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Analysis of wine primary aroma compounds by stir bar sorptive extraction

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Abstract

Due to the great importance of some primary aroma compounds on wine quality, these compounds which includes terpenes, C_{13}-norisoprenoids and C_6 compounds, have been analyzed by stir bar sorptive extraction (SBSE) followed by a thermal desorption-gas chromatography–mass spectrometry analysis. The stir bar sorptive extraction method was optimized in terms of temperature, time, pH and NaCl addition. The best SBSE sorption kinetics for the target analytes were obtained after submitting the solutions to 60 °C during 90 min. The addition of sodium chloride did not enhance the volatile extraction. The method proposed showed good linearity over the concentration range tested, with correlation coefficients higher than 0.98 for all the analytes. The reproducibility and repeatability of the method was estimated between 0.22 and 9.11%. The detection and quantification limits of all analytes were lower than their respective olfactory threshold values. The application of this SBSE method revealed that monovarietal white wines were clearly separated by two canonic discriminating functions when grape varieties were used as differentiating variable, the first of which explained 98.4% of the variance. The compounds which contributed most to the differentiation were limonene, linalool, nerolidol and 1-hexanol.

Keywords: Stir bar sorptive extraction (SBSE); Primary aroma; Wines

1. Introduction

Analysis of aroma compounds is one of the most important steps in the evaluation of wine quality. The low concentration of the volatile compounds responsible of wine aroma makes enrichment as a basis for identification and quantification [1], among them liquid–liquid extraction [2–4] or solid phase extraction [5,6] using organic solvents prior to analysis by GC–MS have been the most widely used. These analytical methods have some drawbacks such as the possibility of contamination with solvents and later solvent concentration, generation of artifacts and the length of time of analysis. Techniques which requires neither solvents nor sample preparation such as solid phase microextraction (SPME) [7–10] or a most powerful technique, the stir bar sportive extraction (SBSE) [11], have been successfully been applied for flavour profiling of different types of matrix because it combines ease of use, ruggedness, precision and sensitivity [12–18].

Today, there is an increasing demand for wines with a fresh and fuity aroma, which can also be used to identify the *Vitis vinifera* used for winemaking. One of the most important factors in determining wine typicity and quality. Wine primary aroma compounds, which are also defined as varietal aroma compounds and represent the typical aroma of the grapes noted in wines, are present as free forms, which may contributed directly to odour and, in much larger concentrations as non-volatile forms, among them the sugar-bound conjugates being the most abundant. The hydrolysis of these glycoconjugates mainly by acids, enzymes or while wine aging, can yield odour-active aglycones such as terpenes, C_{13}-norisoprenids, benzene derivates, and aliphatic alcohols [19,20] that are not always present in all *V. vinifera* varieties. These compounds have a great importance because they play a key role in the differentiation of the wines according to the different grape varieties used for winemaking [19,21].

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However, not all compounds contribute to the same extent to wine aroma. In fact, if the concentration/olfactory threshold ratio of each compound known as the "odour activity value" (OAV) is ≥1, this allows estimating the contribution of each compound to the wine aroma. According to Güth [22] this concept is therefore necessary to quantify the levels of flavour differences between wines obtained from the different grape varieties or origins.

The analysis of some wine aroma compounds by SBSE was first carried out by Hayasaka et al. [23], although there are only few papers that optimized the sorptive extraction procedure in wine matrixes for cork taint, oak volatiles and pesticides [14,16,24]. In this paper, the wine primary aroma compounds analysis by SBSE have been optimized for first time in terms of ionic strength, temperature and extraction time. The primary aroma compounds that can have a great contribution on wine and are closely related to quality in white wines and may be used to differentiate monovarietal wines are: terpenes such as limonene, linalool, α-terpineol, β-citronellol, nerol, geraniol, nerolidol; C_6 compounds such as *trans*-2-hexenal, 1-hexanol, *cis*-3-hexen-1-ol, *trans*-2-hexen-1-ol; and C_{13}-norisoprenoids such as β-damascenone, α and β-ionone. As well, this SBSE method has been applied to the differentiation of six different monovarietal white commercial wines elaborated with Chardonnay, Macabeo, Muscat, Eva, Cayetana and Pardina varieties from Extremadura, in the Spanish Southwest region.

2. Experimental

2.1. Chemicals and reagents

Standards: β-citronellol, geraniol, α and β-ionone, limonene, linalool, nerol, nerolidol, γ-hexalactone (IS), 1-hexanol, *trans*-2-hexenal, *trans*-2-hexen-1-ol, *cis*-3-hexen-1-ol, α-terpineol, were obtained from Sigma–Aldrich (Madrid, Spain). β-Damascenone was a gift from Firmenich (Barcelona, Spain). Exact masses of the chemical standards were dissolved in absolute ethanol.

Solvents: ethanol (analytical-reagent grade) was obtained from Merck (Damstard, Germany), while water was purified through a Milli-Q system (Millipore, Bedfords, MA, USA).

Synthetic wine samples were prepared by an ethanol solution at 12% (v/v) to which 5 g/L tartaric acid were added. Solution pH was adjusted to 3.6 with 1 M sodium hydroxide (Panreac, Barcelona, Spain).

2.2. Proposed extraction method

A sample of 25 mL of wine, to which 250 μL of internal standard γ-hexalactone solution at 1 μL/mL in absolute ethanol was added, was poured into a 25 mL volumetric flask. Compounds were extracted by introducing the polydimethylsiloxane coated stir bar (0.5 mm film thickness, 10 mm length, Twister, Gerstel GmbH, Mülheim and der Ruhr, Germany) into the sample (either commercial wine or synthetic wine solution). Samples were stirred at 700 rpm at 60 °C for 90 min. The stir bar was then removed from the sample, rinsed with distilled water and dried with a cellulose tissue, and later transferred into a thermal desorption tube for GC/MS analysis.

2.3. GC/MS analysis

In the thermal desorption tube, the volatile compounds were desorbed from the stir bar at the following conditions: oven temperature at 290 °C; desorption time, 4 min; cold trap temperature, −30 °C; helium inlet flow, 45 mL/min. The compounds were transferred into the Hewlett-Packard 6890 gas chromatograph coupled to an Hewlett-Packard LC 3D mass detector (Palo Alto, USA) with a fused silica capillary column (BP21 stationary phase 50 m length, 0.22 mm i.d., and 0.25 μm film thickness) (SGE, Ringwood, Australia). The chromatographic program was set at 50 °C (held for 2 min), raised to 230 °C at 12 °C/min and held for 20 min. For mass spectrometry analysis, electron impact mode (EI) at 70 eV was used. The mass range varied from 35 to 500 u and the detector temperature was 150 °C. Identification was carried out using the NIST library and quantification was based on the calibration curves of respective standards in the synthetic wines.

2.4. Analytical method validation

For linearity study, calibration graphs were established with five standard solutions in synthetic wine ranged from their OAV 0.5 to 10 which level of concentration has been included in Table 1. Each level of concentration was analyzed twice with two different stir bars, so there were a total of four replicates.

The detection and quantification limits (LOD and LOQ, respectively) were calculated with the data generated in the linearity studies. LOD was defined as $(a + 3S_a/b)$ and LOQ as $(a + 10S_a/b)$, "a" being the origin ordinate, "S_a" the origin ordinate variance and "b" the slope. The limit of quantification was taken to be validated when within-batch relative standard deviation, using three replicate samples spiked with known LOQs, was fewer than 20% according to Catice methodology [25].

The standard deviation for each compound (square root of the arithmetic mean of the variances) was calculated to obtain the repeatability (%R.S.D.). The standard deviation of the three values for each compound multiplied by the square root of 3 was taken as the reproducibility value (if this value was higher than repeatability; if not, this last value was also taken as reproducibility).

2.5. Wine samples differentiation

Six different commercial monovarietal white wines (Chardonnay, Muscat, Eva, Cayetana and Pardina) from Extremadura (Spanish Southwest region) were analyzed in duplicate in this study following the methodology proposed. Wine sample differentiation was performed with the SPSS Version 11.5 statistical package for Windows (SPSS, Chicago, IL).