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Analytical Methods

Characterisation of tequila according to their major volatile composition using multilayer perceptron neural networks

Silvia G. Ceballos-Magaña [a], Fernando de Pablos [b,*], José Marcos Jurado [b], María Jesús Martín [b], Ángela Alcázar [b], Roberto Muñiz-Valencia [c], Raquel Gonzalo-Lumbreras [d], Roberto Izquierdo-Hornillos [d]

[a] Facultad de Ciencias, Universidad de Colima, c/Bernal Díaz del Castillo 340, 28045 Colima, Mexico
[b] Department of Analytical Chemistry, Faculty of Chemistry, University of Seville, c/Profesor García González 1, E-41012 Seville, Spain
[c] Facultad de Ciencias Químicas, Universidad de Colima, Carretera Colima-Coquimatlán km 9, 28400 Coquimatlán, Colima, Mexico
[d] Department of Analytical Chemistry, Faculty of Chemistry, University Complutense, E-28040 Madrid, Spain

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ABSTRACT

Differentiation of silver, gold, aged and extra-aged tequila using 1-propanol, ethyl acetate, 2-methyl-1-propanol, 3-methyl-1-butanol and 2-methyl-1-butanol and furan derivatives like 5-(hydroxymethyl)-2-furaldehyde and 2-furaldehyde has been carried out. The content of 1-propanol, ethyl acetate, 2-methyl-1-propanol, 3-methyl-1-butanol and 2-methyl-1-butanol was determined by means of head space solid phase microextraction gas chromatography mass-spectrometry. 5-(Hydroxymethyl)-2-furaldehyde and 2-furaldehyde were determined by high performance liquid chromatography with diode array detection. Kruskal–Wallis test was used to highlight significant differences between types of tequila. Principal component analysis was applied as visualisation technique. Linear discriminant analysis and multilayer perceptron artificial neural networks were used to construct classification models. The best classification performance was obtained when multilayer perceptron model was applied.

1. Introduction

Tequila is a beverage made from the juice of *Agave tequilana* Weber var. *azul*. According to current regulations the agave used in the elaboration of tequila must come from defined geographic areas of México, the state of Jalisco and some districts of the states of Tamaulipas, Nayarit, Michoacan and Guanajuato (Mexican Ministry of Commerce, 2006). The manufacturing process involves several steps. The stem of the plant is cut and baked, being the resulting juice fermented, double distilled and diluted to obtain the final product, with or without an ageing period in oak barrels. Two basic categories of the product are considered, 100% agave and mixed tequilas that are produced by adding sugar cane or corn prior to fermentation. According to the ageing time, there are four types, silver, gold, aged and extra-aged. Silver tequila is bottled or stored immediately after distillation. Aged tequila is obtained after an ageing period of two to twelve months and extra-aged is matured during a period of one to three years. Gold tequila is a mixture of silver with aged or extra-aged tequilas and, in this case, caramel colouring, sugar-based syrup, glycerin, and/or oak extract can be added so as to resemble aged tequila. The estimated production of tequila reached 249 million litres in 2009, being more than half of this production for exports (Consejo Regulador del Tequila, 2010). Tequila is protected under the North American Free Trade Agreement (NAFTA) and an agreement between the European Union and the United Mexican States on the mutual recognition and protection of designations for spirit drinks (European Communities, 1997). To avoid fraud and misuse of the name tequila there is a need of effective methods. Within this realm multivariate analysis in combination with pattern recognition methods is a very powerful tool. Classification models using this information, without additional cost, can be useful for companies or governments. They can be a source of information for the consumers about the characteristics of a product and useful tools to preserve the quality of a specific brand, authenticate products, set the specifications of a category and identify geographical origins (Vichi, Riu, Mora, Boxaderas, & López, 2005).

Characterization of tequila has been carried out taking into account $^{13}C/^{12}C$ and $^{18}O/^{16}O$ ratios of ethanol (Aguilar-Cisneros, López, Richling, Heckel, & Schreier, 2002) and the methanol, 2-methyl butanol and 3-methyl butanol contents (Bauer et al., 2003). Differentiation between tequila and other traditional Mexican beverages such as mezcal has been performed considering the presence of terpenes and fatty acids in the Agave species used in the elaboration (Peña et al., 2004). A differentiation based on the

* Corresponding author. Tel.: +34 954557173; fax: +34 954557168.
E-mail address: fpablos@us.es (F. de Pablos).

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anions and volatile composition of traditional Mexican beverages, tequila, sotol, mezcal and bacanora has been reported (Lachenmeier, Sohnius, Attig, & López, 2006). Also, differences in the ethyl esters composition of the four types of tequila have been found (Vallejo, González, & Estrada, 2004). Some of these compounds could be used as chemical descriptors to characterise this product. With this aim, pattern recognition techniques, such as principal component analysis (PCA), linear discriminant analysis (LDA) or artificial neural networks (ANN) are commonly used. In the case of tequila, few previous works have applied pattern recognition methods for tequila characterization. PCA has been used to differentiate authentic tequila samples according to FTIR and ion chromatography data (Lachenmeier, Richling, López, Frank, & Schreier, 2005) and the UV–Vis absorption spectra (Barbosa et al., 2006). LDA and ANN have been applied to differentiate among varieties of tequila and mezcal using metals as chemical descriptors (Ceballos, Jurado, Martín, & Pablos, 2009).

In this work, the differentiation of the four types of tequila, silver, gold, aged and extra-aged, has been carried out using the major components, other than ethanol, of the volatile fraction 1-propanol, ethyl acetate, 2-methyl-1-propanol, 3-methyl-1-butanol and 2-methyl-1-butanol and the aldehydes 5-(hydroxymethyl)-2-furaldehyde, 2-furaldehyde as chemical descriptors. Furaldehydes may help in assessing the ageing conditions as they are produced as degradation products of cellulose and hemicellulose during the heat treatment of the inner part of the wooden barrels used for the ageing process (Muñoz, Wrobel, & Wrobel, 2005). All these compounds are considered by the NOM (Mexican Ministry of Commerce & Industry, 2006) as important parameters for the quality control of tequila. Determination of ethyl acetate and higher alcohols was performed by head space solid phase microextraction gas chromatography-mass spectrometry (HS-SPME-GC–MS). Concentrations of 5-(hydroxymethyl)-2-furaldehyde and 2-furaldehyde were determined by high performance liquid chromatography with diode array detection (HPLC-DAD). PCA was applied in order to visualise possible data trends. LDA and back propagation multilayer perceptrons (BP-MLP) ANN were used to construct classification models.

2. Materials and methods

2.1. Chemicals and reagents

5-(Hydroxymethyl)-2-furaldehyde (99%), 2-furaldehyde (99%), 1-propanol (99.5%), ethyl acetate (99%), 2-methyl-1-propanol (99.5%), 3-methyl-1-butanol (98.5%), 2-methyl-1-butanol (98%), 1-butanol (99.5%) and ethanol (99.5%) were purchased from Sigma–Aldrich (Steinheim, Germany). HPLC grade acetonitrile was from Romil (Cambridge, UK). Analytical grade acetic and phosphoric acids were from Merck (Darmastadt, Germany). Ultrapure water with resistivity higher than 18.2 MΩ/cm was used throughout. For HPLC experiments a 0.035 M acetic acid and 0.004 M phosphoric acid aqueous solution (Solution A) was prepared. Standard solutions (200 mg L^{-1}) of 2-furaldehyde and 5-(hydroxymethyl)-2-furaldehyde were prepared in acetonitrile and those of ethyl acetate and higher alcohols (1000 mg L^{-1}) in ethanol. These solutions were stored a 4 °C. Diluted working solutions were prepared weekly.

2.2. Samples

Sixty-nine commercially available tequilas were purchased in retail liquor stores. Samples were chosen so as to be representative of the types of tequila available to the consumer. The production date was between years 2004 and 2007. They were 100% agave and included 22 silver (S), 12 gold (G), 23 aged (A) and 12 extra-aged (E) tequilas.

2.3. Apparatus and methods

2.3.1. HS-SPME-GC–MS

Due to the high sensitivity, solvent-free, fast sampling and low cost, the determination of higher alcohols and ethyl acetate was carried out with a previous extraction using HS-SPME. Taking into account the results obtained in previous works (Jurado et al., 2007, 2008) a CAR/PDMS fibre was selected for the extraction. Ethanol content, time of extraction, temperature and ionic strength were optimised. GC–MS conditions were fixed considering those applied to determine higher alcohols in distillates (González, González, Pablos, & González, 1999; Jurado et al., 2007). Prior to the GC–MS determination of 1-propanol, ethyl acetate, 2-methyl-1-propanol, 3-methyl-1-butanol and 2-methyl-1-butanol, HS-SPME was used in order to extract these compounds from the sample matrix. A manual fibre-holder for SPME and 85 μm Carboxen/polydimethylsiloxane (CAR/PDMS) fibres were purchased from Supelco (Bellefonte, PA, USA). A SPME inlet guide and pre-drilled Thermogreen LB-2 septa from Supelco were used. Agimatic-N magnetic stirrer and temperature-controlled water bath from JP Selecta (Barcelona, Spain) was also used during the extraction process. The fibres were conditioned before their first use according to specifications of the manufacturer. A fibre blank was run after the conditioning process to confirm that there were no peaks assigned to compounds that could have been introduced during their manufacture process. The proposed sample treatment is as follows: 3 mL of tequila were diluted with 7 mL of water to reduce the ethanol content close to 12% and 1-butanol was added as internal standard with a final concentration of 60 mg L^{-1}. This solution was placed in a 20 mL vial containing a magnetic stirring bar. The vial was hermetically sealed with PTFE faced silicone septum and heated at 35 °C. The CAR/PDMS fibre was exposed into the headspace of the sample during 30 min stirring at 100 rpm. After sampling, the fibre was removed from the sample vial and inserted into the GC injection port for thermal desorption of the analytes at 220 °C during 2 min. The GC–MS system consisted of a GC-8000 series gas chromatograph fitted with a split/splitless injector and a Trio 1000 mass spectrometer detector equipped with an electronic impact source and quadrupole mass analyzer from Fisons Instruments (Milano, Italy). The separation was achieved using a 5% phenyl-95% dimethyl-polysiloxane Zebron ZB-5 capillary column (30 m × 0.25 mm, d_f 0.25 μm) from Phenomenex (Torrance, CA, USA). Helium (purity 99.999%) was used as carrier gas at 1 mL min^{-1} flow-rate and the injection was made in split mode (1:15) at 220 °C. Oven was operated in isothermal mode at 40 °C. After every analysis temperature was increased to 250 °C and kept during 20 min to elute non target compounds. The GC–MS interface and ionisation source temperatures were set at 250 and 300 °C, respectively. The mass detector was operated in the electron impact mode at 70 eV. Identification and quantitation were performed in full scan mode with a mass/charge (m/z) range of 35–250 at 2.5 scan/s scan rate. The compounds were identified by comparison with the Wiley 6th Ed. and NIST-98 mass spectral libraries. Standards and samples were injected and analysed under the same conditions. Triplicate analyses were performed, using 1-butanol as internal standard.

2.3.2. HPLC-DAD

Determination of furaldehydes was performed by applying a reversed phase HPLC method based on the use of C-18 column and acetonitrile–water mobile phase with isocratic elution and detection at 280 nm (Alcázar, Jurado, Pablos, González, & Martín, 2006). The HPLC system consisted of a 1525 Binary HPLC pump,