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Optimization and validation of headspace sorptive extraction for the analysis of volatile compounds in wine vinegars

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ABSTRACT

Quantification of aroma compounds in wine vinegars is challenging due to the complexity of the matrix and the low concentrations expected. A method for the determination of volatile compounds in wine vinegars employing headspace sorptive extraction–thermal desorption–gas chromatography–mass spectrometry (HSSE–TD–GC–MS) was developed. A central composite design was used to optimize the sampling condition. The proposed method was successfully validated and low detection and quantification limits was obtained. The application of the proposed methodology allows the determination of 53 compounds in different wine vinegars (red, Sherry). Five of them have been detected in wine vinegars for the first time.

1. Introduction

The volatile composition of wine vinegars is quite complex, with many compounds accounting for low concentrations (ppm to ppb). There are a number of methods used to extract and/or concentrate aroma compounds from foods prior to analysis. Nowadays, solvent-free extraction techniques are extensively employed, the best known among them are solid-phase microextraction (SPME) [1] and stir bar sorptive extraction (SBSE) [2].

SPME using different type of fibre has been widely applied for the study of aroma in wine [3–6] as well as vinegar [7]. Two types of SPME techniques can be used to extract analytes: headspace (HS) when the fibre is exposed in the vapour phase above a liquid or solid sample or direct immersion (DI) when the fibre is directly immersed in the liquid sample [8]. The most important parameters affecting the SPME method are type of fibre employed, extraction temperature and time, salt concentration and sample volume [9]. The competition between analytes and the saturation of these extraction materials is limiting parameters. In SPME the amount of extraction medium is very small, thus, new strategies are being developed to avoid these limiting aspects of adsorbents [10].

Baltussen et al. [2] developed a new extraction technique known as SBSE, based on the polydimethylsiloxane (PDMS) phase as an extraction medium. The extraction mechanism and advantages are similar to those of SPME. In the technique of SBSE, magnetic stirring rods are incorporated in glass jackets and coated with a layer of polydimethylsiloxane phase. These coated stir bars are commercially known as "Twister". Several different types of Twisters are available depending on the length (10–20 mm) and phase thickness (0.5–1.0), typically, the 10 mm stir bar is used for 1–50 mL sample volumes. SBSE has shown a much higher sensitivity than SPME by a factor within 100 and 1000 due to the higher content of PDMS (50–300 μL). The amount of analyte extracted is proportional to the coating thickness thus increasing the limit of detection of ultra trace compounds [11]. Different comparison studies between the use of headspace-SPME and SBSE [2,12] or immersion-SPME and SBSE [13,14] showed the higher capability, greater accuracy and sensitivity of SBSE.

SBSE technique has been successfully applied to the analyses of aroma compounds in wine [15–17]. In a recent work a comparison of SBSE and SPME for the analysis of volatile compounds in vinegar was carried out, SBSE technique was capable of determining a higher amount of compounds, showing a better sensitivity and reproducibility values [18].

The headspace sorptive extraction (HSSE) technique introduced by Bicchi et al. [19] and Tienpont et al. [20] is an extension of SBSE for headspace sampling, which results in a high solute concentration

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capability. In HSSE, a Twister is placed in an open glass adapter inside a closed headspace vial. Thus, analytes are extracted from the vapour phase above the sample.

In this case HSSE recovery depends on the overall partition coefficient, *K*. In turn, *K* depends on the analyte partition coefficient between PDMS stir bar and sample headspace, K_1, and on the partition coefficient between the headspace and the sample matrix, K_2 [21]. HSSE was successfully applied for the analysis of solid and liquid matrices [21–25]. Although in general direct immersion techniques are more sensible, extraction in the vapour phase generally is preferred [10]. This method presents as advantage to reduce the risk of contamination and to increase the stir bar lifetime [25].

Various parameters affecting the extraction kinetics have to be optimized when developing an extraction method. In similar way as occur with SPME, extraction temperature and time, salt concentration and sample volume are features (or experimental parameters) influencing the analyte equilibrium between the sample and the fibre. These factors have to be optimized to obtain the best extraction condition.

Many independent parameters such as the nature of extraction material, physicochemical characteristics of the matrix (pH, salt content, temperature, etc.), and time can affect the extraction of volatiles and it is likely that the operational variables interact and influence each other's effects on the response. Therefore, it is necessary to use an optimization method that can determine all the factors as well as the possible interactions between these independent variables, so that a set of optimal experimental conditions can be determined [26]. Optimization through factorial design and response surface analysis particularly fulfils this requirement [27].

Response surface methodology (RSM) is a collection of statistical and mathematical techniques useful for developing, improving and optimizing process [28]. The main advantage of RSM is to reduce the number of experimental trials needed to evaluate multiple variables and its interactions; it is less laborious and time-consuming than other approaches [29]. In general, it applies an experimental design such as central composite design (CCD) to fit a second-order polynomial by a least squares technique. An equation is used to describe how the test variables affect the response and determine the interrelationship among the variables [27].

RSM has been extensively used to optimize SPME [8,30,31] and SBSE [32] conditions for the analysis of volatile compounds in water, milk and vinegar.

The aim of this work is the optimization of a method for the determination of volatile compounds in wine vinegar employing headspace sorptive extraction–thermal desorption–gas chromatography–mass spectrometry (HSSE–TD–GC–MS). A CCD was used to optimize the sampling condition: sampling time, temperature and sample volume. The method is then applied to different wine vinegar samples and compared with previous reported methodologies based on SPME and SBSE.

2. Experimental

2.1. Reagents and chemicals

The standards of 57 aroma compounds were obtained from the commercial sources as follows: 3, 4, 9, 10, 12, 15, 16, 19, 20, 22, 24–27, 29, 31, 33, 34, 36, 38–40, 42–46, 48–56 (Sigma–Aldrich, Madrid, Spain); 1, 2, 5, 6, 13, 14, 17, 18, 21, 23, 32, 47, 57 (Merck, Darmstadt, Germany); 7, 8, 11, 30, 35, 37, 41 (Fluka, Madrid, Spain). 4-Methyl-2-pentanol (Merck) was employed as internal standards (IS). Numbers corresponding to different standards are given in tables. Sodium chloride, acetic acid and ethanol were supplied by Merck, and all of them were of analytical quality. Water was obtained from a Milli-Q purification system (Millipore, USA). A hydroaceticalcoholic solution with a mix of different standards was prepared for the optimization of sampling condition (Table 1). Volatile substances for the optimization were selected on the basis of their previously reported presence in different vinegar samples and their different volatilities. For most of the compounds, concentrations were chosen taking into account minimum amounts expected according to published data [7,33–35].

Table 1
Chemical composition of the solution employed in optimization experiments

Compound	Concentration ($\mu g\,L^{-1}$)
Benzaldehyde	621
Diacetyl ($mg\,L^{-1}$)	80
α-Ionone	50
Isovaleric acid	225
Octanoic acid	120
Propanoic acid	112
Ethyl acetate	1000
Methyl acetate	3000
Isoamyl acetate	153
Ethyl lactate	4210
Eugenol	109
Guaiacol	113
Isobutanol	2000
2-Methyl-1-butanol	193
3-Methyl-1-butanol	374
2-Phenylethanol	300
γ-Butyrolactone	221
trans-β-Methyl-γ-octalactone	89
cis-β-Methyl-γ-octalactone	80
Vanillin ($mg\,L^{-1}$)	30
2-Furfuraldehyde	50
Diethyl succinate	76.4
Acetoin ($mg\,L^{-1}$)	150
Acetic acid (g/L^{-1})	70
Ethanol (g/L^{-1})	20

2.2. Samples

A commercial wine vinegar was used to validate the analytical method. 4 red wine vinegars and 3 Sherry wine vinegars were used to test the suitability of the method and perform the comparative study. Red wine vinegars were produced by traditional fermentation (surface culture) methods in oak wood barrels (VRW1–VRW4). Sherry wine vinegars belong to the three commercial types recognised in the Denomination of Origin, named "Vinagre de Jerez" (VJ), "Vinagre de Jerez Reserva" (VJR) and "Vinagre de Jerez Gran Reserva" (VJGR), accounting for 6 months, 2 years and at least 10 years ageing in wood barrels, respectively. Each sample was analysed by triplicate.

2.3. HSSE sampling

The HSSE of samples was carried out employing special 20 mL headspace vials with open glass adapters provided by Gerstel (Müllheim an der Ruhr, Germany). 33% ca of NaCl was added to the sample. 10 mm long stir bar coated with 0.5 mm PDMS layer (Twister, Gerstel, Müllheim an der Ruhr, Germany) was put into the glass insert. The vial was tightly capped and was heated in a thermostatic bath. When the vial was at room temperature, the stir bar was removed with tweezers, rinsed with Milli-Q water and dried with a lintfree tissue paper. Finally, the stir bar was put into a glass tube of 60 mm in length, 6 mm o.d. and 4 mm i.d., which was placed in the autosampler tray of the thermo desorption unit for GC–MS analysis.