

# Stir bar sorptive extraction for the determination of volatile compounds in oak-aged wines

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## Abstract

Stir bar sorptive extraction (SBSE) followed by a thermal desorption-gas chromatography–mass spectrometry analysis has been optimized for the determination of 13 of the most important oak volatiles in wine samples, all in a single run. The stir bar sorptive extraction method was optimized in terms of temperature, time, pH and NaCl addition, and the best results were obtained after stirring the wine sample with the polydimethylsiloxane stir bar during 90 min at room temperature. 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 ranging from 0.92 to 0.99 for all the analytes. The reproducibility and repeatability of the method was estimated between 0.11 and 5.45%. The detection and quantification limits of all analytes were lower than their respective olfactory threshold values, and, most importantly, no artifacts have been observed during the analysis as described in most extractions using other current methodologies.

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## 1. Introduction

When aging wines, wood releases some compounds that may have a great influence on the final wine characteristics, and it also permits atmospheric oxygen to pass through its pores, leading to the gentle oxidation of certain wine components, a reduction in astringency, and changes in color and taste [1–3]. Oak is the most commonly used wood for wine aging, and its effect on wine quality depends, among other factors, on the species and geographical origin, usually American oak (*Quercus alba* L.) or French oak (*Quercus sessilis* L.); its manufactured process and the aging time [4–9].

According to the bibliography, hundreds of oak compounds have been identified, although only several are considered to make a significant contribution to wine flavor and wine quality [10,11]. Some of the compounds that may be released with an important effect on wine aroma composition due to their

low olfactory threshold are: vanillin, *trans*- and *cis*- $\beta$ -methyl- $\gamma$ -octolactone (whiskylactone) and eugenol.

Vanillin is an aromatic aldehyde which has a wine olfactory threshold of about 320  $\mu\text{g/L}$  [10]. It contributes to oak-aged alcoholic beverages with aromatic notes similar to vanilla. It is still not clear whether this aromatic note is due solely to this compound or also to others, as it is quite difficult to analyze [2,12]. *Cis*- and *trans*-whiskylactone isomers are specific from oak wood [13] and the ratio between them has been used to differentiate wines that have been in contact with other species used in wood barrels [6,14]. Associate aroma descriptors are fresh oak and coconut depending on their concentration, the *cis*-isomer being much more aromatic than the *trans*-isomer (the olfactory threshold of *cis* and *trans* racemic forms of whiskylactones in red wine is 74 and 320  $\mu\text{g/L}$ , respectively [15]). Eugenol is a genuine wood-extractable compound, not solely due to barrel toasting, and its concentration increases with the toasting temperature during barrel making [16–18]. Eugenol has a spicy, clove-like aroma descriptor and its olfactory threshold is 15  $\mu\text{g/L}$  [10].

Other volatile compounds that can be released from wood into wine are: furfural, 5-methylfurfural, as they are generated

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during barrel toasting and contribute with an almond-like aroma. The furfural olfactory threshold is 20 mg/L, while for 5-methylfurfural, it is 45 mg/L [19]; guaiacol and 4-methylguaiacol have smoky descriptors and 25 and 65  $\mu\text{g/L}$  olfactory threshold values, respectively [20]; 4-ethylguaiacol with 33  $\mu\text{g/L}$  olfactory threshold [21] and 40  $\mu\text{g/L}$  for vinylguaiacol [22] with aromatic notes of carnation flower and pepper; the 4-vinylphenol aroma is associated to phenolic and medicinal descriptors and its olfactory threshold is 180  $\mu\text{g/L}$  [23]; the horsy, leather and sweaty-saddle-like descriptors are due to 4-ethylphenol when its concentration is above 425  $\mu\text{g/L}$  [20,24]. All these compounds increase with oak-aging time, but also 4-ethylphenol and 4-ethylguaiacol may increase as phenolic acids degrade due to the action of some undesirable microorganism present in oak barrels [24]. Finally, syringaldehyde is a genuine wood compound that does not have a direct incidence on wine aroma composition as its olfactory threshold is higher than the rest of the compounds described (25 mg/L) [25]. However, it is commonly analyzed to differentiate from oak-aged and non-aged wines [2] and its degradation may increase the phenolic precursors of several ethylphenol compounds [26].

The chromatographic analysis of volatile wine compounds is a powerful tool to determine their quality and to understand their sensory impact. A detailed volatile analysis is quite complex and expensive; hence, there is a logical interest in developing reliable, quick and less expensive quantitative methods that allow us to study the volatile composition of wines. The compounds responsible for wine aroma are generally present in extremely low concentrations (sometimes lower than  $\mu\text{g/L}$ ) [27–31]. For this reason, a pre-concentration step of the target compounds such as liquid/liquid or solid/liquid extractions [6,20,32–35] is always carried out prior to their analysis by GC/MS. As several authors affirm [20,36], generally little or no consideration is given to the possibility that extraction methods could increase the risk of artifact formation during analysis. The relatively polar solvents (dichloromethane, ethanol/toluene) used by some authors could well extract precursors to the volatiles of interest that may then generate those volatiles during the analysis. An environmentally friendly approach has therefore been considered to avoid sample manipulation and solvent consumption, together with lower analysis time, by using thermally desorbed solid-phase microextraction (SPME) fiber techniques [20,33,34,37–41]. With the same SPME extraction theory but increasing its sensitivity by a factor of more than 100 [42], a magnetic stir bar covered with a polydimethylsiloxane coating (Twister, Gerstel, GmbH) has been successfully assayed in different matrixes [43–50].

The main aim of our research was to develop a fast and simple method able to quantify in wines the volatile composition related to oak wood aging in a single chromatographic run based on thermal desorption-gas chromatography–mass spectrometry of the analytes absorbed by the stir bar sorptive extraction (SBSE). The analyte extraction has been optimized in terms of ionic strength, temperature and extraction time. The compounds can have a great aromatic contribution and are closely related to quality in aged wine are: furfural,

5-methylfurfural, guaiacol, 4-methylguaiacol, 4-ethylguaiacol, 4-ethylphenol, 4-vinylguaiacol, 4-vinylphenol, eugenol, *cis*- and *trans*-whiskylactone, vanillin and syringaldehyde.

## 2. Experimental

### 2.1. Chemicals and reagents

#### 2.1.1. Standards

2-Furaldehyde (furfural), 5-methylfurfural, guaiacol (2-methoxyphenol), 4-methylguaiacol, 4-ethylguaiacol, 4-ethylphenol, 4-vinylguaiacol, 4-vinylphenol, eugenol (4-allyl-2-methoxyphenol), whiskylactone (*cis*- and *trans*- $\beta$ -methyl- $\gamma$ -octalactone isomers), vanillin and syringaldehyde were obtained from Sigma–Aldrich (Madrid, Spain). Exact masses of the chemical standards were dissolved in absolute ethanol.

#### 2.1.2. Solvents

Ethanol (analytical-reagent grade) was obtained from Merck (Darmstadt, Germany), while water was purified through a Milli-Q system (Millipore, Bedford, MA, USA).

### 2.2. Proposed extraction method

A sample of 25 mL of wine, to which 250  $\mu\text{L}$  of internal standard  $\gamma$ -hexalactone solution at 1  $\mu\text{L/mL}$  in absolute ethanol was added, was poured into a 25 mL aforesaid-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 room temperature 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  $^{\circ}\text{C}$ ; desorption time, 4 min; cold trap temperature,  $-30^{\circ}\text{C}$ ; helium inlet flow, 45 mL/min. The compounds were transferred into the Hewlett-Packard 6890 gas chromatograph coupled to a 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  $\mu\text{m}$  film thickness) (SGE, Ringwood, Australia). The chromatographic program was set at 50  $^{\circ}\text{C}$  (held for 2 min), raised to 230  $^{\circ}\text{C}$  at 12  $^{\circ}\text{C}/\text{min}$  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  $^{\circ}\text{C}$ . 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 a red wine solution spiked with five different concentrations of each analyte. Each level of concentration was analyzed twice with two different stir bars, so there were a total of four replicates. The concentration ranges were from 25 to 1000  $\mu\text{g/L}$ . The detection and quantification limits (LOD and LOQ, respectively) were calculated with the data generated in the linearity studies. LOD was defined as  $(a + 3Sa/b)$  and LOQ as  $(a + 10Sa/b)$ , “a” being the origin ordinate, “Sa” 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 [51].

The standard deviation for each compound (square root of the arithmetic mean of the variances) was calculated to obtain the repeatability (%RSD). 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) [52].

## 3. Results and discussion

In order to optimize the best extraction conditions for oak wine volatile compounds using SBSE, factors such as temperature, agitation time and ionic strength were considered. After absorption in the stir bar, the analytes were thermally desorbed and determined by GC/MS. To avoid wine matrix interferences between the aromatic compounds, the MS analysis was carried out in the single ion monitoring (SIM) mode using their characteristic  $m/z$  values. The internal standard was also quantified in the single ion monitoring (SIM) mode at its  $m/z$  85. A typical total ion chromatogram using the experimental conditions discussed below is shown in Fig. 1. Extraction conditions were studied by comparing the relative peak area compound (peak area of the target analyte/I.S. peak area ratio). The influence of extraction time was evaluated by immersing the stir bar into the sample for 30, 60 and 90 min at room temperature and 60 °C. As

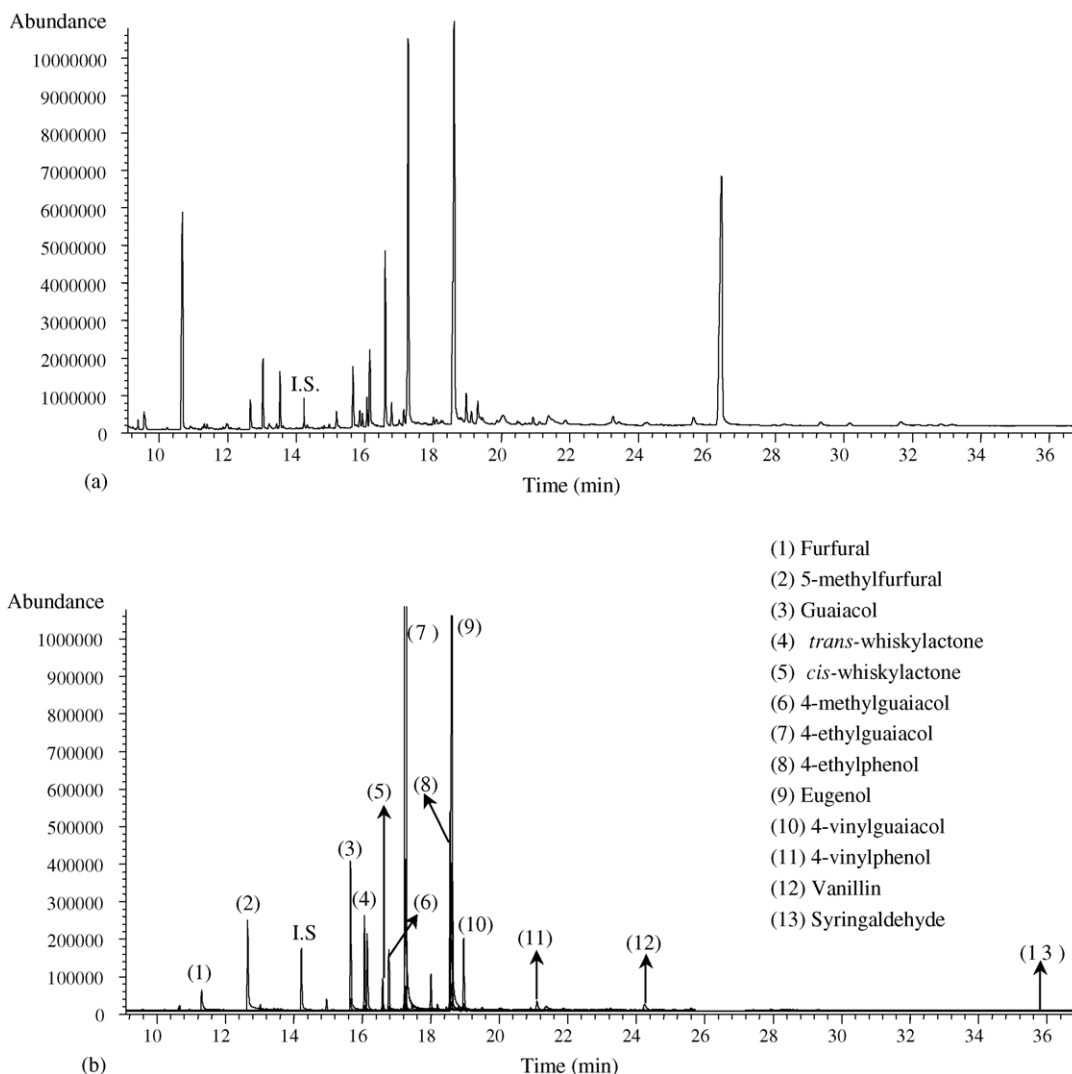


Fig. 1. (a) Red wine chromatogram analyzed by stir bar sorptive extraction (SBSE) with gas chromatography–mass spectrometry technique; (b) overlaid selected ion chromatograms of the 13 target compounds at 2  $\mu\text{g/L}$  in red wine.

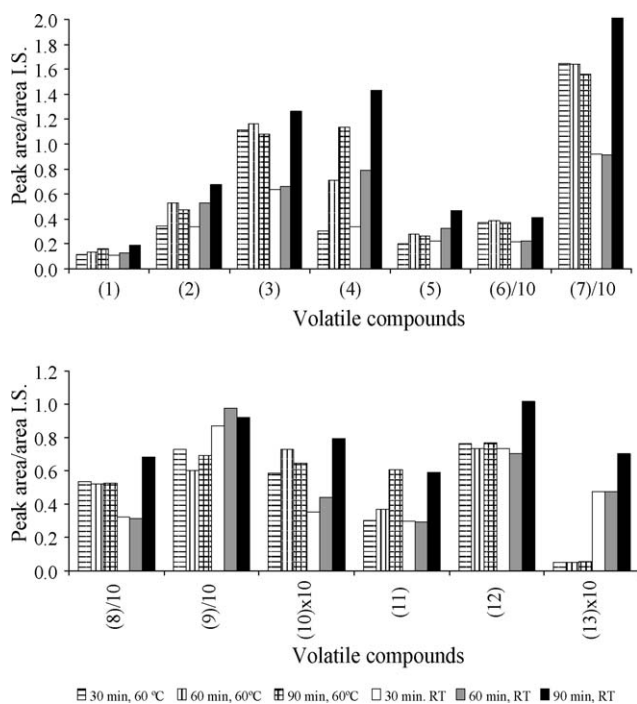


Fig. 2. Peak area vs. I.S. area at different conditions. Note: volatile compound (1) furfural; (2) 5-methylfurfural; (3) guaiacol; (4) *trans*-whiskylactone; (5) *cis*-whiskylactone; (6) 4-methylguaiacol; (7) 4-ethylguaiacol; (8) 4-ethylphenol; (9) Eugenol; (10) 4-vinylguaiacol; (11) 4-vinylphenol; (12) vanillin; (13) syringaldehyde.

can be observed in Fig. 2, the higher the extraction time (90 min) at room temperature, the higher the concentration of compounds such as 5-methylfurfural, *cis*- and *trans*-whickilactone, 4-methylguaiacol, 4-ethylguaiacol, 4-ethylphenol, eugenol, 4-vinylguaiacol, vanillin and syringaldehyde. No significant differences were observed in the case of guaiacol, 4-vinylphenol and furfural. The influence of ionic strength was evaluated by addition of two different amounts of sodium chloride (0 or 1 g) but no significant differences were observed (data not shown).

The optimum stir bar sorptive extraction conditions were therefore fixed as: 25 mL of the sample spiked with the target analytes and stirred at 700 rpm with a stir bar at room temperature for 90 min. A saturation effect on the polydimethylsiloxane

stir bar was observed for several compounds when assaying higher concentration levels (up to 1 mg/L).

Different calibration curves were made in a wide concentration range, including olfactory threshold values of the analytes commonly found in wine samples [2,6,24,53]. Since the recoveries of all analyzed compounds were higher than 98%, the calibration curves were carried out on wine samples and not on synthetic wines. The method showed good linearity over the concentration ranges tested and the correlation coefficients were higher than 0.98 for all the analytes, except 4-vinylphenol (0.92) (Table 1). It is important to point out the excellent signal-to-noise ratio observed for the individual ions. Blank runs of the stir bar were made before and after each analysis and no memory effect occurred for the target solutions. The precision of the experimental procedure was also evaluated. For the reproducibility of a method (%RSD) to be considered acceptable, its value should be less than 20%. As can be seen in the results summarized in Table 2, this parameter ranged from 0.026% (4-vinylguaiacol) to 5.45% (4-vinylphenol). The same limit (20%) was taken to represent good repeatability; in this case ranging from 0.015% (4-vinylguaiacol) to 3.55% (*cis*-whiskylactone). In the literature, many analytical methods have been described to determine oak volatiles, but only a few show the method validation parameters. In this case, the authors want to point out that the method proposed by SBSE shows very good repeatability and reproducibility parameters. Moreover, the quantification and detection limits were always lower than their known olfactory thresholds. The methodology proposed in this paper allowed us to determine, in a quick and easy procedure with a low sample volume and no manipulation, the 13 most important volatile compounds responsible for oak-aged wines.

Upon examining in detail, each volatile compound studied in this paper, we can see, for example, that vanillin determination is known to be quite problematic due to the acetal formation from wine glycols during the liquid–liquid extraction and concentration steps required for its analysis [12]. Some authors persist in using a high pH [54], although an important artifact formation has been observed [12]. Others try to overcome this artifact by using stable isotope dilution analysis (SIDA) [20,55] followed by a headspace-SPME analysis, even though this generally shows relatively poor precision and sensitivity,

Table 1  
*m/z* values and the linearity data obtained for the identified compounds

Volatile compound	Selected ions ( <i>m/z</i> )	Slope ( $\times 10^{-3}$ )	Intercept	Correlation coefficient ( $r^2$ )
Furfural	96	0.03	0.0179	0.983
5-Methylfurfural	110	0.4	0.0466	0.986
Guaiacol	124	0.5	-0.0148	0.999
<i>Trans</i> -whiskylactone	99	0.3	-0.0119	0.999
<i>Cis</i> -whiskylactone	99	0.1	0.058	0.944
4-Methylguaiacol	138	1.5	-0.0605	0.999
4-Ethylguaiacol	137	2.9	0.3748	0.999
4-Ethylphenol	107	1.0	-0.0193	0.999
Eugenol	164	3.6	0.055	0.999
4-Vinylguaiacol	151	0.3	0.0896	0.949
4-Vinylphenol	120	0.07	0.1023	0.922
Vanillin	152	0.1	0.0237	0.987
Syringaldehyde	182	0.04	0.0046	0.995

Table 2  
Precision of the experimental procedure

Volatile compounds	LOD	LOQ	Reproducibility (%)	Repeatability (%)
Furfural (ng/L)	3.26	3.40	4.10	2.39
5-Methylfurfural (ng/L)	0.10	0.11	0.87	0.50
Guaiacol ( $\mu\text{g/L}$ )	38.93	39.23	0.80	0.46
<i>Trans</i> -whiskylactone (ng/L)	0.40	0.41	0.88	0.51
<i>Cis</i> -whiskylactone (ng/L)	0.61	0.69	5.08	3.35
4-Methylguaiacol ( $\mu\text{g/L}$ )	38.99	39.03	0.35	0.20
4-Ethylguaiacol (ng/L)	0.12	0.13	1.42	0.82
4-Ethylphenol ( $\mu\text{g/L}$ )	21.00	20.99	0.16	0.093
Eugenol ( $\mu\text{g/L}$ )	14.78	14.79	0.31	0.18
4-Vinylguaiacol (ng/L)	0.29	0.30	0.026	0.015
4-Vinylphenol (ng/L)	1.24	1.34	5.45	3.15
Vanillin (ng/L)	0.24	0.24	0.44	0.255
Syringaldehyde (ng/L)	0.13	0.14	0.11	0.065

presumably due to the low volatility of vanillin and its preference for the liquid phase. López et al. [56] proposed the solid-phase extraction methodology of vanillin with a %RSD of 15% while Díaz-Maroto [40] showed %RSD values of 4.8 and 9.8% when analyzing different oak chips.

With the use of stir bar sorptive extraction, no artifacts related to vanillin have been detected as the conditions proposed within this method do not require any pH modification, nor the use of polar solvents or any concentration process. Thus, detection limits lower than its olfactory threshold as well as a very good reproducibility (0.44%) and repeatability (0.255%) have been obtained (Table 2).

In the case of whiskylactones, the same artifact effect has been observed with the numerous analytical methodologies proposed in the literature [20,33,57]. Hayasaka et al. [58] applied SBSE to the analysis of volatile compounds in wine and detected the whiskylactones in scan mode, although investigations into oak aging were not the focus of this paper. The paper [58] was more concerned with identification of wine components in general, with little focus on quantitative method validation. The detection and quantification limits for *trans*-whiskylactone are 0.40 and 0.41 ng/L, respectively. The *cis*-isomer showed LOD and LOQs values of 0.61 and 0.69 ng/L. In both cases, values lower than their respective olfactory threshold and lower than the 1  $\mu\text{g/L}$  found when liquid–liquid or SPME extraction techniques have been assayed [33]. The reproducibility (0.88%) and repeatability (0.51%) values of this method for this compound were much lower than the ones found by using SPME [33,40].

As for the volatile phenols such as: 4-ethylguaiacol, 4-ethylphenol, eugenol, 4-methylguaiacol, guaiacol, 4-vinylguaiacol and 4-vinylphenol, only some of them have been identified in a single run. Pollnitz et al. [34] demonstrated the use of deuterated 4-ethylphenol to determine 4-ethylphenol and 4-ethylguaiacol in oak-aged wines followed by its extraction with diethyl ether: pentane and identification by GC/MS and quantified concentrations down to 1  $\mu\text{g/L}$  and often lower. Values that can be easily detected for 4-ethylguaiacol when SBSE was used, but not for 4-ethylphenol although values lower than its olfactory threshold. With this methodology, RSD values of 1.35% for 4-ethylphenol and 1.31% for 4-ethylguaiacol were

calculated. The same compounds have been analyzed by Martorell et al. [59] with headspace-SPME resulting in detection and quantification limits lower than their respective olfactory thresholds and a %RSD in both cases of 10%. The same conclusions can be reached when eugenol, 4-vinylguaiacol and 4-vinylphenol and syringaldehyde were extracted with other volatile compounds by solid-phase extraction [40,56] and the %RSD values ranged between 1.7 and 12.5%. The identification and quantification of 4-ethylguaiacol, 4-ethylphenol, 4-vinylguaiacol and 4-vinylphenol by SBSE was studied by Díez et al. [47], but with different extraction parameters and the limits ranged from 373  $\mu\text{g/L}$  (4-vinylphenol) to 6  $\mu\text{g/L}$  (4-ethylphenol). In this paper, the results of 4-ethylguaiacol was of the same order, but for 4-vinylguaiacol and 4-vinylphenol were between 3 and 4 times lower; 4-ethylphenol values were higher although lower than its olfactory threshold, and the RSD values ranging from 0.065 to 0.46% (Table 2).

In relation to furfural and 5-methylfurfural, López et al. [56] found a %RSD of 10.5 and 12.3%, respectively, using the solid-phase extraction methodology, whereas with HS-SPME [40] values between 2.0 and 9.7% were found, depending on the type of oak. With SBSE, RSD (%) values were 2.39 and 0.50%, respectively, and the detection and quantification limits, for the rest of compounds as well, were lower than the olfactory thresholds.

The results presented in this report for the analysis of oak volatile compounds in wine submitted to oak aging indicate that the stir bar sorptive extraction technique is an excellent technique, due to the fact that all compounds are clearly and easily detected. With no pre-concentration step and a much reduced analysis time, all the analyzed compounds, except for 4-ethylphenol, showed detection and quantification limits that were lower than those observed with other methods found in the bibliography and, more importantly, with concentrations lower than their olfactory threshold values including the 4-ethylphenol.

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