Artificially-aged cachaça samples characterised by direct infusion electrospray ionisation mass spectrometry

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A B S T R A C T

Direct infusion electrospray ionisation mass spectrometry in the negative ion mode [ESI(−)-MS] was employed to evaluate the authenticity of aged cachaças, a traditional and valuable Brazilian alcoholic beverage prepared from the distillation of brewed sugarcane juice and aged in barrels made of common woods. Counterfeit samples were prepared by adding dyes, sawdust or essences to a freshly-distilled, much less valuable sample (white cachaça) to simulate the 1–2 years long natural ageing in wooden barrels. A simple visual inspection revealed remarkable differences between the ESI(−)-MS of the authentic samples (aged in oak or amburana casks) and the artificially-aged counterfeit samples. A set of diagnostic ions were detectable in the ESI(−)-MS of the authentic samples aged in oak (m/z 197, 241, 301 and 307) and amburana (m/z 271 and 377/379). This fast and direct methodology seems useful as a routine procedure to monitor this highly profitable and common counterfeit practice.

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1. Introduction

Cachaça, the most traditional Brazilian alcoholic beverage, is made by the distillation of brewed sugarcane juice. In Brazil, Minas Gerais is the state with the highest production, but several other regions are also becoming large producers (Badotti, Belloch, Rosa, Barrio, & Querol, 2010). Cachaça can be commercialised right after distillation (white cachaça) but may also be aged in wooden barrels. During ageing, the alcohol content is reduced and numerous chemical reactions occur leading to the enhancement of the organoleptic properties of cachaça. The colour also changes since the beverage becomes enriched with chemicals extracted from the wood. The nature and concentration of such compounds are therefore related to the type of wood and the ageing time (de Souza et al., 2007). The ageing process also reduces defects introduced during fermentation (mainly the formation of an excessive amount of acidic compounds) and distillation, is crucial to improve palatability, and causes the attainment of a beverage with much higher market value as well as notable appreciation. Oak casks have been extensively and preferentially employed in cachaça ageing due mainly to their well-known and valuable characteristics. But since oak has become scarce, other woods have been alternatively used, such as amburana (Amburana cearensis), balsam (Myroxylon peruiferaum), jequitibá (Cariniana estrellensis), oak (Quercus spp.), jatobá (Hymenaea spp.), and ipê (Tabebuia spp.) (Lea & Piggott, 2003).

The current Brazilian legislation requires that aged cachaças must undergo a period of at least one year in wooden barrels (Ministry of Agriculture Livestock and Food Supply of Brazil, 2005). The Brazilian legislation also prohibits the use of any sort of dyes, extracts, sawdust or other substances for correction or modification of the original colour of the stored or aged product. It is also forbidden to add any substance or ingredient that alters the natural sensory characteristics of the final product, excepting caramel that is used for colour correction/standardization (Ministry of Agriculture Livestock and Food Supply of Brazil, 2007). Despite these restrictive directives, counterfeiters have usually made use of sawdust to simulate ageing in oak casks and therefore to illicitly produce a beverage with much higher commercial value (Sanza, Dominguez, & Merino, 2004).

Direct infusion electrospray ionisation mass spectrometry (ESI-MS) is a powerful, fast and robust technique that in recent years has been employed for the chemical typification of alcoholic beverages, such as beer, wine, whisky and sake (Araujo et al., 2005;
Artificially-aged samples of cachaça aged in oak and amburana barrels were collected directly at local trustworthy producers (9 of each group) from several locations of the State of Minas Gerais and used as control. The mass spectra of the samples within each group showed to be quite similar. Because of that, only one mass spectrum (characteristic of each group) was selected as illustrative example to be displayed in the present manuscript.

2.2. Mass spectrometry

Mass spectra 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. To avoid cross-interference between samples, HPLC-grade methanol (Merck, Germany) was injected between each infusion. Each analysis required about 60 s and the mass spectra were scanned in the m/z 50–500 range.

3. Results and discussion

Although ESI(+)–MS were also acquired, only ESI(−)–MS (negative ion mode) yielded fingerprints able to characterise all the types of adulteration described in this paper. Therefore, only the ESI(−)–MS data is presented and discussed herein.

3.1. Differentiating authentic and counterfeit samples

Fig. 2 shows that the ESI(−)–MS of an authentic cachaça sample (aged in oak cask) and a counterfeit sample (artificially-aged with oak essence) are remarkably different. Whereas for the authentic cachaça a quite complex ESI(−)–MS is recorded (Fig. 2a), in which the ions of m/z 197, 241, 301 and 307 are predominant, the counterfeit sample yields a ESI(−)–MS with a much less diverse set of diagnostic ions, mainly those of m/z 255, 283 and 301 (Fig. 2d). The ESI(−)–MS of the freshly-distilled white cachaça (Fig. 2b, major diagnostic ions of m/z 255 and 283) and that of an aqueous solution of the oak essence (Fig. 2c, m/z 255 and 301) undoubtedly reveal the origin of the ions observed in the ESI(−)–MS of the artificially-aged sample (Fig. 2d). The quite distinct mass spectra recorded for the authentic and counterfeit samples can be
explained considering that during the 1 year-long natural ageing time in the oak cask, a variety of compounds is continuously and slowly extracted from the inner surface of the cask or slowly formed via a series of chemical reactions. Recent reports have also shown that the nature and concentration of many chemicals in cachaça and other aged spirits are related to the type of wooden cask and period of ageing (Mosedale & Puech, 1998).

Since oak has become increasingly rare and expensive, producers are looking for alternative woods, and amburana (a native tree from Brazil) has become a common and highly appreciate wood for cachaça ageing casks. Counterfeit samples of cachaça that simulate ageing in amburana casks were therefore also tested. The ESI(−)-MS of the counterfeit sample (Fig. 3c) prepared by adding amburana essence to the white cachaça displays very abundant ions of m/z 195, 255, 281, and 341, whereas ions of m/z 271, 283, 313 and 377 dominate the ESI(−)-MS of the authentic cachaça sample aged for 1 year in amburana casks (Fig. 3a). The ions of m/z 195, 281, and 341 are therefore promptly associated to the use of amburana essence (Fig. 3b and c). Although less common, counterfeiting of aged cachaça using other types of wood essences such as jequitiba (C. estrellensis) and balm (M. peruiferum), which also display very characteristic sets of diagnostic ions (de Souza et al., 2007), could also be promptly recognised by ESI(−)-MS fingerprinting.

Fig. 2. ESI(−)-MS of: (a) cachaça aged for 1 year in an oak barrel; (b) freshly-distilled white cachaça; (c) aqueous solution of oak essence; (d) white cachaça spiked with the oak essence. The inset in (a) refers to the product ion mass spectra (MS/MS) of the ion of m/z 255. Similar MS/MS were recorded for this ion arising from all other samples.

Fig. 3. ESI(−)-MS of: (a) cachaça aged for 1 year in an amburana barrel; (b) aqueous solution of amburana essence; (c) white cachaça spiked with the amburana essence.
Different chemical profiles as compared to natural ageing have also been found for other spirits artificially aged via the use of wood sawdust. For instance, Sanza and coworkers (Sanza et al., 2004) found that Spanish red wine in contact with oak sawdust experienced a quicker ageing (and also a less diverse chemical composition) in comparison to the traditional ageing process in wood barrels. Morales and coworkers (Morales, Benitez, & Troncoso, 2004) also observed a remarkable increase in the concentration of vanillin when wine vinegars were aged in the presence of oak sawdust (for 15 days) in comparison with the traditional ageing in oak barrels for 180 days. For this counterfeit method, ESI(−)-MS has also been found to provide proper monitoring. Hence, the ESI(−)-MS of the white cachaça sacked with oak sawdust (Fig. 4) is promptly distinguishable from the authentic sample aged for 1 year (Fig. 2a). Note the presence of a possible diagnostic ion (m/z 191) in the mass spectrum of the counterfeit sample (Fig. 4) that can possibly be used to characterise this type of adulteration. For this sample, the fast and less selective extraction of compounds from the sawdust evidently lead to contrasting chemical profile in comparison to the genuine aged sample (Fig. 2a).

The ability of ESI-MS to detect the addition of illegal dyes to simulate natural ageing was also investigated. The yellow and brown dyes, the two most common dyes used for this purpose, were therefore added to the fresh white cachaça (Fig. 5a and b). Note in the spectra a series of ions (m/z 171, 172, 198 and m/z 195, 243, 367 for the brown and yellow dyes, respectively) that clearly serve as diagnostic for the use of each dye. Note that these mass spectra can be promptly distinguished of the mass spectra of the genuine samples, aged either in oak (Fig. 2a) or in amburana (Fig. 3a) casks.

3.2. Identification of diagnostic ions and other remarkable details of the mass spectra

As above demonstrated, the identification of the compounds that yield the respective diagnostic ions in the mass spectra of all samples (cachaças and aqueous solutions of essences) is not mandatory to a classification purpose. However, possible attributions for the main ions detected in each mass spectrum (Table 1) are proposed based on previous investigations reported in the literature. For instance, the ion of m/z 255 was proposed to be the deprotonated form of palmitic acid. This ion was detected in the following mass spectra: cachaça aged in oak cask for one year (Fig. 2a), freshly-distilled white cachaça (Fig. 2b), aqueous solution of oak essence (Fig. 2c), and in several counterfeit cachaças (with oak essence, Fig. 2d, with amburana essence, Fig. 3c, and with yellow dye, Fig. 5b). Furthermore, as all samples yield ions of m/z 255 that fragmented to generate almost identical profiles (see, for instance, the inset of Fig. 2a), one can reasonably concluded that palmitic acid was present in all of these samples. The fact that this ion is also detected in the MS of the sample aged in oak barrel (Fig. 2a) indicates that palmitic acid was not completely depleted during the ageing time.

Note that the ion of m/z 283, ascribed to be the deprotonated form of stearic acid (Table 1), is also typical of freshly-distilled cachaca (Fig. 2b). This ion was, however, undetectable in the sample adulterated with the amburana essence (Fig. 3c). This apparently inconsistent result can be explained taking into account that the compounds from the amburana essence are possibly much more acid than those of the white cachaça. As a consequence, the compounds from the amburana essence are preferentially ionised and therefore suppress the ionisation of the compounds (including stearic acid) from white cachaca. This effect, known as ionisation suppression, is quite common in ESI and has been profusely reported in the literature. (Gosetti, Mazzucco, Zampieri, & Gennaro, 2010) Another relevant aspect observed in the mass spectra of these samples is that the ion of m/z 281, the deprotonated form of oleic acid (Table 1) typical of oak (Fig. 2c and d) and amburana (Fig. 3b and c) essences was not detected in the authentic samples aged in oak (Fig. 2a) and amburana (Fig. 3a) barrels. This finding can be conveniently rationalised considering that the ageing process could cause the total depletion of oleic acid or that the
products generated after the ageing time could suppress the ionisation of such a compound. The same sort of arguments can be postulated to justify the presence of the ion of m/z 191 (deprotonated form of triminioacid) in the ESI (−)-MS of the white cachaca sacked with oak sawdust (Fig. 4) whereas in the ESI (+)-MS of the spirit aged for one year in an oak barrel (Fig. 2a) this ion is not detected at all.

4. Conclusions

Direct infusion ESI (−)–MS is shown to function as an efficient method to monitor aged cachaca, not only to identify the type of wood used in the ageing process but also to detect illegal and quite common practices of artificially ageing, such as the addition of essences, dyes and sawdust to the fresh product. ESI (−)–MS therefore provides a simple, fast and direct method to certify the origin and ageing of cachaca based on a diverse and hard to imitate set of diagnostic ions that guarantee the superior organoleptical properties of the highly valuable and appreciated aged cachaca produced in Brazil.

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