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Analytical Methods

Solid-phase microextraction gas chromatography–mass spectrometry (HS-SPME-GC–MS) determination of volatile compounds in *orujo* spirits: Multivariate chemometric characterisation

S. García-Martín *, C. Herrero, R.M. Peña, J. Barciela

Departamento de Química Analítica, Nutrición y Bromatología, Facultad de Ciencias, Universidad de Santiago de Compostela, Av. Alfonso X El Sabio s/n, 27002 Lugo, Spain

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ABSTRACT

A headspace solid-phase microextraction gas chromatography mass spectrometric procedure (HS-SPME-GC–MS) was developed and applied in order to determine 22 volatile compounds (including alcohols, esters, aldehydes and terpenes) in different *orujo* spirit samples from the Geographic Denomination "*Orujo de Galicia/Augardente de Galicia*". The *orujo* samples considered in this study were elaborated from *Albariño* variety grapes grown in the *Rías Baixas* restricted geographical area, and *Albariño* variety grapes grown in other geographical areas of Galicia (NW Spain) using two of the traditional distillation techniques: alembic and steam distillation. HS-SPME adequate results were obtained using a 65 μm carbowax-divinylbencene fibre during a headspace extraction at 40 °C with constant magnetic stirring for 15 min, and after a 5 min period of pre-equilibrium time. Desorption was performed directly in the gas chromatograph injector port for 5 min at 250 °C using the splitless mode. The applied method was demonstrated to be sensible, accurate, precise, and linear over more than one order of magnitude. Multivariate chemometric techniques (such as cluster analysis, principal component analysis and linear discriminant analysis) were used to characterise the *orujo* samples according to the geographical origin of the grapes and the distillation system employed in their elaboration on the basis of the chemical information provided for their volatile composition data.

1. Introduction

The alcoholic beverage *orujo* is produced in Galicia (NW Spain) by distillation of fermented grape pomace. Galicia is the only region in Spain which could obtain a geographic denomination for *orujo* alcoholic distillates, in the same category as French *marcs*, Italian *grappas*, Portuguese *bagaceiras* and Greek *tsipouros* (European Regulation, EEC, 1989). This beverage has come to be a product of an important economic interest for producers and not merely as a complementary activity to wine elaboration.

The resulting *orujo* distillates undergo strict quality controls established by the Directive Council of the Geographic Denomination "*Orujo de Galicia/Augardente de Galicia*" (DOG, 1993). This Council defined the origin of the raw material, the authorised grape varieties, the geographical production areas, and several other characteristics which are necessary in order to be classified as a quality product under the brand "*Orujo de Galicia*". Among these, the concentration limits of different volatile compounds in the distilled spirit (ethanol, methanol, the total content of higher alcohols, ethyl acetate, and acetaldehyde) must be determined to ensure the product meets these standards.

More than 300 different compounds are present in *orujo* distillates, mainly alcohols, esters, carboxylic acids, aldehydes and acetals. These compounds produce key notes which characterise the flavour and aroma of these beverages. There are many factors in the production of distillates which influence the sensorial characteristics appreciated in the final commercial products: the raw material, the different distillation systems and the distillate maturation in wood (when this takes place) (Silva, Macedo, & Malcata, 2000). In the case of an unaged distillate, this set of compounds is composed of the volatile substances present in the grapes and of the ones formed either during fermentation or generated during distillation (Silva & Malcata, 1999; Silva, Malcata, & De Revel, 1996). Some of these compounds are present in relatively large amounts and can be determined by direct gas chromatography. Other compounds are present at much lower concentrations. Therefore, the determination of these constituents often requires the use of sophisticated preconcentration procedures before gas chromatography.

Chemometric pattern recognition techniques have been widely applied in food chemistry (Brown, Bear, & Blank, 1992; Forina & Lanteri, 1984) to elucidate the chemical information provided for

* Corresponding author. Tel.: +34 982 285900; fax: +34 982 285872.
E-mail address: sagrario.garcia.martin@usc.es (S. García-Martín).

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the different multicomponent analytical techniques. Since the pioneering works done by Kwan, Kowalski, and Skogerboe (1979) and Kwan and Kowalski (1980) on the classification of wines of *Vitis vinifera* cv. Pinot Noir from France and the United States, a number of examples reported in previous studies demonstrate the useful combination of chemical analysis and chemometrics to solve different problems in a variety of food products. These include the characterisation of the geographic classification of olives (Pinheiro & Esteves da Silva, 2005), ciders (Alonso-Salces, Guyot, et al., 2004; Alonso-Salces, Herrero, et al., 2004), coffees (Rocha, Maeztu, Barros, Cid, & Coimbra, 2003; Zambonin, Balest, De Benedetto, & Palmisano, 2005) and wines (Jurado et al., 2008; Martí, Busto, & Guasch, 2004), etc.

In this work, a headspace solid-phase microextraction (HS-SPME) and gas chromatography-selective ion monitoring/mass spectrometry (GC-SIM/MS) procedure was applied in order to determine 22 volatile compounds (alcohols, esters, aldehydes and terpenes) in *orujo* spirit samples from the Geographic Denomination "*Orujo de Galicia/Augardente de Galicia*". Using the chemical information obtained and applying multivariate chemometrical tools–such as cluster analysis, principal component analysis and linear discriminant analysis–the *orujo* samples were characterised according to their geographical and botanical origins, and also to the distillation system employed in their elaboration.

2. Experimental

2.1. Orujo samples

Thirty commercial *orujo* spirit samples that were provided by the Directive Council of the Geographic Denomination "*Orujo de Galicia/Augardente de Galicia*" in order to guarantee their geographical origin and authenticity were used in this study. All samples were *orujo* monovarietal spirits (2004 vintage) obtained from the distillation of *Albariño* variety grapes. Nine of the samples were obtained from the alembic distillation of grapes grown in the *Rías Baixas* restricted geographical area (for identification purposes, these were coded as class 1). The remaining twenty one samples were obtained using steam distillation: 11 of them (coded as class 2) from grapes grown in the *Rías Baixas* restricted geographical area and the other 10 (coded as class 3) from grapes grown any place in Galicia other than the *Rías Baixas* area. All samples were collected in 400 mL glass bottles and stored at 4 °C before analysis.

2.2. Reagents

All volatile compound standards such as alcohols: 1-propanol, 2-methyl-1-propanol, 3-methyl-1-butanol, 2-phenylethanol, 3-octanol (used as an internal standard); esters: ethyl hexanoate, ethyl lactate, ethyl octanoate, ethyl decanoate, diethyl succinate, 2-phenyl ethyl acetate, ethyl dodecanoate, ethyl tetradecanoate; acids: acetic, hexanoic, octanoic, decanoic; aldehydes: benzaldehyde; and terpenes: linalool, α-terpineol, citronellol, nerol, geraniol, were supplied by Aldrich Flavor and Fragrances (Alcobendas, Madrid, Spain). The sodium chloride, used to control the ionic strength, was supplied by Panreac (Barcelona, Spain). Absolute ethanol (Panreac, Barcelona, Spain) and ultra-pure Milli-Q water (Millipore Co., Bedford, USA) were used as solvents. All solvents and reagents used were analytical grade.

Stock standard solutions of 10^3 or 10^4 mg $L^{-1}$of each component were prepared by dissolving the pure standards in 40% (v/v) ethanol. Then they were stored at 4 °C. Working standard solutions of each compound were prepared daily by mixing an aliquot of each individual solution and diluting them with ultrapure water to obtain a final ethanol content of 10% (v/v).

2.3. Apparatus and methods

2.3.1. Analytical determinations

Twenty two volatile compounds such as alcohols: 1-propanol, 2-methyl-1-propanol, 3-methyl-1-butanol, 2-phenylethanol, 3-octanol (used as an internal standard); esters: ethyl hexanoate, ethyl lactate, ethyl octanoate, ethyl decanoate, diethyl succinate, 2-phenyl ethyl acetate, ethyl dodecanoate, ethyl tetradecanoate; acids: acetic, hexanoic, octanoic, decanoic; aldehydes: benzaldehyde; and terpenes: linalool, α-terpineol, citronellol, nerol, geraniol were analysed using HS-SPME-GC–MS All determinations were carried out in triplicate and the average values were calculated.

2.3.2. HS-SPME

A manual fibre-holder and two types of fibres–100 μm polydimethylsiloxane (PDMS) and 65 μm carbowax-divinylbenzene (CW-DVB)–were obtained from Supelco (Bellefonte, PA, USA). In all cases, the fibres were conditioned before use by inserting them into the GC injector port for 1 h at 250 °C (PDMS) and for 30 min at 220 °C (CW-DVB). Between injections, the fibres were desorbed for 10 min at 250 °C in split mode in order to prevent any contamination. Due the high ethanol content of the *orujo* spirits (40% v/v) a dilution to 10% ethanol before solid-phase microextraction was necessary. The SPME process was carried out by introducing 6 mL of diluted *orujo* spirit into a 12 mL PTFE coated septum-closed vial; 1.5 g of sodium chloride and 20 mg L^{-1} of the internal standard (3-octanol) were added. SPME extractions were performed by inserting the fibre in the headspace for 15 min at 40 °C using continuous magnetic stirring of the liquid phase at 1100 rpm. Before the extraction, the sample with 25% (w/v) of NaCl was maintained at 40 °C for 5 min in order to establish equilibrium between headspace and sample. After each extraction, the fibre was inserted into the GC injector port using a 0.75 mm i.d. liner (in order to improve the GC resolution). Desorption time and temperature were 5 min and 250 °C, respectively. All experiments and sample measurements were carried out in triplicate and the average values were calculated.

2.3.3. GC–MS

Chromatographic analysis were performed using an Agilent 6890 gas chromatograph equipped with a mass spectrometric detector (MSD) model 5973 N (Agilent Technologies Deutschland Gmbh, Waldbronn, Germany). The capillary column used was a HP-Innowax (30 m × 0.25 mm i.d., film thickness 0.25 μm) from Agilent Technologies. The gas chromatographic operation conditions were as follows. The injector temperature was 250 °C; the carrier gas employed was Helium (purity 99.9995%) at a constant flow rate of 1 mL min^{-1}. The oven temperature program was 5 min at 40 °C, then 1.5 °C min^{-1} up to 80 °C, and finally 5 °C min^{-1} up to 200 °C. This final temperature was maintained for 0.5 min. The injection was made in splitless mode for 2 min at a temperature of 250 °C and using a SPME inlet guide and pre-drilled Thermogreen LB-2 septa from Supelco (Bellefonte, PA, USA).

The mass spectrometer was operated in electron impact mode with the following conditions. The source temperature was 230 °C; the quadrupole temperature selected was 150 °C and the relative electron multiplier voltage (EM) applied was 400 V with a resulting voltage of 1553 V. In order to improve the detection limits, the selected ion monitoring (SIM) mode was used. The data acquisition was carried out with the HP-Chemstation software version D.00.00.38 (Agilent Technologies). The compounds were identified using the NIST98 version 2.0 mass spectra library. Each compound was further confirmed by comparing its mass spectra,