Food Research International 60 (2014) 212–217

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Feasibility of different chemometric techniques to differentiate commercial Brazilian sugarcane spirits based on chemical markers

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ARTICLE INFO

Article history:
Received 21 May 2013
Revised 21 September 2013
Accepted 27 September 2013
Available online 4 October 2013

Keywords:
Hierarchical cluster analysis
Principal component analysis
Linear discriminant analysis
Contaminants
Cachaça

ABSTRACT

Chemometric techniques were used to assess the quality of 51 commercial Brazilian sugarcane spirits (*cachaça*) based on chemical markers. Benzo(a)pyrene, methanol, 2,3-methyl-1-butanol, acetaldehyde, isobutyl alcohol, n-propanol, density, alcoholic strength, and higher alcohols were quantified using chromatographic methods and results were subjected to Principal Component Analysis (PCA), Hierarchical Cluster Analysis (HCA), and Linear Discriminant Analysis (LDA). No significant differences ($p \geq 0.071$) were observed in the chemical markers of Brazilian aged and non-aged *cachaça* samples. Besides non-significant ($p = 0.922$), the content of benzo(a) pyrene in aged sugarcane spirits was 1.83 times higher than in non-aged ones. Differences in alcoholic strength ($p = 0.001$) and n-propanol ($p = 0.015$) were observed among *cachaças* produced by double distilling, alembic and in stainless steel columns. PCA was not suitable to separate the samples according to the provenance, aging and distilling process, while HCA was effective in separating alembic *cachaças* produced by from two distinct producing regions. LDA seemed to be very suitable to assess not only the provenance but also the distilling and aging processes that *cachaça* undergoes, yielding about 91% accuracy to discriminate non-aged from aged *cachaça*, 81.82% and 86.61% accuracy to discriminate samples from Minas Gerais and São Paulo, respectively.

1. Introduction

Sugarcane spirit (*cachaça*) is a typical Brazilian alcoholic beverage obtained from the distillation of fermented sugarcane juice, and its alcoholic strength varies from 38 to 48 mL·100 mL^{-1} at 20 °C (MAPA, 2005). After beer, *cachaça* is the most consumed alcoholic beverage in Brazil (ABRABE, 2013), and its annual production reaches up to 1.2 billion liters; however, only 1% of this total is exported, mainly to Germany, United States, Portugal and France. *Cachaça* is widely used for the preparation of *caipirinha* (lemon juice added with ice and *cachaça*) and also some dishes (MAPA, 2005).

In Brazil, there are two main processes to produce sugarcane spirits: alembic or industrial *cachaça*. In accordance with Souza et al. (2009), when the distillation process is performed in alembics, three main fractions are separated on the basis of their alcoholic contents: the head, heart, and tail. The heart fraction presents an alcoholic degree of 38–50% (v/v) and represents approximately 80–85% (v/v) of the total volume of the distilled, and is commonly known as alembic. When industrial stainless steel columns are employed, a continuous distillation process yields a homogeneous fraction with an alcoholic degree of 35–65% (v/v). Generally, *cachaça* produced by copper pot stills (alembics) is obtained from small field crops, where the cane harvest is manual, while industrial *cachaças* (distilled in stainless steel columns) are obtained from field crops, usually with mechanized harvesting after burning the straw (Caruso, Nagato, & Alaburda, 2010).

Ethanol is the main product of fermentation of sugar cane; however, the minority or secondary compounds (volatile acids, aldehydes, esters and other types of alcohol) are responsible for the characteristic aroma and flavor of the beverage. These compounds are produced by the degradation of some amino acids and once they have a considerable molecular weight, they are concentrated mainly in the "tail". According to the Normative Instruction n° 13/05 (MAPA, 2005), higher alcohols present in *cachaças* can be estimated by summing the content of n-propanol, 2-methyl-1-butanol, 3-methyl-1-butanol, and isobutanol, and the maximum allowed concentration is 360 mg·100 mL^{-1} of absolute alcohol.

Table 1
The limit of quantification, limit of detection, regression equation and the coefficients of determination for the chemical markers of Brazilian sugarcane spirits.

Chemical markers	LOQ	LOD	Regression equation	R^2
Benzo(a)pyrene (ng · mL^{-1})	0.1	0.03	y = 331,713 × −626	0.9995
Methanol (mg · 100 mL^{-1} AA)	2.0	0.67	y = 0.4723 × −0.0061	0.9998
Acetaldehyde (mg · 100 mL^{-1} AA)	4.0	1.33	y = 0.1008 × −0.0008	0.9994
n-Propanol (mg · 100 mL^{-1} AA)	5.0	1.67	y = 0.8608 × −0.0272	0.9985
Isobutanol (mg · 100 mL^{-1} AA)	5.0	1.67	y = 1.0342 × −0.0172	0.9994
2,3-Methylbutanol (mg · 100 mL^{-1} AA)	6.0	2.00	y = 1.0621 × −0.0221	0.9993

Note: LOQ = limit of quantification; LOD = limit of detection.

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http://dx.doi.org/10.1016/j.foodres.2013.09.044

Table 2
Statistical comparison between aged and non-aged *cachaças* marketed in Brazil.

Chemical markers	Aged *cachaça* (n = 18)	Non-aged *cachaça* (n = 33)	p-Value[a]
Benzo(a)pyrene (ng · mL^{-1})	0.0897 (0.0656)	0.0489 (0.1718)	0.922
Alcoholic strength at 20 °C (mL · 100 mL^{-1})	40.45 (3.15)	40.18 (2.73)	0.758
Methanol (mg · 100 mL^{-1} AA)	5.02 (1.93)	5.68 (1.29)	0.292
Acetaldehyde (mg · 100 mL^{-1} AA)	32.61 (15.90)	43.01 (23.10)	0.071
n-Propanol (mg · 100 mL^{-1} AA)	85.12 (96.03)	76.98 (67.71)	0.514
Isobutyl alcohol (mg · 100 mL^{-1} AA)	47.05 (12.49)	49.88 (17.23)	0.503
2,3-Methyl-1-butanol (mg · 100 mL^{-1} AA)	142.20 (28.92)	159.83 (53.29)	0.484
Higher alcohols (mg · 100 mL^{-1} AA)	274.37 (100.14)	286.69 (91.14)	0.588

Note: Values expressed as mean (SD). AA: absolute alcohol.
[a] Probability values obtained by Student's-t test for independent samples or Mann–Whitney test.

Methanol is produced in small concentrations during fermentation because of the hydrolysis of pectins, and its content is normally below 20 mg·100 mL^{-1} absolute alcohol (MAPA, 2005). The benzo(a)pyrene (BaP) is a polycyclic aromatic hydrocarbon compound that has been considered as carcinogenic to humans (IARC, 2012). Its formation can be associated with the incomplete combustion of organic matter and its presence in *cachaças* can be explained by several factors: production during the burning of sugarcane; by contamination during the period in which the industrial *cachaças* are stored in tanks coated with asphalt resins while waiting to be bottled; or by contamination during the aging process in wood casks (previously burned almost up to carbonization) (Caruso & Alaburda, 2009). Although the evaluation of BaP in *cachaças* is demanding to assure the safety of such products, there is no specific legislation in Brazil regarding the maximum content of BaP in *cachaça*.

The statistical evaluation of chemical, sensory and other quality parameters of a wide range of foodstuffs, ingredients and beverages by using multivariate techniques is well recognized and accepted worldwide. These techniques have gained much attention and have been more widely used in Food Science and Technology because they provide not only a better visualization and interpretation of experimental data but also they allow the analyst to draw more conclusive assumptions of some food components when many samples are evaluated (Pereira et al., 2012; Souza et al., 2011). In this sense, the objective of this study was to evaluate the feasibility of different chemometric techniques in assessing the provenance, type of aging and distilling processes of commercial Brazilian sugarcane spirits based on chemical markers.

2. Material and methods

2.1. Sampling

Commercial *cachaça* samples (n = 51) from different distilling processes, producing regions and aging, were acquired in the commerce of São Paulo, Brazil. A total of 51 samples of different batches and/or brands were analyzed, in which 33 were non-aged and 18 were aged *cachaças*. With regard to the producing region, samples from São Paulo (n = 23), Minas Gerais (n = 22), Mato Grosso (n = 2), Ceará (n = 2) and Pernambuco (n = 2) were analyzed. It is noteworthy that São Paulo and Minas Gerais are the largest Brazilian producers of sugarcane spirits.

2.2. Chemicals

Ethyl alcohol (Merck, Germany), methanol (Merck, Germany), acetaldehyde (Fluka, USA), n-propanol and isobutyl alcohol (Sigma, USA), 3-pentanol (Merk, Germany), 2-methyl-1-butanol (Aldrich, USA), 3-methyl-1-butanol (Aldrich, USA), Benzo(a)pyrene (BaP) (Sigma Aldrich, USA), cyclohexane (Merk, Germany), and acetonitrile (J.T. Baker, USA) were used in the experiment.

2.3. Analytical determinations

The alcoholic strength was obtained from the conversion of the value of relative density at 20 °C/20 °C in alcoholic strength volume percent using a digital densimeter (Mettler DA-300, Brazil) (Nagato, Caruso, Duran, Carvalho, & Cano, 2005).

The contents of methanol, acetaldehyde, n-propanol, isobutanol, 2-methyl-1-butanol, and 3-methyl-1-butanol were quantified in triplicate according to the procedures outlined by Nagato et al. (2005) by gas chromatography coupled with a flame ionization detector (model 9001GC, Finnigan, USA), using hydrogen as gas carrier and a capillary column (CP-Wax 52 CB, 30 m × 0.25 mm × 0.25 μm, Varian, Australia). A calibration curve for internal standardization employing 3-pentanol as internal standard was built and used for quantification. Higher alcohol contents were estimated by the sum of n-propanol, isobutanol, 2-methyl-1-butanol and 3-methyl-1-butanol. Results were expressed as mg·100 mL^{-1} of absolute alcohol (AA). The solutions of chemical compounds were prepared using ethyl alcohol.

Benzo(a)pyrene (BaP), which is an organic contaminant present in sugarcane spirits, was extracted by solid phase extraction with cyclohexane in a Accubond SPE C-18 cartridge (500 mg, 6 mL) and quantification was carried out, in triplicate, using an HPLC system coupled with a fluorescence detector (Shimadzu, Japan) set at λ_{exc} = 295 and λ_{em} = 405 nm. The system was composed of a reversed-phase C-18 column (Varian, Australia, 250 mm × 4.6 mm × 5 μm) and a guard column C-18 (Varian, Australia, 20 mm × 4.6 mm × 5 μm). The mobile phase was composed of acetonitrile:water (70:30) with isocratic elution. The BaP quantification was performed by a calibration curve with a high-purity BaP standard.

The limit of quantification, regression equation and the coefficients of determination of the selected chemical markers evaluated in this study are presented in Table 1.

Table 3
Statistical comparison among sugarcane spirits produced by double distilling, alembic, or stainless steel columns marketed in Brazil.

Chemical markers	Column	Alembic	Double distilling	p-Value[a]	p-Value[b]
Benzo(a)pyrene (ng · mL^{-1})	0.1725 (0.2259)	0.0293 (0.0338)	0.0400 (0.0652)	0.001	0.100
Alcoholic strength, 20 °C (mL · 100 mL^{-1})	38.82 (1.08)[b]	41.54 (3.11)[a]	38.14 (1.04)[b]	0.001	0.001
Methanol (mg · 100 mL^{-1} AA)	5.19 (1.58)	5.20 (1.42)	5.76 (2.43)	0.452	0.753
Acetaldehyde (mg · 100 mL^{-1} AA)	38.83 (21.44)	36.78 (18.46)	25.14 (15.06)	0.668	0.378
n-Propanol (mg · 100 mL^{-1} AA)	47.72 (15.53)[b]	104.24 (107.28)[a]	60.78 (11.41)[b]	0.001	0.015
Isobutanol (mg · 100 mL^{-1} AA)	53.36 (16.21)	45.14 (12.60)	48.52 (14.85)	0.499	0.177
2,3-Methyl-1-butanol (mg · 100 mL^{-1} AA)	159.31 (37.48)	140.62 (36.86)	160.44 (58.98)	0.253	0.605
Higher alcohols (mg · 100 mL^{-1} AA)	260.38 (65.69)	290.00 (112.85)	269.74 (71.15)	0.334	0.248

Note: Values expressed as mean (SD).
[a] Probability values obtained by Levene test for homogeneity of variances.
[b] Probability values obtained by one-way ANOVA or Welch–ANOVA test. Different capital letters in the same line represent statistical different results ($p < 0.05$) according to the LSD Fisher or multiple comparison Kruskal–Wallis Z-test.