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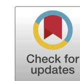

A two-run heart-cut multidimensional gas chromatography method using flame ionization and mass spectrometry for automated and robust determination of nearly complete wine aroma-volatile profiles

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ABSTRACT

A quantitative analytical method capable of determining the concentrations of 81 aroma-relevant wine volatiles covering nine orders of magnitude was developed and validated in this study. The method is based on stir bar sorptive extraction (SBSE) of 200 μL of wine diluted with 1.8 mL NaCl brine with pH 3.5. Volatiles thermally desorbed from the stir bars were separated in two runs in a heart-cut multidimensional gas chromatographic system and quantified using either a flame ionization detector (FID) in the first dimension (27 aroma compounds) or a mass spectrometer in the second dimension (54 aroma compounds, transferred to 22 cuts). Typical limits of compound detection lay around 0.02 mg/L by FID or ranged from 0.001 to 0.30 μg/L by mass spectrometry detector, liying below the corresponding odor thresholds in all cases. Linearity, reproducibility, and recovery were considered satisfactory for most compounds, with typical R^2 values of 0.989–0.999, relative standard deviation below 10 % for 37 compounds and between 10 and 20 % for 44 compounds, and recovery rates of approximately 100 % (85–109 %) for all but acetaldehyde. An analysis of 20 wine samples completed our validation of the method, showing that a single-sample preparation procedure combined with heart-cut multidimensional two-detector gas chromatography can determine wine volatile concentrations ranging from 350 mg/L of isoamyl alcohol to 3.8 ng/L of 3-isobutyl-2-methoxypyrazine.

1. Introduction

In the delicate, intricate task of wine aroma modeling, it is essential for researchers and wine producers to be able to determine all the relevant aroma that act alone or in concert and which are responsible for a wide range of aromatic sensory perceptions. The pool of aroma-relevant volatiles only constitutes a minor subset of a wine's overall volatile profile, which can contain several hundreds of additional volatile constituents: more than one thousand have been identified [1]. Strategies for the assessment of the potential relevance of aroma volatiles have improved through decades of research. At the dawn of the field of aroma analysis, only volatile compounds were analyzed [2]; later, concentration data were normalized by applying odor thresholds to obtain corresponding odor activity values [3]. Recent interpretations attempt to take perceptual interactions among odorants into account [4]. This nevertheless demands a somewhat holistic approach since all relevant aroma vectors need to be measured, requiring, in some instances, the quantification of several sub-threshold aroma compounds [5].

According to current scientific consensus, a wine's positive aroma notes essentially depends on a combination of up to 80 different volatile compounds, some of which are present above their odor threshold [6]. In-depth inspection of this group of odorants as analytical targets has revealed a considerably degree of heterogeneity in terms of chemical functionalities, chemophysical properties and ranges of occurrence. This diversity jeopardizes the possibilities of using broad-scope analytical strategies and makes it necessary to complement data from general gas chromatograph with flame ionization detection (GC-FID) or comprehensive two-dimensional gas chromatography (GC–MS) methods with highly specific analytical methodologies for the quantitative determination of those aroma compounds that are the most difficult to detect.

Generic analytical strategies are based on nonselective extraction methods and subsequent GC, GC–MS, or comprehensive 2-D GC (GCxGC) analysis of the extract with the aim of obtaining quantitative

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data from as many volatile compounds as possible in a single chromatographic run. Such strategies are convenient for wine components considered “easy to analyze”, as defined by [6], that is, compounds present in all wines at concentrations above μg/L and lacking any further special requirements in terms of stability, detectability, or matrix interactions. With adequate sample preparation, the most affordable chromatographic system, a GC-FID, can adequately serve the purpose of quantifying major secondary metabolites from alcoholic fermentation (range 0.2–400 mg/L), such as higher alcohols and their acetates, volatile fatty acids and their ethyl esters, 2,3-butanodione, 3-hydroxybutanone, and acetaldehyde. Many different preconcentration techniques have been combined with GC-FID to achieve this. Liquid-liquid extraction has probably been the most frequently used technique: for example, Lilly et al. [7] applied it to obtain data regarding 21 major compounds. Liquid-liquid microextraction to determine 21 volatile compounds [8] and headspace solid-phase microextraction (HS-SPME) to quantify 18 major components [9] have also been used. Static headspace extraction is also viable [10]. Even direct injection, although not very friendly to the inlet system, has been applied for this type of analysis [11]. The next predictable step in this generic strategy would be to use GC–MS, not only because the improved sensitivity of such a system allows for the determination of smaller concentrations, but also because spectrometric data can help avoid misidentifications. In combination with the same sample preparation techniques as those mentioned above, GC–MS systems are capable of determining minor volatile compounds (concentrations below 0.2 mg/L), but at the cost of losing the quantification of major compounds in order to protect the MS detector. For example, Mihnea et al. [12] reported the quantification of 42 volatiles using liquid-liquid extraction, Lopez et al. [13] applied solid phase extraction (SPE) to 29 compounds in the range of 0.1–600 μg/L, Fang et al. [14] preconcentrated with stir bar sorptive extraction (SBSE) to likewise determine 29 volatiles in the same range, and HS-SPME was used to quantify 47 volatile components with multivariate calibration [15]. All these methodologies have been recently reviewed [6,16–18].

An obvious way to expand the number and ranges of occurrence of analytes targeted in the same analytical method is by using multidimensional chromatographic techniques combined with a non-selective extraction strategy. Among the sample preparation techniques mentioned above, SBSE has several advantages that make it a good choice for a global analysis of wine aroma. SBSE has a substantial amount of sorbent, resulting in a high sample extraction capacity; moreover, the technique can be automated and requires no solvent, thereby facilitating GC injection. The risk of column overloading and peak interference associated with this approach could be addressed by coupling SBSE with multidimensional chromatography. GC × GC coupled with SBSE has been used to study large numbers of wine volatiles [19,20]; however, as a non-target metabolic strategy, it lacks the necessary quantitative information to understand those volatiles’ impact on wine aroma. Moreover, due to the detector’s limitations, compounds determined in the same run cannot lie further apart from one another than 5 orders of concentration magnitude.

On the other hand, SBSE combined with two-dimensional gas chromatography, specifically in a heart-cutting format (GC-GC), has proven highly useful in solving a series of particularly difficult targets in wine aroma analysis [21–24]. As reflected in the latter references, GC-GC has typically been applied to resolve the co-elutions of certain compounds. However, GC-GC could also serve as a tool designed to increase the number of compounds quantifiable with non-selective preconcentration technique by preventing the major compounds from reaching the MS detector and causing saturation. Furthermore, a multidimensional setup could allow researchers to quantify those major compounds by redirecting them to a less sensitive detector.

Our study’s aim was thus to evaluate the performance of an alternative method based on a heart-cut multidimensional automated thermal desorption (TD) GC-GC-FID/MS system that took advantage of the combined performance of the FID and MS detectors to expand the range of concentrations and quantifiable components in the test while employing a well-established, robust SBSE extraction strategy.

2. Material and methods

2.1. Reagents and standards

We purchased the chemical standards for this study from Chem Service (West Chester, PA), PolyScience (Niles, IL, USA), Fluka (Buchs, Switzerland), Sigma-Aldrich (Munich, Germany), Merck (Darmstadt, Germany), Panreac (Barcelona, Spain), Probus (Barcelona, Spain), Lancaster (Strasbourg, France), Sugelabor (Madrid, Spain), Firmenich (Geneva, Switzerland), and SAFC (Steinheim, Germany).

Stir bars coated with 126 μL polydimethylsiloxane (PDMS, 20 mm length x 0.1 mm thickness) were obtained from Gerstel (Müllheim an der Ruhr, Germany). Before first use, each stir bar was conditioned at 300 °C under constant helium flow for 2 h. Then, before each use, we inspected each stir bar under a magnifiying glass to check for any cracks or signs of deterioration that could affect extraction efficiency.

2.2. Wine samples and solutions

We prepared each chemical standard in individual ethanol solutions, which, in turn, were used to group the chemical standards into a series of intermediate stock solutions destined to be mixed and diluted to final concentrations in the synthetic wine. The synthetic wine used in this study consisted of a solution of water/ethanol 12 % (v/v) with 3.5 g/L of tartaric acid and pH adjusted to 3.2. The buffer solution was prepared with 200 g/L NaCl and 5 g/L tartaric acid adjusted to pH 3.5 with NaOH. Two internal standard solutions were used: solution A contained 4-methyl-2-pentanol, 2-octanol, ethyl heptanoate, heptanoic acid, and nonanoic acid, each of them in a quantity of 15 mg per L of ethanol. Solution B contained β-damascone (500 μg/L) and 3,4-dimethylphenol (60 μg/L) in ethanol. To validate the method, we used two Spanish young wines, one red and one white.

To validate the proposed method, we used it to analyze twenty Spanish white wines stemming from nine different Spanish Denominations of Origin: Rueda, Ribeiro, Catalunya, Allela, Navarra, Rioja, Bierzo, La Mancha, and Penedés, elaborated in the vintages 2017 to 2019 with several different grape varieties (Macabeo, Godello, Verdejo, Xarel·lo, Garnacha Blanca, Parellada, Malvasía, Palomino, Godello, Zalema, Airen). All wines were purchased from a local retail outlet; none of them presented any noticeable off-flavors.

2.3. Stir bar sorptive extraction procedure

For each analysis, 200 μL of the sample, 1.8 mL of buffer solution, and 20 μL of each internal standard solution were transferred into a clean 25 mL Erlenmeyer flask, to which a previously conditioned stir bar was added. The closed flask was placed in a 20-position magnetic stirrer (Gerstel). Extraction conditions were: stirring at room temperature and 400 rpm for 60 min. After sampling, the PDMS-coated stir bar was removed with a magnetic bar, rinsed in ultrapure water, and dried with lint-free tissue. Each stir bar was inserted into a thermal desorption tube (60 mm length and 5 mm internal diameter) and placed in the autosampler tray for analysis. This procedure was performed twice for each sample (Runs 1 and 2).

2.4. Instrumental set-up

Analyses were performed using a GC-GC system equipped with a thermal desorption injection TD-30R system, a Deans switch device coupled to a FID in the first dimension, and a QP 2010plus quadrupole mass spectrometer in the second dimension (all systems from Shimadzu, Tokyo, Japan). The analytical column set consisted of a 30 m x 0.25 mm x 0.25 μm ZB-FFAP column (Phenomenex, Alcobendas, Spain) for the

first dimension and a 30 m x 0.25 mm x 1 μm ZB-5MSplus column (Phenomenex, Alcobendas, Spain) for the second dimension.

Volatiles were desorbed from the stir bar on the thermal desorption unit at 300 °C for 15 min with a 50 mL/min He flow and focused on an internal Tenax trap at −15 °C. The volatiles were desorbed from that internal trap at 300 °C for 10 min and then transferred to the first column through a transfer line kept at 250 °C.

The analyses were performed in two consecutive runs, Runs 1 and 2, which were differentiated by their heart-cutting window times. The first column oven program was the same for both runs: after an initial period of 5 min at 40 °C, oven temperature was raised by a rate of 2 °C/min to 85 °C, then at 4 °C/min up to 145 °C, and then at 8 °C/min to 240 °C held for 30 min. The second column oven program for both runs started at 35 °C for 55 min then rose by a rate of 3 °C/min up to 190 °C for Run 1 and to 175 °C for Run 2. Finally, in both runs, oven temperature in the second dimension increased at 20 °C/min up to 300 °C and was held for 5 min. Heart-cutting windows times for Run 1 were as follows. Cut 1: 35.0–39.4 min, Cut 2: 40.1–41.0 min, Cut 3: 44.3–44.8 min, Cut 4: 45.6–47.0 min, Cut 5: 51.4–52.5 min, Cut 6: 53.1–54.1, Cut 7: 54.9–55.7 min, Cut 8: 56.2–62.0 min, Cut 9: 67.0–69.0. Windows times for Run 2 were as follows. Cut 1: 6.9–8.5 min, Cut 2: 9.1–11.7 min, Cut 3: 15.5–18.7 min, Cut 4: 27.0–28.3 min, Cut 5: 32.5–32.8 min, Cut 6: 34.1–34.8 min, Cut 7: 41.3–42.0 min, Cut 8: 48.1–48.6 min, Cut 9: 48.7–49.2 min, Cut 10: 49.3–50.0 min, Cut 11: 50.2–51.5 min, Cut 12: 52.5–52.6 min, Cut 13: 54.1–54.3 min. Helium was used as carrier gas with a constant linear velocity of 22.4 cm/s in the first dimension and 50.5 cm/s in the second dimension. The FID was kept at 270 °C. The single quadrupole mass detector was operated in selected ion monitoring mode (SIM). The temperature of the ion source and quadrupole were set at 200 °C, and the transfer line was kept at 240 °C. Details of retention times, internal standards, chromatographic cuts, and quantifier and qualifier ions are detailed in Tables 1 and 2.

2.5. Blanks

To prevent contamination by carryover, analysis of blanks of the previously described synthetic wine was carried out once every ten samples. Additionally, stir bars were visually inspected to check for potential cracks in the PDMS phase; if detected, a blank analysis of the stir bar was performed to ensure complete desorption of the volatiles.

2.6. Method validation

To assess the method's performance, we used the following quality parameters: linearity was tested using the previously prepared working solutions at five concentration levels in triplicate. The relative peak areas of A_0/A_i (where A_0 is the peak area of the target compound and A_i is the peak area of the internal standard) were used for linear regression analysis. The limit of detection (LOD) was determined as three times the signal-to-noise ratio ($S/N = 3$). The rate of inter-day precision was determined by analyzing the spiked red and white wines on six different days. We examined the method's accuracy by applying a standard addition procedure to analyze the same wines in triplicate with and without spiking with the standard solutions in typical wine quantities and calculating the obtained concentration with respect to the expected concentration.

Table 1
Compounds and internal standards analyzed in the first column using GC-FID. Run number, retention times (R_t), and assigned internal standard.

Compound	Run	R_t (min)	I.S.[a]
Acetaldehyde	run 1	3.02	4M
Ethyl acetate	run 1	4.66	4M
Ethyl propanoate	run 1	6.60	4M
2,3-Butanedione	run 1	7.33	2O
Ethyl butyrate	run 1	9.21	2O
2-Methylpropanol	run 1	11.73	2O
Isoamyl acetate	run 1	13.54	2O
1-Butanol	run 1	14.78	4M
Isoamyl alcohol	run 1	18.89	2O
4-Methyl-2-pentanol	run 1	17.71	I.S.
Ethyl hexanoate	run 1	20.51	EH
Hexyl acetate	run 1	23.27	EH
3-Hydroxybutanone	run 1	25.82	2O
Ethyl heptanoate	run 1	27.20	I.S.
Ethyl lactate	run 1	27.76	2O
1-Hexanol	run 1	28.43	2O
cis-3-hexenol	run 1	30.47	2O
2-Octanol	run 1	32.77	I.S.
Ethyl octanoate	run 1	33.51	2O
Acetic acid	run 1	34.74	HA
2-Methylpropanoic acid	run 1	39.69	HA
γ-Butyrolactone	run 1	41.15	2O
Butyric acid	run 1	41.81	HA
Ethyl decanoate	run 1	42.27	2O
3-Methylbutyric acid	run 1	43.44	HA
Diethyl succinate	run 1	43.72	2O
Methionol	run 1	45.06	2O
Hexanoic acid	run 1	48.50	HA
Benzyl alcohol	run 1	49.24	2O
β-Phenylethanol	run 1	49.98	2O
Heptanoic acid	run 1	50.98	I.S.
Octanoic acid	run 1	52.63	NA
Nonanoic acid	run 1	54.30	I.S.
Decanoic acid	run 1	55.79	NA

[a] Internal standard. 4M: 4-methyl-2-pentanol; 2O: 2-octanol; EH: ethyl heptanoate; HA: heptanoic acid; NA: nonanoic acid.

3. Results and discussion

3.1. Heart-cut multidimensional gas chromatography separation

This study aimed to develop a complete analytical method for the quantitative determination of as many as possible of the relevant volatile compounds involved in wine aroma. Given the wide range of concentrations at which these compounds appear in wine and the large number of potential coelutions – many of which cannot be satisfactorily solved by mass spectrometry – we studied the use of heart-cut multidimensional gas chromatography with an FID detector in the first dimension for the determination of well-separated major compounds, and a mass spectrometer in the second for the determination of the remaining compounds. Considering that the predominant volatiles in wine are alcohols and fatty acids, we chose a terephthalic-acid-modified carbowax (FFAP) for the first dimension in order to limit the typical tailing and fronting problems observed with polar compounds in non-polar phases. For the second chromatographic dimension, we chose a non-polar phase of 5 %-phenyl-arylene-95 %-dimethylpolysiloxane column in order to obtain maximum orthogonality in the separation [25]. To ensure a certain degree of retention of the most volatile compounds in the second dimension, we chose to use a relatively thick phase (1 μm of thickness).

Once we had decided upon the combination of columns, the first step in the procedure consisted in ascertaining the most adequate set of GC-GC conditions. As mentioned above, in FID in the first dimension we quantified major volatiles, present in wine in the range 0.2–400 mg/L and transferred minor compounds (levels lying below 0.2 mg/L) to the second dimension for them to be determined with the MS detector. We initially spiked the target compounds into red wine at typical wine concentrations with the aim of selecting the most adequate chromatographic cuts that would allow for complete transfer of trace compounds to the second dimension without affecting the major compounds. However, after several trials, it became clear that it was impossible to transfer all the targeted trace compounds while maintaining the integrity of the peaks of the major compounds in the first dimension. We therefore split our analysis into two runs. In the first run (Run 1), all relevant major compounds were separated in the first dimension using the FID as detector, and only those trace compounds whose cuts did not affect any major compound were selectively transferred to the second

Table 2
Compounds and internal standards analyzed in the second column, run, chromatographic cuts in the first column, retention times (R_t) in the second column, selected *m/z,* and assigned internal standard.

Compound	Run	Cut (min)	R_t (min)	Quantifier ions *m/z*	Qualifier ions *m/z*	I.S. [a]
Ethyl 2-methylpropanoate	run 2	6.90–8.50	17.08	116	71, 88	EH
Isobutyl acetate			20.60	73	43, 56	EH
Butyl acetate	run 2	9.10–11.70	19.66	73	115, 56	EH
Ethyl 2-methylbutyrate			32.83	102	115, 85	EH
Ethyl 3-methylbutyrate			34.84	88	85, 115	EH
Limonene	run 2	15.50–18.70	72.40	136	93, 107	BD
1,8-Cineole			72.63	154	108, 139	BD
Ethyl heptanoate	run 2	27.00–28.30	78.12	88	113, 101	I.S.
cis-Rose oxide			78.93	139	83, 154	BD
Ethyl cyclohexanoate			80.53	156	101, 111	EH
3-Isopropyl-2-methoxypyrazine	run 2	32.5–32.80	77.70	137	152, 124	BD
Methional	run 2	34.05–34.80	64.01	104	61, 76	BD
Linalool			78.68	136	93, 121	DM
Linalool oxide			76.64	94	59, 93	BD
Benzaldehyde	run 1	35.00–39.40	69.31	106	105, 77	DM
Ethyl 2-hydroxy-4-methylpentanoate			75.98	104	69, 87	DM
3-Nonen-2-one			81.66	125	97, 111	DM
3-sec-butyl-2-methoxypyrazine			82.41	138	124, 151	BD
3-Isobutyl-2-methoxypyrazine			82.99	124	94, 151	BD
Phenylacetaldehyde	run 2	41.25–42.00	75.28	91	92, 120	BD
β-Damascone			94.85	177	69, 123	I.S.
Geosmine			95.42	112	125, 149	BD
α-Terpineol	run 1	44.30–44.80	84.29	136	121, 93	DM
TDN			92.41	157	142, 172	DM
β-Citronellol	run 1	45.60–47.00	85.78	156	138, 123	DM
Phenylethyl acetate	run 2	48.05–48.55	87.30	104	93, 121	EH
β-Damascenone			93.47	190	69, 121	BD
α-Ionone			95.46	192	121, 136	BD
Guaiacol	run 2	48.65–49.24	78.21	124	109, 81	DM
Geraniol			87.01	121	93, 136	BD
trans-Whiskylactone	run 2	49.31–50.00	89.05	99	87, 71	BD
Ethyl dihydrocinnamate	run 2	50.20–51.50	91.93	178	104, 91	DM
cis-Whiskylactone			90.64	99	71, 87	BD
o-cresol	run 1	51.35–52.45	76.40	108	107, 79	DM
4-Ethylguaiacol			88.31	152	137, 122	DM
γ-Nonalactone			92.51	85	128, 100	DM
β-Ionone	run 2	52.45–52.60	98.02	177	43, 135	BD
m-Cresol	run 1	53.10–54.05	77.70	108	107, 79	DM
Ethyl cinnamate			97.50	176	131, 103	DM
Eugenol			92.16	164	149, 131	DM
4-Propylguaiacol			92.64	166	137, 122	DM
γ-Decalactone	run 2	54.05–54.25	97.40	85	128, 100	DM
4-Ethylphenol	run 1	54.90–55.65	82.64	107	122, 77	DM
3,4-Dimethylphenol			84.05	107	122,77	I.S.
4-Vinylguaiacol			90.16	150	135, 107	DM
Massoia lactone			97.79	97	68, 108	DM
δ-Decalactone			98.58	99	71, 114	DM
4-Vinylphenol	run 1	56.20–62.00	85.38	120	91	DM
2,6-Dimethoxyphenol			91.84	154	139, 111	DM
Vanillin			94.26	152	151, 123	DM
trans-Isoeugenol			96.60	164	149, 131	DM
Acetovanillone			98.17	166	151, 123	DM
Methyl vanillate			99.47	182	151, 123	DM
Ethyl vanillate			102.43	196	151, 168	DM
4-Allyl-2,6-dimethoxyphenol			102.86	194	91, 179	DM
Linalyl acetate	run 1	65.00–39.40	86.88	136	93, 121	DM
Syringaldehyde	run 1	67.00–69.00	105.33	182	181, 167	DM

[a] Internal standard. DM: 3,4-dimethylphenol; BD: β-damascone; EH: ethyl heptanoate.

dimension for MS detection (Fig. 1). The 29 major compounds analyzed with FID are reported in Table 1; for the most part, they are secondary metabolites of alcoholic fermentation that commonly appear in wine at concentrations liying above their odor thresholds [6]. In the time windows of the chromatogram where none of those analytes eluted, we programmed the Deans switch system for nine different cuts in Run 1, as detailed in Table 2. Those nine cuts transferred 28 targeted trace compounds to the second column, where they were analyzed in SIM mode with the MS detector. The retention times in the second dimension for those compounds, together with their detection conditions, are also shown in Table 2. Remarkably, we were able to analyze the heaviest and most polar trace compounds, such as phenols and vanillin derivatives, in Run 1, due to their elution at higher retention times in the polar column containing less interesting major compounds. Other relevant aroma compounds, such as alkylmethoxypyrazines, terpenes, and lactones, were also analyzed in this run. In total, 57 compounds of interest were analyzed in Run 1.

The remaining trace compounds reported in Table 2 were analyzed in Run 2. In this second run, the Deans switch valve was programmed for 16 heart-cutting windows to transfer 25 additional trace aroma compounds to the second dimension (Fig. 2), where they were further quantified with the MS detector in SIM mode (Table 2). Light and less polar trace aroma compounds were mostly transferred to the second column in this run, since most of them elute close to or coelute with

Fig. 1. Top: Chromatogram of the first column (GC-FID) Run 1 of a wine sample. Bottom: TIC chromatogram of the Run 1 of the second column of a wine sample (GC-GC–MS).

Fig. 2. Top: Chromatogram of the first column (GC-FID) Run 2 of a wine sample. Bottom: TIC chromatogram of the Run 2 of the second column of a wine sample (GC-GC–MS).

some of the relevant major aroma compounds analyzed in the first dimension. This is the case for ethyl esters of branched acids or ethyl cyclohexanoate which co-elute with major esters, acetates, and alcohols in polar columns [25]. Typical GC-FID and GC–MS chromatograms are shown in Fig. 1. Overall, taking into account all compounds separated and measured in the two runs, the system provided analytical signals for 83 targeted analytes plus 7 internal standards.

3.2. Method development

Our method of choice for sample preparation was SBSE extraction, considering its advantages in terms of automatization, preconcentration ability, and setup simplicity. However, as wine is a product extremely rich in volatiles, even the relatively large sample capacity of the largest extractive bars can be easily surpassed by with relatively low quantities of wine. Furthermore, even if the capacity of the SBSE is not surpassed, the chromatographic system can be easily overloaded by the major volatiles present in wine, which can cause chromatographic distortions and shifts in retention times, which need to be avoided in heart-cut multidimensional GC. For all these reasons, we kept the volume of wine to be extracted to a minimum, selecting the lowest amount of sample capable of providing a quantifiable response for trace target volatiles. In view of this requirement, we selected the conditions for the SBSE procedure by applying a method for wine volatiles previously validated in our laboratory [22], with the only exception that we reduced the amount of sample from 10 mL in the original method to 200 μL in the method proposed herein. We found that this amount was sufficient to obtain acceptable limits of detection that allowed us to quantify trace compounds at normal levels in wine. To facilitate sample handing and extraction while reducing the influence of the matrix during the extraction, we diluted the sample with 1.8 mL of a NaCl brine adjusted with tartaric acid to pH 3.5. This procedure allowed us to minimize differences between samples in terms of alcohol content; furthermore, the procedure standardized the matrix and its pH while considerably improving the extraction of polar compounds. This even made it possible for us to extract short chain fatty acids to the non-polar PDMS phase, thus ensuring that the extract contained nearly all relevant wine aroma volatiles and that matrix effects were kept to a minimum [24]. We also contemplated the use of the most polar type of EG/Silicone stir bar as an alternative that might have allowed us to improve the extraction of polar compounds, but found that it extracted too much ethanol, making the chromatographic process poorly repetitive.

Therefore, our final extraction procedure used two standard PDMS 20 mm x 0.1 mm stir bars, each extracting 2 mL of a 1:10 wine-pH-regulated brine dilution for 1 h at 400 rpm. The two stir bars were then independently desorbed in the TD-GC-GC–MS system under the conditions described for Runs 1 and 2, respectively. We proceeded to evaluate this method's analytical performance.

3.3. Method validation

We used an external calibration method with internal standards to assess linearity and to calculate the response factors with the purpose of estimating the concentrations of the analytes. Method linearity was evaluated by using model wines spiked at five different concentration levels according to the typical range of occurrence of each volatile compound. We assessed the linear regression's validity by visually examining the distributions of the residuals vs. estimates plots obtained in the calibration curves, as well as through the determination coefficients. We did not find any significant linearity deviations. The smallest determination coefficients (0.9714 for acetaldehyde and 0.9808 for acetic acid) were mainly caused by the poor repeatability observed for these polar compounds, but not by lack of fit to the linear model. Therefore, for the 83 target compounds, the response (FID or selected ion areas normalized by the corresponding internal standard given in Table 2) remained linear in all ranges under study (as reported in Table 3), which cover the natural ranges of occurrence in wine. Determination coefficients lay above 0.99 in most cases. Determination coefficients were only lower than 0.989 in certain poorly extracted or poorly chromatographed compounds, such as diacetyl, methionol, ethyl vanillate, ethyl 2-hydroxy-4-methylpentanoate, and 3-Isopropyl-2-methoxypyrazine. To estimate concentrations, we used the slopes obtained in the least square linear regression models. As weighted regression models provided slopes not significantly different from those obtained with the standard model, we retained the latter.

We estimated the limits of detection (LODs) by analyzing real wines, and the figures we obtained corresponded to the concentration at which the signal-to-noise ratio was 3. As expected, LODs were generally worse for compounds analyzed with GC-FID (Table 3). Although LODs are irrelevant for most of these compounds as they are always present at significant concentrations in all wine samples, LODs can nevertheless be used to estimate the method's overall efficiency. This can be defined as its ability to produce a quantifiable signal, and depends on all variables that exert an effect on the analyte's signal, including recovery in the extraction, efficiency in the transference to the column, quality (shape and resolution) of the chromatographic peak, and signal-to-noise ratio. Typical values for major compounds for which there were no major problems in terms of extraction, transference, chromatographic, or detection lay around 0.02 mg/L: such values can be considered quite satisfactory for the FID detection of such a complex mixture. As expected, LODs were higher for compounds that are more polar, such as acids: this can be attributed to a less satisfactory extraction performance to the PDMS phase. This was especially noticeable in the case of acetic acid, which, with a LOD of 1.2 mg/L, was the compound least extracted. Furthermore, this small compound was even difficult to re-trap in the internal trap of the TD unit. Nonetheless, the LODs provided by the method were still sufficient for the determining major compounds, and they were comparable to or better than those reported for similar methods [8,10].

LODs of the 54 trace compounds analyzed by GC-GC–MS, were also satisfactory (Table 3). The selectivity and sensitivity of the SIM-MS detection combined with the separation power of the multidimensional chromatography and the high recovery efficiency of the SBSE provided very good LODs. In general, LODs ranged from 0.01 to 0.30 μg/L, although in 16 cases, LODs lay below 0.02 ug/L and, in four cases (the three methoxypyrazines plus ethyl cyclohexanoate), they were below 0.001 μg/L. Therefore, in spite of the simplicity and broad scope of the sample isolation we applied in this study, the method's quantitative performance was comparable, in the best cases, to that of an ultratrace analysis. Differences in LODs values could be attributed to the aforementioned factors (extractability, thermal transference, chromatographic quality, and noise), as well as to the compound's specific detectability by EI mass spectrometry. An extreme, rather frustrating case was that of the three polyfunctional mercaptans: 3-mercaptohexanol, 3-mercaptohexyl acetate, and 4-methyl-4-mercapto-2-pentanone, all of which are key aroma compounds that frequently play a determining role in wine aroma character. However, because of their poor mass spectrometric properties, their generally low activity toward active points in the chromatographic path, and, in the case of 3-mercaptohexanol, poor extractability, the corresponding LODs obtained in the system were too high (above 0.03 μg/L for 4MMP, 0.05 for 3MHA and 0.5 μg/L for 3MH) for wine aroma analysis. We therefore did not include these compounds in the validation process. Apart from the latter, acetovanillone and syringaldehyde were the compounds with the least satisfactory LODs (1.30 and 2.06 μg/L, respectively). This was due to several reasons, including poor extractability, poor thermal transference and late elution from the column in a noisy area combined with broader peaks. On the other hand, we were able to detect compounds such as alkylmethoxypyrazines and ethyl cyclohexanoate in sub-nanogram per liter amounts thanks to the extremely low baseline noise at their selected m/z ions during SIM acquisition. The 0.5 ng/LOD of ethyl cyclohexanoate is even more satisfactory than the 0.76 ng/L reported in a

Table 3
Linearity, limits of detection (LOD), reproducibility ($n = 12$), and recovery of the SBSE-GC-GC-FID-MS method.

Chemical family	Compound	R^2	LOD	Linear range	Reproducibility RSD (%)	Recovery (%)	±RSD (%)	Units
Carbonyls	Acetaldehyde	0.9714	0.032	1.66 - 83.11	38	133 %	11 %	mg/L
	Benzaldehyde	0.9909	0.054	1.57 - 236	4	108 %	5 %	µg/L
	Methional	0.9997	0.021	0.22 - 33.2	8	97 %	9 %	µg/L
	Phenylacetaldehyde	0.9989	0.074	0.32 - 15.9	14	93 %	5 %	µg/L
	Syringaldehyde	0.9996	2.06	7.10 - 1686	18	97 %	8 %	µg/L
	2,3-Butanedione	0.9879	0.011	0.043 - 7.91	9	85 %	5 %	mg/L
	3-Hydroxybutanone	0.9898	0.036	0.155 - 15.5	12	92 %	8 %	mg/L
	3-Nonen-2-one	0.9998	0.031	0.160 - 16.2	8	89 %	4 %	µg/L
Alcohols	2-Methylpropanol	0.9946	0.128	0.42 - 421	10	98 %	3 %	mg/L
	1-Butanol	0.9921	0.084	0.260 - 69.4	16	93 %	12 %	mg/L
	Isoamyl alcohol	0.9916	0.030	0.680 - 1020	9	94 %	3 %	mg/L
	1-Hexanol	0.9954	0.007	0.170 - 70.8	5	97 %	3 %	mg/L
	cis-3-Hexenol	0.9961	0.024	0.080 - 7.12	7	102 %	3 %	mg/L
	Methionol	0.9887	0.024	0.081 - 41.9	15	100 %	10 %	mg/L
	Benzyl alcohol	0.9948	0.029	0.097 - 95.1	15	101 %	2 %	mg/L
	β-Phenylethanol	0.9940	0.022	0.43 - 440	11	101 %	1 %	mg/L
Acids	Acetic acid	0.9808	1.20	142.2 - 2133	50	5 %	14 %	mg/L
	2-Methylpropanoic acid	0.9968	0.207	0.690 - 84.7	18	102 %	11 %	mg/L
	Butyric acid	0.9975	0.240	0.810 - 31.5	13	102 %	3 %	mg/L
	3-Methylbutyric acid	0.9915	0.238	0.794 - 41.5	19	107 %	9 %	mg/L
	Hexanoic acid	0.9973	0.114	0.470 - 99.8	17	107 %	3 %	mg/L
	Octanoic acid	0.9995	0.021	0.330 - 249	4	90 %	2 %	mg/L
	Decanoic acid	0.9971	0.010	0.140 - 61.6	3	96 %	3 %	mg/L
Phenolic compounds	4-Vinylphenol	0.9917	0.407	3.38 - 507	8	95 %	3 %	µg/L
	4-Ethylphenol	0.9931	0.034	0.246 - 369	4	100 %	1 %	µg/L
	4-Ethylguaiacol	0.9994	0.030	0.248 - 372	6	90 %	1 %	µg/L
	4-Vinylguaiacol	0.9957	0.393	2.41 - 361	7	92 %	2 %	µg/L
	2,6-Dimethoxyphenol	0.9995	0.455	1.52 - 362	14	100 %	6 %	µg/L
	Eugenol	0.9891	0.039	0.129 - 68.0	13	91 %	2 %	µg/L
	4-Propylguaiacol	0.9999	0.024	0.080 - 76.0	10	101 %	2 %	µg/L
	trans-Isoeugenol	0.9935	0.040	0.450 - 67.2	14	94 %	2 %	µg/L
	4-Allyl-2,6-dimethoxyphenol	0.9950	0.167	0.556 - 442	15	96 %	2 %	µg/L
	Guaiacol	0.9995	0.149	1.51 - 227	13	105 %	19 %	µg/L
	o-Cresol	0.9988	0.098	0.240 - 51.1	7	105 %	6 %	µg/L
	m-Cresol	0.9934	0.082	0.207 - 48.7	6	98 %	7 %	µg/L
	Vanillin	0.9908	0.525	3.92 - 587	14	102 %	9 %	µg/L
	Acetovainillone	0.9973	1.30	4.60 - 690	13	98 %	4 %	µg/L
	Methyl vanillate	0.9871	0.159	0.531 - 121	13	90 %	4 %	µg/L
	Ethyl vanillate	0.9906	0.087	2.76 - 337	14	109 %	1 %	µg/L
Terpenes	Limonene	0.9920	0.147	0.960 - 144	8	102 %	9 %	µg/L
	1,8-Cineole	0.9898	0.045	1.04 - 155	7	92 %	5 %	µg/L
	Linalool	0.9999	0.296	0.980 - 142	7	96 %	3 %	µg/L
	α-Terpineol	0.9932	0.172	1.59 - 79.4	14	103 %	3 %	µg/L
	β-Citronellol	0.9921	0.437	1.42 - 141	16	97 %	12 %	µg/L
	Linalool oxide	0.9969	0.235	1.40 - 210	7	93 %	7 %	µg/L
	Geraniol	0.9894	0.344	1.14 - 135	6	89 %	8 %	µg/L
	Linalyl acetate	0.9967	0.009	0.060 - 8.56	12	97 %	11 %	µg/L
	cis-Rose oxide	0.9947	0.009	0.130 - 20.2	7	97 %	8 %	µg/L
Esters	Ethyl acetate	0.9955	0.011	3.14 - 471	11	107 %	9 %	mg/L
	Ethyl propanoate	0.9942	0.005	0.040 - 6.46	10	92 %	6 %	mg/L
	Ethyl butyrate	0.9947	0.003	0.050 - 7.08	8	105 %	8 %	mg/L
	Isoamyl acetate	0.9980	0.006	0.056 - 83.7	7	108 %	9 %	mg/L
	Ethyl hexanoate	0.9952	0.004	0.013 - 16.6	4	107 %	6 %	mg/L
	Hexyl acetate	0.9943	0.011	0.040 - 5.26	4	103 %	5 %	mg/L
	Ethyl lactate	0.9973	0.231	4.37 - 1255	10	101 %	4 %	mg/L
	Ethyl octanoate	0.9960	0.002	0.030 - 45.4	9	104 %	7 %	mg/L
	Ethyl decanoate	0.9982	0.004	0.030 - 8.14	18	98 %	3 %	mg/L
	Diethyl succinate	0.9997	0.003	0.390 - 58.6	11	96 %	3 %	mg/L
	Ethyl 2-hydroxy-4-methylpentanoate	0.9878	0.084	1.74 - 261	7	99 %	5 %	µg/L
	Ethyl 2-methylpropanoate	0.9977	0.224	4.64 - 696	12	105 %	7 %	µg/L
	Butyl acetate	0.9980	0.063	0.430 - 64.8	11	106 %	11 %	µg/L
	Isobutyl acetate	0.9895	0.086	1.24 - 454	10	109 %	9 %	µg/L
	Ethyl 2-methylbutyrate	0.9976	0.104	2.39 - 359	10	105 %	14 %	µg/L
	Ethyl 3-methylbutyrate	0.9988	0.110	0.970 - 196	10	103 %	13 %	µg/L
	Ethyl cyclohexanoate	0.9955	0.0005	0.040 - 1.90	9	89 %	8 %	µg/L
	Phenylethyl acetate	0.9937	0.011	4.66 - 3330	9	90 %	6 %	µg/L
	Ethyl cinnamate	0.9998	0.037	0.123 - 68.2	12	87 %	3 %	µg/L
	Ethyl dihydrocinnamate	0.9951	0.008	0.026 - 30.2	3	93 %	6 %	µg/L
Norisoprenoids	TDN	0.9997	0.069	1.38 - 138	13	98 %	3 %	µg/L
	β-Damascenone	0.9976	0.032	0.380 - 57.2	8	97 %	4 %	µg/L
	β-Ionone	0.9946	0.015	0.051 - 13.8	5	99 %	3 %	µg/L

(*continued on next page*)

Table 3 (*continued*)

Chemical family	Compound	R^2	LOD	Linear range	Reproducibility RSD (%)	Recovery (%)	±RSD (%)	Units
	α-Ionone	0.9904	0.088	0.290 - 30.2	16	96 %	9 %	μg/L
Alkylmethoxypyrazines	3-Isopropyl-2-methoxypyrazine	0.9839	0.959	4.36 - 218	12	103 %	37 %	ng/L
	3-sec-butyl-2-methoxypyrazine	0.9920	0.955	4.31 - 647	5	98 %	17 %	ng/L
	3-Isobutyl-2-methoxypyrazine	0.9970	0.983	4.36 - 515	5	87 %	5 %	ng/L
Lactones	γ-Butyrolactone	0.9971	0.235	0.860 - 129	17	109 %	26 %	mg/L
	trans-Whiskylactone	0.9989	0.016	0.345 - 346	8	109 %	9 %	μg/L
	cis-Whiskylactone	0.9998	0.014	0.345 - 346	9	89 %	10 %	μg/L
	Massoia lactone	0.9992	0.007	0.104 - 157	11	88 %	6 %	μg/L
	γ-Nonalactone	0.9964	0.015	0.137 - 205	8	94 %	1 %	μg/L
	γ-Decalactone	0.9998	0.0024	0.104 - 52.8	14	101 %	28 %	μg/L
	δ-Decalactone	0.9995	0.005	0.050 - 7.95	14	97 %	0 %	μg/L
Miscelaneous	Geosmin	0.9890	0.013	0.030 - 2.08	13	91 %	9 %	μg/L

previous method based on SPE followed by GC-GC–MS [25]. As can be seen in Table 3, the best LODs were obtained for non-polar compounds with good *m/z* ions, such as ethyl cyclohexanoate, phenylethyl acetate, ethyl dihydrocinnamate, linalyl acetate, cis-rose oxide, β-ionone, and all the lactones, while the worse LODs were obtained for volatile phenols and terpenols. In comparison with other analytical methods based on SBSE and MS detection [14,26], the LODs in the present method are similar or better for most compounds. Of course, other analytical procedures, likewise based on SBSE or MDGC, provide better LODs for specific families of compounds. For example, LODs below 0.5 ng/L can be achieved for alkylmethoxypyrazines [22,27], norisoprenoids can be determined with LODs ranging from 3 to 9 ng/L [28], geosmin can be detected at 3.3 ng/L [26], and whiskylactones and guaiacols with LODs below 1 ng/L have also been reported [29]. However, all those extremely low LODs were mainly obtained at the expense of extracting considerably greater amounts of sample in the stir bar, which, in turn, would prevent the analysis of major compounds in the first GC dimension, thereby rendering the procedure susceptible to matrix effects, reducing its universality. We therefore find that the procedure proposed herein represents an optimal compromise between the number of measured analytes and overall sensitivity.

We calculated method reproducibility via repeated analysis of spiked red and white wines on six different days, spanning a total of three weeks. Reproducibility data, expressed as the average relative standard deviation, are given in Table 3. These figures are quite satisfactory: 37 compounds feature relative standard deviations (RSDs) below 10 %, 37 more have RSDs between 10 and 15 %, 7 have RSDs between 15 and 20 % and only two compounds feature RSDs above 20 %: acetaldehyde (35 %) and acetic acid (50 %). Considering the concentration levels involved and the number of heart-cutting windows we applied, reproducibility can be considered satisfactory and in line with similar methods [24,26].

We determined the method's accuracy by conducting a recovery experiment on two different commercial spiked wines. Those two experiments' results are listed in Table 3. In nearly all cases, the average recovery rate was approximately 100 % (85–109 %). Again, only two exceptions were observed: acetaldehyde and acetic acid. Acetaldehyde showed an excess error (recovery of 133 %), partly due to the high irreproducibility of the determination and partly due to the cleavage of some of its adducts with sulfur dioxide induced by the spiking [30]. The low recovery rate of acetic acid should be attributed to its poor extractability to the PDMS, which seems to be strongly affected by the presence of other wine components. Therefore, the method featured in this study cannot provide reliable data for this compound. Data for acetaldehyde can be considered semiquantitative. For the remaining analytes, recovery data confirmed that was accurate and free from matrix effects.

3.4. Wine analysis

To further evaluate the method's viability and performance, we applied it for the analysis of 20 Spanish wine samples. A summary of the results obtained from those analyses is presented in Table 4. As shown in the table, most of the concentrations of the volatile compounds determined in the wines lay within the calibrated intervals, and only 5 out of the 83 calibrated compounds were not detected in any wine. In those five cases, we expected very low concentrations. Geosmine is an off-flavor and, therefore, seldom found in faultless wines [31], Moreover, owing to climatic condictions, we only expected to find 3-isopropyl- and 3-sec-butyl-2-methoxypyrazine in extremely low levels in Spanish wines [22]. We likewise expected to find ethyl cyclohexanoate and 1, 8-cineole at extremely low levels in young wines [25,32]. Finally, 3-nonen-2-one, has only been previously detected by olfactometry in Madeira wines [33]. Apart from those compounds, our analysis provided concentration data for the rest of the analytes, most of which were involved in the aroma of wine, with sufficient sensitivity to cover concentrations lying around or below their odor thresholds in wine. Offering the advantage of straightforward sample preparation, the method featured herein is capable of quantifying a large number of relevant components in concentrations ranging from 350 mg/L of isoamyl alcohol to the 3.8 ng/L of 3-isobutyl-2-methoxypyrazine we were able to detect. Leaving aside polyfunctional mercaptans and certain polar trace aroma compounds, this method therefore provides a remarkably comprehensive analysis of wine aroma composition. Offering the further advantage of a relatively simple, semiautomated sample setup, it yields reliable quantitative data stemming from more than 80 aroma chemicals differing in concentration ranges close to nine orders of magnitude.

4. Conclusions

A semiautomated analytical method capable of quantifying 81 target aroma molecules in two chromatographic runs was developed and validated in this study. The method uses SBSE extraction of brine-diluted wine and the stir bars are further desorbed in a TD-GC-GC-FID/MS system in two different runs in which 28 major aroma compounds are quantified in the FID in the first dimension and 54 trace aroma compounds are transferred to 22 cuts and further quantified by GC–MS in the second dimension. Leaving aside acetic acid, the extraction of which was erratic, and apart from polyfunctional mercaptans, which are poorly detectable ultratrace odorants, the method featured in this study is capable of providing robust, reliable results of the most relevant wine aroma components, ranging from methional at low μg/L levels and methoxypyrazines at low ng/L levels to major fermentative aroma compounds at quantities up to several hundreds mg/L. Calibration was carried out by transforming normalized peak areas (with 7 different internal standards) into concentrations with response factors estimated in the analysis of wine models. Except for acetaldehyde, with 135 %, all recovery rates lay between 85 % and 109 %. Reproducibilities for 37 compounds were better than 10 %; for other 37 between 10 and 15 %, and, except for acetaldehyde, better than 20 %. Linearities were, better than 0.989 (as R^2), except for acetaldehyde. Overall, this method provides a nearly complete wine aroma-volatile profile in the two runs, quantifying analytes that differ by nearly 9 orders of concentration

Table 4
Minimum, maximum, and mean concentrations found in wine sample analyses.

Chemical family	Compound	Minimum	Maximum	Mean[1]	Odor threshold[2]	Units
Carbonyls	Acetaldehyde	3.06	12.3	7.58	0.50	mg/L
	Benzaldehyde	1.89	9.93	2.43	2	μg/L
	Methional	1.03	8.17	4.80	0.50	μg/L
	Phenylacetaldehyde	0.56	5.07	2.06	1	μg/L
	Syringaldehyde	7.14	1601	135	25,000	μg/L
	2,3-Butanodione	0.04	0.17	0.05	100	mg/L
	3-Hydroxybutanone	0.29	14.4	2.83	150	mg/L
	3-Nonen-2-one	N.D.	N.D.	N.D.		μg/L
Alcohols	2-methylpropanol	2.31	130	41.5	40	mg/L
	1-Butanol	0.270	0.766	0.396	150	mg/L
	Isoamyl alcohol	125	350	196	30	mg/L
	1-Hexanol	0.440	2.96	1.35	8	mg/L
	cis-3-hexenol	0.072	0.373	0.150	0.4	mg/L
	Methionol	0.301	1.30	0.964	1	mg/L
	Benzyl alcohol	0.053	1.14	0.538	200	mg/L
	β-phenylethanol	15.2	106	52.7	14	mg/L
Acids	Acetic acid	115	857	378	300	mg/L
	2-Methylpropanoic acid	0.114	5.43	1.77	0.05	mg/L
	Butyric acid	0.592	17.3	5.07	0.173	mg/L
	3-Methylbutyric acid	1.24	31.2	6.70	0.033	mg/L
	Hexanoic acid	1.87	11.0	5.61	0.42	mg/L
	Octanoic acid	2.18	17.9	8.15	0.5	mg/L
	Decanoic acid	0.385	5.85	2.26	1	mg/L
Terpenes	Limonene	N.D.	1.17	1.17	200	μg/L
	1,8-Cineole	N.D.	N.D.	N.D.	1.3	μg/L
	Linalool	0.711	31.8	6.99	25	μg/L
	α-Terpineol	1.94	47.8	5.07	250	μg/L
	β-Citronellol	0.264	7.23	1.18	100	μg/L
	Linalool oxide	N.D.	3.42	1.75	3000	μg/L
	Geraniol	28.5	41.7	32.8	20	μg/L
	Linalyl acetate	N.D.	0.892	0.315	50	μg/L
	cis-Rose oxide	N.D.	0.716	0.344	0.2	μg/L
Norisoprenoids	TDN	N.D.	7.87	1.24	2	μg/L
	β-Damascenone	1.10	17.6	4.80	0.05	μg/L
	β-ionone	0.0445	0.452	0.157	0.09	μg/L
	α-ionone	N.D.	5.32	1.13	2.60	μg/L
Phenolic compounds	4-Vinylphenol	37.4	590	142	180	μg/L
	4-Ethylphenol	0.330	1016	136	35	μg/L
	4-Ethylguaiacol	0.140	434.3	55.7	33	μg/L
	4-Vinylguaiacol	6.68	194	34.8	40	μg/L
	2,6-Dimethoxyphenol	1.75	44.3	7.81	560	μg/L
	Eugenol	0.361	12.7	2.96	6	μg/L
	4-Propylguaiacol	0.084	3.52	1.53	10	μg/L
	trans-Isoeugenol	0.690	2.29	0.289	6	μg/L
	4-Allyl-2,6-dimethoxyphenol	0.752	25.4	5.54	1200	μg/L
	Guaiacol	1.55	21.4	4.62	9.5	μg/L
	o-Cresol	0.151	1.99	0.780	31	μg/L
	m-Cresol	0.132	1.37	0.284	68	μg/L
	Vanillin	4.45	111.05	21.4	995	μg/L
	Acetovainillone	18.1	87.6	57.3	1000	μg/L
	Methyl vanillate	0.560	4.12	1.06	990	μg/L
	Ethyl vanillate	2.74	22.7	8.15	3000	μg/L
Esters	Ethyl acetate	12.1	175	57.6	12.3	mg/L
	Ethyl propanoate	0.0380	0.863	0.272	5.5	mg/L
	Ethyl butyrate	0.0554	0.337	0.153	0.125	mg/L
	Isoamyl acetate	0.0919	1.64	0.728	0.03	mg/L
	Ethyl hexanoate	0.210	0.767	0.297	0.062	mg/L
	Hexyl acetate	0.0497	0.510	0.162	1.5	mg/L
	Ethyl lactate	5.92	689	142	154	mg/L
	Ethyl octanoate	0.149	0.485	0.240	0.58	mg/L
	Ethyl decanoate	0.033	0.245	0.145	0.2	mg/L
	Diethyl succinate	1.11	27.3	9.75	200	mg/L
	Ethyl 2-hydroxy-4-methylpentanoate	15.7	33.2	24.7	300	μg/L
	Ethyl 2-methylpropanoate	67.2	371	161	15	μg/L
	Butyl acetate	1.26	5.24	2.70	1800	μg/L
	Isobutyl acetate	2.04	36.6	14.6	1600	μg/L
	Ethyl 2-methylbutyrate	2.89	28.3	13.4	18	μg/L
	Ethyl 3-methylbutyrate	9.96	91.2	35.1	3	μg/L
	Ethyl cyclohexanoate	N.D.	N.D.	N.D.	0.001	μg/L
	Phenylethyl acetate	4.87	94.7	28.0	250	μg/L
	Ethyl cinnamate	0.305	6.87	2.77	1.1	μg/L

(continued on next page)

Table 4 (*continued*)

Chemical family	Compound	Minimum	Maximum	Mean[1]	Odor threshold[2]	Units
	Ethyl dihydrocinnamate	0.090	18.8	1.14	1.6	μg/L
Alkyl-methoxypyrazines	3-Isopropyl-2-methoxypyrazine	N.D.	N.D.	N.D.	1	ng/L
	3-sec-Butyl-2-methoxypyrazine	N.D.	N.D.	N.D.	1	ng/L
	3-Isobutyl-2-methoxypyrazine	N.D.	4.93	4.18	15	ng/L
Lactones	γ-butyrolactone	2.38	13.8	3.45	35	mg/L
	cis-Whiskylactone	5.11	78.6	7.31	67	μg/L
	trans-Whiskylactone	0.232	36.1	4.78	790	μg/L
	Massoia lactone	0.148	2.93	1.16	56	μg/L
	γ-Nonalactone	0.187	4.10	1.02	25	μg/L
	γ-Decalactone	0.752	4.10	1.82	0.7	μg/L
	δ-Decalactone	0.296	3.26	1.43	386	μg/L
Miscellaneous	Geosmin	N.D.	N.D.	N.D.	0.0011	μg/L

[1] Average concentration calculated only with the values of samples above the limit of detection.
[2] Threshold values have been taken from [5]
N.D.: Not detected, below limit of detection given in Table 3.

magnitude.

CRediT authorship contribution statement

Oscar Castejón-Musulén: Methodology, Investigation, Writing – review & editing. **Ricardo Lopez:** Conceptualization, Methodology, Formal analysis, Writing – original draft, Writing – review & editing. **Ignacio Ontañón:** Methodology, Investigation, Formal analysis, Writing – review & editing. **Vicente Ferreira:** Conceptualization, Resources, Funding acquisition, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

Data availability

Data will be made available on request.

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