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Rum classification using fingerprinting analysis of volatile fraction by headspace solid phase microextraction coupled to gas chromatography-mass spectrometry

José Raúl Belmonte-Sánchez[a], Simona Gherghel[a,b,c], Javier Arrebola-Liébanas[a], Roberto Romero González[a], José Luis Martínez Vidal[a], Ivan Parkin[c], Antonia Garrido Frenich[a,*]

[a] Research Group "Analytical Chemistry of Contaminants", Department of Chemistry and Physics, Research Centre for Agricultural and Food Biotechnology (BITAL), University of Almería, Agrifood Campus of International Excellence, ceiA3, E-04120 Almería, Spain
[b] UCL Department of Security and Crime Science, 35 Tavistock Square, London WC1H 9EZ, United Kingdom
[c] UCL Department of Chemistry, 20 Gordon Street, London WC1H 0AJ, United Kingdom

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ABSTRACT

In this study, targeted and untargeted analyses based on headspace solid phase microextraction coupled to gas chromatography-mass spectrometry (HS-SPME-GC-MS) method were developed for classifying 33 different commercial rums. Targeted analysis showed correlation of ethyl acetate and ethyl esters of carboxylic acids with aging when rums of the same brand were studied, but presented certain limitations when the comparison was carried out between different brands. To overcome these limitations, untargeted strategies based on unsupervised treatments, such as hierarchical cluster analysis (HCA) and principal component analysis (PCA), as well as supervised methods, such as linear discriminant analysis (LDA) were applied. HCA allowed distinguishing main groups (with and without additives), while the PCA method indicated 40 ions corresponding to 13 discriminant compounds as relevant chemical descriptors for the correct rum classification (PCA variance of 88%). The compounds were confirmed based on the combination of retention indexes and low and high-resolution mass spectrometry (HRMS). Using the obtained results, LDA was carried out for the analytical discrimination of the remaining rums based on manufacturing country, raw material type, distillation method, wood barrel type and aging period and 94%, 91%, 92%, 95% and 94% of rums, respectively, were correctly classified. The proposed methodology has led to a robust analytical strategy for the classification of rums as a function of different parameters depending on the rum production process.

1. Introduction

Rum is a fairly aromatic spirit, obtained exclusively from sugar cane juice or molasses, and then subjected to the processes of alcoholic fermentation, distillation and aging. This spirit represents a widely popular alcoholic beverage with a high world consumption rate (more than 1 billion of litres per year) and an expected increase of 1.9% in volume terms over 2016–2021. [1,2].

The complex elaboration of this type of alcoholic beverage makes it an attractive object of study. Differences in the production process are known to lead to wide variability in its composition, although this variation has not been fully understood yet [3,4]. The production process begins with the fermentation of the chosen raw material, which leads to the formation of a number of volatile compounds, such as alcohols, ethyl esters and aldehydes, among others [5]. The resulting mash is distilled using heat in copper pot stills or in stainless steel columns to obtain a high content of ethanol, which inevitably leads to the loss of some aroma compounds [6,7]. Additionally, different distillation methods can be applied, such as continuous and batch distillation (e.g. Jamaican "heavy rums" typically made by batch distillation) [8]. The resulting distillate is diluted with pure demineralized water to obtain an alcohol percentage of around 35–40%, which is then aged in oak barrels previously used for whiskey or brandy production [9,10]. The aging step gives rum its characteristic flavor as a large number of new compounds emerge. Ethyl esters are generated as a result of the high percentage of ethanol, while a number of different compounds such as whiskey lactone, vanillin and 2-methoxyphenol can form because of the interaction with the wood barrels [3]. Additionally, as rum matures, it generally gains golden hues as a result of the tannins from the barrel staves [11]. After an aging period, typically of at least 1

* Corresponding author.
E-mail address: agarrido@ual.es (A. Garrido Frenich).

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year, the containers are opened for an optional blending step, where rums of different ages are mixed to obtain specific organoleptic characteristics. Lastly, the colour and flavor of rums can be further modified by adding colorants and flavorings. Therefore, rums can be classified depending on the raw material, fermentation process, distillation process, aging period, type of barrel used, blending technique, alcohol strength and possible addition of additives.

Because of a lack of clear legislation around labelling, terms loosely related to aging periods, such as “Añejo”, “Dorado”, “Premium”, “Super Premium” or “Reserve” are often used by rum manufacturers without an actual quantitative/qualitative justification. Moreover, the age statement on labels is often not representative of the actual age, as blending of rums of different ages is carried out. According to legislation from both the European Union and the United States, the age statement on the label needs to refer to the youngest rum in the bottle [12,13]. However, in other countries, such as Canada, it can refer to the oldest rum [14]. Therefore, the development of methods that allow the reliable characterization of rums and an increased confidence of the consumers in this type of products in terms of authenticity is needed.

Nowadays, numerous methods have been described for the classification of alcoholic beverages based on the analysis of the volatile composition [9]. For that purpose, gas chromatography (GC) coupled to mass spectrometry (MS) has been one of the most frequently used technique [9]. In recent years, headspace solid phase microextraction (HS-SPME) has become the extraction method of choice. The combination of HS-SPME and GC-MS has been applied to different matrices such as wine [15–19], beer [20–24], tea beers [25] and other popular spirit beverages, such as whiskey [26,27], gin [28], or cocktail bitters [29].

However, to our knowledge, rum studies are less frequent and they have been generally limited to the comparison of this type of spirit with their South American analogue (cachaça) [30], to ascertain a specific geographic origin (Cuban rums from non-Cuban rums) [31] or to the identification of some aroma indicators [6,32–35]. Due to the complexity and variability of rum preparation, their classification represents an analytical challenge.

To overcome this, multivariate analysis has been commonly employed for other such complex matrices in order to take advantage of the huge amount of data obtained from the GC-MS analysis. Unsupervised chemometric techniques as principal component analysis (PCA) [36,37] as well as hierarchical cluster analysis (HCA) [38,39] have been commonly used for a preliminary inspection of the data. Further supervised classification methods, such as linear discriminant analysis (LDA) [40,41] have been successfully applied for chemometric analysis, as well as for the classification of different types of beverages or foods [42].

The aim of this study has been the classification of various types of rums by developing a comprehensive and robust analytical strategy for the analysis of the volatile/semi-volatile compounds. After simple and completely automated HS − SPME − GC − MS analyses, the raw data were processed applying available statistical tools for targeted and untargeted analysis. For exploratory data analysis, unsupervised chemometric techniques using unlabelled data were applied. Afterwards, supervised techniques were applied to achieve rums classification based on the chemical correlations between samples.

2. Materials and methods

2.1. Reagents

Ethanol HPLC grade was obtained from J.T. Baker (Deventer, Holland). C7-C40 saturated alkanes standard mix (1000 μg/mL in *n*-hexane) were supplied by Supelco (Bellefonte, PA, USA).

2.2. Samples

For this study, a total of 33 commercial rums were purchased from different local liquor stores (Almería, Spain). The rums were manufactured in 10 different countries: Cuba (5 samples), Dominican Republic (8 samples), Grenade (1 sample), Guatemala (3 samples), Jamaica (2 samples), Nicaragua (3 samples), Republic of Mauritius (2 samples), Spain (6 samples), Trinidad & Tobago (1 sample) and Venezuela (2 samples). All samples were stored in a refrigerator (4 °C) prior to analysis, in their original glass bottles. Information about the rum production from the official website of rum manufacturers as well as from the label, and assigned codes for each rum are summarized in Table 1. It should be pointed out that information about aging, raw material and distillation process was not provided by all manufacturers. When the information was not available, this was recorded as NA.

2.3. Sample preparation and HS-SPME procedure

Prior to HS-SPME-GC-MS analysis, the rum bottles were left to reach room temperature for 1 h. After that, they were opened for the first time. Rums with common origin were analysed equally across the sampling sequence according to a block design in order to guarantee their comparability and lack of potential analytical bias. Three replicates of each bottle were analysed.

Blanks which consisted of a mixture of Milli-Q water (J.T. Baker) and ethanol (Sigma-Aldrich; San Louis, MO, USA) at a ratio of 63:37 v/v were prepared to simulate the alcohol content in a typical commercial rum. Blanks were analysed between each brand for various specific purposes: (i) to check the potential contamination generated by the septum (blank correction during the statistical treatment of the data), (ii) to evaluate potential carry over effect in the fiber and (iii) for additional cleaning up of the fiber.

For SPME extraction, different combinations of the selected parameters that are known to affect the fiber performance (sample volume, incubation time, extraction temperature, extraction time, and stirring speed) were applied in order to maximize the number and the intensity of volatile compounds extracted. Finally, ten mL of each rum sample were placed into a 20-mL glass vial fitted with a magnetic cap and a PTFE/silicone septum of 1.5 mm thickness. After 5 min of preheating the sample at 65 °C (continuous stirring, 250 rpm), the SPME fiber was exposed to the sample headspace for an adsorption time of 30 min with constant stirring (250 rpm).

After extraction, the fiber was inserted into the GC injector using a 0.8 mm dedicated SPME liner to allow thermal desorption of the analytes at a temperature of 250 °C for 2 min. The compounds were desorbed into the injector in splitless mode for 2 min, prior to the GC-MS analysis. After desorption, a fiber cleaning step was carried out for 6 additional min with an increased split rate of 100:1.

2.4. GC-QqQ-MS analysis

A Scion GC system equipped with an autosampler (Bruker Corporation, Freemont, CA, USA) was used for chromatographic analyses. Polydimethylsiloxane (100 μm film thickness) SPME fibers were obtained from Supelco (Bellefonte, Pennsylvania, USA). After their conditioning following manufacturer's recommendations, the fibers were used without any further modification. A VF-5 ms capillary column (30 m x 0.25 mm i.d. x 0.25 μm film thickness) from Varian (Palo Alto, California, USA) was utilized for GC separation. Helium was used as carrier gas at a constant flow rate of 1 mL/min (36.7 cm/s linear velocity). An untreated fused silica capillary column (2 m x 0.25 mm) from Supelco was used as pre-column.

Mass spectrometric detection was performed by a triple quadrupole Scion QqQ-MS/MS (Bruker) operating in electron ionization mode (EI, 70 eV). Mass spectral data of the total ion chromatograms (TICs) and Kovats retention index (KI) of rum samples were compared to the NIST