}]^-$ adduct of m/z 377/379 in the ESI(–)–MS of Fig. 3, contrarily to that observed in the ESI(–)–MS of both the sweetened and unsweetened industrial cachaças (Fig. 2), can be rationalized by the favorable formation of Cl^- and Cu^{2+} complexes (generated under alembic distillation by oxidation of metallic copper), such as CuCl^+ and CuCl_2 . These complexes could sequester Cl^- from the solution and thus suppress the formation of the $[\text{sucrose} + \text{Cl}]^-$ adduct. Therefore, the relatively high abundance of the isotopologue anions of m/z 377 and 379 from $[\text{sucrose} + \text{Cl}]^-$ are diagnostic of sweetened and unsweetened industrial cachaças and are nearly absent in the ESI(–)–MS of the alembic cachaças (compare Fig. 2 with Figs. 1 and 3). Adulteration of alembic cachaças by sugar addition can also be promptly recognized by a simple visual comparison of the ESI(–)–MS of both the genuine and counterfeit spirits (Figs. 1 and 3, respectively).

3.3. Chemometry

Although simple visual inspection of the ESI(–)–MS fingerprints allows secure distinction, the data were also treated (and better evaluated) by using the PCA methodology. Fig. 4 shows the PCA scores plot clearly splitting the alembic and industrial samples into two distinct groups. The PC1 and PC2 were found to account for 63.56% and 12.92% of the total variance, respectively. Therefore, the total variance explained by the two first PC's is 76.48% at a confidence level of 95%, see dot line in Fig. 4. Note also that the 14

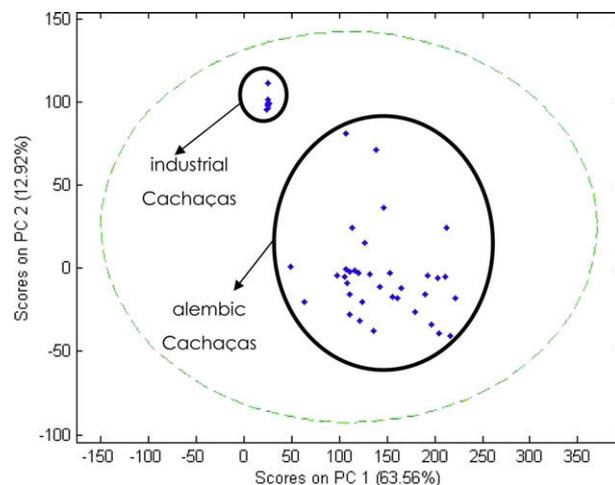


Fig. 4. PCA score plot (PC1 \times PC2) for the ESI(–)–MS data of the alembic and industrial cachaças.

industrial cachaça samples (including the sugar-free sample) seem to form a more uniform group in comparison to the cluster formed by the alembic cachaças. This is probably because the production of industrial cachaças makes use of much more uniform and controlled experimental conditions than those regularly employed for the alembic spirits manufacture (Maia et al., 2006; Piggott & Lea, 2003; Reche et al., 2007). Finally note that in the PCA treatment no clear separation among samples as a function of the geographical region of production could be noted.

4. Conclusions

Direct infusion ESI(–)–MS provides “single shot” distinction of the two major types of Brazilian cachaças. ESI(–)–MS is reproducible, requires no sample preparation or pre-separation, and is very fast (less than 1 min/sample). Furthermore, the consistency and reproducibility of the method presented herein were also demonstrated as a given sample furnished quite similar mass spectra when acquired in different days and even when different mass spectrometers were used. This is a fortunate result as the differentiation between these samples is important from the economical point of view. This method can assist therefore the Brazilian producers to certify their distinct product as an authentic (and more commercially valuable) alembic cachaça. ESI(–)–MS fingerprinting can also be used by the alembic cachaça producers to fabricate spirits within pre-established quality parameters and to identify undesirable practices such as sugar addition. As we have shown before (de Souza et al., 2007b), ageing parameters can also be properly monitored.

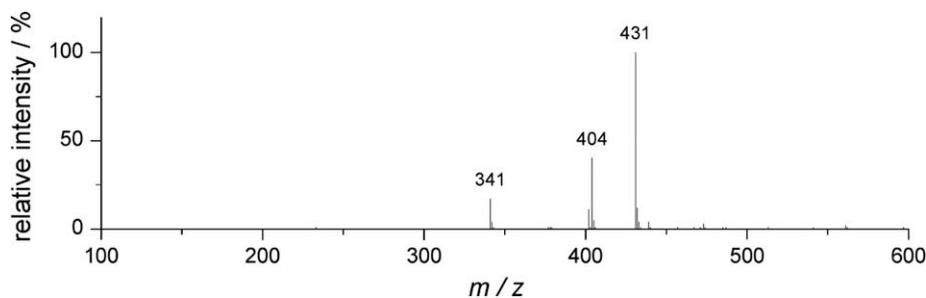


Fig. 3. ESI(–)–MS of a fresh typical alembic cachaça sample spiked with sugar at a concentration of 6 g L^{-1} .

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