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Headspace Solid-Phase Microextraction: A Useful and Quick Tool for the Traceability and Quality Assessment of Wine Cork Stoppers

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Abstract: Natural cork remains a favored option for sealing high-quality wine bottles, despite its high cost for wineries. The cork industry faces the challenge of certifying the quality and traceability of these corks, with physical–chemical characterization being a valuable tool in establishing these parameters. While cork taint compounds must be absent or in low concentrations, the volatile fraction of cork contains numerous compounds that, even in small amounts, can impact the wine's final aroma. Moreover, these volatile compounds are indicative of the geographical origin of the cork planks used to make the stoppers. In this work, a total of 68 volatile compounds (alkanes, terpenes, benzenic compounds, aldehydes, ketones, acids, esters, alcohols and furanic and pyranic compounds) from natural corks of different qualities and origins were identified, using a fast and sensitive technique: headspace solid-phase microextraction coupled to gas chromatography–mass spectrometry (HS-SPME-GC-MS). Based on these volatile compounds, it was possible to establish differences between corks of different origins, although no discernible differences were detected in the samples of differing visual qualities, as this is a subjective parameter largely dependent on the cork's external appearance. These findings show that the analysis of the volatile composition of corks via HS-SPME-GC-MS can be used as a quick tool for tracking their traceability and selecting the most appropriate parameters at each stage of processing to minimize the increase in unwanted compounds.

Keywords: natural corks; volatile compounds; traceability; sensory quality; HS-SPME

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1. Introduction

Cork is a natural material that possesses unique physical properties (impermeability, elasticity, and inertia) that make it an ideal choice for wine bottle closures. However, the cork is in contact with the wine; therefore, it is of great importance to establish the chemical compounds that could be passed to wine and their sensory involvement. The most important thing is to ensure that the sensory attributes of wine are not modified negatively. For this, adequate quality control measures must be in place, especially for natural corks, which are typically the most expensive.

While there are other types of wine bottle closures available, such as synthetic corks, screw caps, and glass stoppers, natural cork stoppers are still widely used for the bottling of quality wines. They are one of the most important factors that influence the quality of wine, which is a great cost for wineries. The certification of their quality and traceability is a great challenge for the cork industry, with their physical–chemical characterization

being a very useful tool when establishing these parameters. Natural cork is mostly formed by two large polymers, suberin and lignin, and to a lesser extent, polysaccharides and a removable fraction with different solvents mostly formed by volatile compounds and phenolic compounds [1]. In natural corks, the qualities are visually established based on their external structure, with stoppers with the lowest number of external imperfections and/or lenticels being those of higher quality and, consequently, greater economic value. However, the migration of different organic compounds (volatile and phenolic compounds) to wine during its storage or aging is demonstrated [2–5].

The volatile fraction of a cork is constituted by a high number of compounds of different chemical natures, which come from the degradation of wood components such as fatty acids or lignin, or by the effect of microbial metabolism, mainly being molds that can grow in cork planks [6–8]. Among them, haloanisoles and halophenols are the most studied due to their implication in the "cork taint", although other volatile compounds such as geosmin, 1-octen-3-ol, 2-methyl-isoborneol and 2-methoxy-3,5-dimethylpyrazine are also responsible for negative attributes in wines, such as "moldy", "green" and "chemical" odors [5,7,9,10].

However, without considering the compounds responsible for the "cork taint" in wines, the volatile fractions of corks include many compounds that, even in small concentrations, could influence the final aroma of wines [11]. On the other hand, these volatile compounds come from cork planks and could be good indicators of their geographical origin [12–14]. Thus, the study of the volatile fraction of corks is presented as a new tool for the cork industry, which will allow it to establish the traceability of cork stoppers, as well as monitor the production process and the implementation of quality standards.

Different analysis techniques have been used to analyze the volatile compounds of corks, normally based on extraction with organic solvents or on maceration in hydroalcoholic solutions similar to wine. However, headspace solid-phase microextraction (HS-SPME) is a fast and simple method that allows the analysis of the volatile fraction of solid samples without prior preparation. This technique has been used by our research group for the fast screening of the volatile compounds of oak wood [15] and has also been applied to cork stoppers [13,16].

The objective of this work is to analyze the volatile fraction of natural corks of different visual qualities (flower, superior, second, third and fourth) and geographical origins (south Spain, northeast Spain and Portugal), without prior maceration, using a fast and sensitive technique: headspace solid-phase microextraction coupled with gas chromatography–mass spectrometry (HS-SPME-GC-MS). The study aims to provide a complete characterization of the volatile compounds present in natural corks, which will enable the establishment of their sensory quality and traceability.

2. Materials and Methods

2.1. Samples

Five types of commercially available natural corks from *Quercus suber* L. oak trees were provided by three different manufacturers. The cork stoppers were manufactured following the standard practices specified in the International Code of Cork Stopper Manufacturing Practices [17]. Table 1 provides information on their mixed origins, characteristics, and visual quality grades. To ensure uniformity, the corks were ground using a cutting mill SM 100 (Retsch GmbH, Verder Scientific, Haan, Germany) to achieve a particle size of less than 5 mm.

Duplicate measurements were taken to determine the moisture content of the samples, following the UNE 56921 standard [18]. Homogeneous samples weighing 2 g were dried using a laboratory oven (Selecta, Barcelona, Spain) at 100 °C until the weight remained constant over two consecutive measurements (Table 1).

Table 1. Characteristics of the cork samples used in the study.

Sample Key	Geographic Origin	Quality Grade [1]	Measurement	Moisture (%) Mean ± SD
SS1	South Spain	Flower	49 × 24 mm	3.82 ± 0.50
SS2	South Spain	Second	49 × 24 mm	3.79 ± 0.48
NS1	Northeast Spain	Superior	49 × 24 mm	3.57 ± 0.36
NS2	Northeast Spain	Fourth	49 × 24 mm	3.78 ± 0.45
P1	Portugal	Third	44 × 24 mm	3.65 ± 0.15

[1] According to manufacturer.

2.2. Headspace Solid-Phase Microextraction (HS-SPME)

The HS-SPME analysis was carried out using a CombiPal G6500-CTC (Agilent Technologies, Inc., Santa Clara, CA, USA). The extraction conditions were previously optimized by our research group [15]. Homogenous cork samples (1 g) and 20 µL of 4-nonanol (0.05 g/L in absolute ethanol) used as an internal standard were placed in a 20 mL vial sealed with a screw-capped top containing a Teflon-lined septum. A triple SPME fiber, previously conditioned according to the manufacturer and coated with 50/30 µm of divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) was exposed to the headspace of the sample at 70 °C for 40 min under continuous stirring (250 rpm). The fiber was then retracted and inserted into the gas chromatograph injector. Volatile compounds absorbed in the SPME fiber were thermally desorbed at 250 °C for 5 min (splitless mode for 0.5 min). This desorption time allowed the fiber to be left clean for the next extraction. Each extraction was performed in duplicate.

2.3. GC-MS Analysis

A 6890 N Agilent gas chromatograph coupled to a 5973 N Agilent Mass Detector and fitted with a DB-WAX ultra-inert column, 60 m × 0.25 mm i.d.; 0.25 µm film thickness (Agilent Technologies, Inc., Santa Clara, CA, USA), was used, following the procedure previously described by Soriano et al. [19] with minor modifications. The oven temperature was set at 40 °C for 10 min; then, the temperature was firstly programmed at 3 °C/min to 200 °C and then at 5 °C/min until 230 °C, which was held for 10 min. The carrier gas was helium at a flow of 1 mL/min. Compounds were detected via mass spectrometry in the electron impact (EI) ionization mode at 70 eV, and spectra were recorded in the SCAN mode (45 to 550 a.m.u.). The ion source temperature was 230 °C.

Identification of the volatile components was performed by comparing them with authentic standards from Sigma-Aldrich (Tres Cantos, Madrid, Spain). The tentative identification of compounds for which it was not possible to find reference volatiles was carried out via the comparison of their mass spectra with spectral data from the Wiley G 1035 A, NBS75K and NIST14 libraries and via linear retention index (LRI) comparison. The semiquantitative analysis of volatile compounds was performed by dividing their peak areas by that of the internal standard (4-nonanol) and multiplying this ratio by the initial concentration of the internal standard. The peak areas were measured from the full scan chromatograph using the total ion current (TIC). The concentration of each compound was calculated in ng/g dry weight.

2.4. Statistical Analysis

One-way analysis of variance (ANOVA) was applied to the results, using the Student–Newman–Keuls test to determine statistical differences among natural corks from different geographic origins and quality grades. The IBM SPSS software version 24.0 for Windows statistical package was utilized for the statistical analysis.

3. Results and Discussion

Table 2 displays the volatile compounds found in the natural corks analyzed. A total of 68 volatile compounds were identified and classified into various chemical families, in-

cluding alkanes, terpenes, benzenic compounds, aldehydes, ketones, acids, esters, alcohols and furanic and pyranic compounds. It is worth noting that many of these compounds have not been previously identified in corks.

The alkanes were one of the major groups, with 11 identified compounds. Alkanes are present in different types of wood and have been identified in corks [20]. The SS1 and SS2 samples, both from the same geographical origin, were the ones that showed significantly higher concentrations of alkanes (Figure 1), with tetradecane, dodecane and hexadecane being the majority in all samples. The NS1, NS2 and P1 samples, although they were of different geographical origin, did not present statistically significant differences in the total concentration of alkanes.

Table 2. Mean and standard deviation of volatile compounds (ng/g DW) of natural corks analyzed via HS-SPME-GC-MS.

Volatile Compounds	SS1	SS2	NS1	NS2	P1
Alkanes					
Decane	7.1 ± 1.7	8.1 ± 2.2	6.6 ± 1.4	5.9 ± 1.4	7.5 ± 0.5
Undecane	10.7^{c} ± 2.2	7.5^{b} ± 1.5	6.3^{ab} ± 2.6	4.7^{ab} ± 0.7	3.5^{a} ± 1.0
Dodecane	74.9^{a} ± 11.9	87.1^{a} ± 5.9	87.6^{b} ± 6.2	80.8^{a} ± 5.9	79.6^{b} ± 3.8
Tridecane	30.1^{b} ± 3.9	40.4^{c} ± 5.8	29.3^{b} ± 4.5	21.9^{ab} ± 4.4	20.2^{a} ± 5.2
Tetradecane	167.9^{bc} ± 33.7	181.4^{c} ± 20.3	131.9^{ab} ± 7.8	137.1^{ab} ± 12.2	121.8^{a} ± 13.1
Hexadecane	83.0^{c} ± 4.3	77.0^{c} ± 6.7	40.3^{b} ± 4.3	43.7^{b} ± 10.7	28.9^{a} ± 6.2
Heptadecane	46.0^{c} ± 9.1	31.9^{b} ± 9.5	13.4^{a} ± 3.5	13.2^{a} ± 3.2	10.6^{a} ± 1.4
Octadecane	33.3^{b} ± 11.6	24.8^{ab} ± 5.8	14.7^{a} ± 2.6	15.4^{a} ± 2.0	14.6^{a} ± 1.1
Nonadecane	11.2^{a} ± 5.0	9.9^{a} ± 2.5	7.0^{a} ± 1.5	6.3^{a} ± 1.2	20.5^{b} ± 6.8
Eicosane	11.5^{b} ± 2.7	9.0^{ab} ± 2.4	6.0^{a} ± 0.8	7.8^{ab} ± 0.3	20.6^{c} ± 4.5
Docosane	13.5 ± 8.5	7.9 ± 3.2	6.7 ± 0.7	12.5 ± 7.7	19.4 ± 6.2
Terpenes					
α-Pinene	1.4^{a} ± 0.3	1.6^{a} ± 0.7	1.7^{a} ± 0.3	7.0^{b} ± 2.3	1.8^{a} ± 1.0
Camphene	17.1^{a} ± 3.5	9.3^{a} ± 2.4	8.1^{a} ± 4.1	36.4^{b} ± 12.7	10.0^{a} ± 3.3
Limonene	11.3^{a} ± 2.7	11.5^{a} ± 1.0	74.6^{b} ± 10.3	75.1^{b} ± 24.9	14.6^{a} ± 8.2
Eucalyptol	0.9^{a} ± 0.3	1.0^{a} ± 0.3	3.3^{a} ± 0.9	12.8^{b} ± 3.0	3.0^{a} ± 0.9
α-Copaene *	10.0^{a} ± 2.6	24.8^{a} ± 16.1	322.2^{b} ± 158.2	382.1^{b} ± 76.9	14.9^{a} ± 3.5
Camphor	21.7^{a} ± 3.8	21.9^{a} ± 4.8	18.4^{a} ± 5.7	84.0^{b} ± 21.7	20.2^{a} ± 3.6
Thymol methyl ether *	3.2^{a} ± 0.8	5.6^{a} ± 2.1	25.8^{b} ± 0.6	25.4^{b} ± 5.1	6.0^{a} ± 1.5
α-Terpineol	8.6^{a} ± 1.9	12.1^{a} ± 1.4	13.4^{a} ± 1.0	23.8^{b} ± 4.1	12.3^{a} ± 5.3
Isothymol methyl ether *	5.7^{a} ± 3.1	4.3^{a} ± 0.8	5.5^{a} ± 3.2	22.3^{b} ± 7.9	4.9^{a} ± 2.6
α-Muurolene *	3.2^{a} ± 0.4	8.0^{b} ± 1.8	9.6^{b} ± 1.6	9.9^{b} ± 4.5	6.6^{ab} ± 1.5
Cadalene *	16.2 ± 8.5	7.6 ± 2.2	8.0 ± 1.0	9.4 ± 7.1	7.5 ± 5.2
Geraniol	10.4 ± 4.2	8.2 ± 3.0	5.5 ± 0.7	5.7 ± 1.8	6.6 ± 2.4
Methyl dihydrojasmonate	22.5 ± 8.6	31.9 ± 16.7	18.2 ± 1.3	15.8 ± 2.8	28.2 ± 9.1
p-Cymene	15.5 ± 7.2	12.5 ± 2.8	16.9 ± 3.9	12.4 ± 3.7	12.0 ± 2.9
(E)-Geranyl acetone	45.8 ± 17.1	24.0 ± 5.0	39.2 ± 6.9	44.5 ± 15.1	27.7 ± 2.7
Acids					
Acetic acid	397.0^{b} ± 65.5	327.4^{b} ± 31.7	167.1^{a} ± 12.9	147.2^{a} ± 24.8	524.4^{c} ± 65.8
Butanoic acid	9.6^{c} ± 1.2	7.7^{b} ± 1.6	5.2^{a} ± 0.4	4.7^{a} ± 0.7	6.8^{ab} ± 1.3
Hexanoic acid	90.7^{c} ± 13.3	74.9^{b} ± 5.8	46.5^{a} ± 6.1	47.4^{a} ± 1.5	56.9^{a} ± 6.3
2-Ethylhexanoic acid	34.3^{a} ± 4.4	49.5^{a} ± 28.1	6.7^{a} ± 1.2	4.5^{a} ± 0.7	190.9^{b} ± 70.6
Octanoic acid	57.8^{b} ± 21.7	81.7^{c} ± 17.0	28.0^{a} ± 3.5	29.8^{a} ± 2.1	44.8^{ab} ± 6.9
Nonanoic acid	164.1^{bc} ± 81.9	228.4^{c} ± 63.5	53.0^{a} ± 13.7	55.6^{a} ± 14.9	98.3^{ab} ± 32.1
Decanoic acid	33.9 ± 11.6	49.5 ± 23.3	24.5 ± 6.1	23.1 ± 3.6	33.1 ± 5.5

Table 2. *Cont.*

Volatile Compounds	SS1	SS2	NS1	NS2	P1
Aldehydes					
Hexanal	25.9 ^{b} ± 1.8	19.3 ^{b} ± 6.2	21.4 ^{b} ± 5.2	24.6 ^{b} ± 1.9	13.1 ^{a} ± 2.8
Heptanal	11.7 ^{d} ± 0.8	7.8 ^{bc} ± 1.9	9.2 ^{c} ± 1.6	6.4 ^{b} ± 1.2	4.3 ^{a} ± 0.8
Nonanal	212.5 ^{a} ± 76.5	206.3 ^{a} ± 68.5	351.7 ^{b} ± 24.3	242.7 ^{a} ± 38.4	219.2 ^{a} ± 26.4
(E)-2-Octenal	17.7 ± 3.8	15.6 ± 8.2	15.6 ± 2.7	20.8 ± 2.9	14.9 ± 2.4
Decanal	120.3 ^{bc} ± 25.1	107.4 ^{b} ± 6.5	141.7 ^{c} ± 12.2	114.8 ^{bc} ± 17.8	79.6 ^{a} ± 8.2
(E)-2-Nonenal	49.8 ^{b} ± 8.9	51.5 ^{b} ± 13.2	54.0 ^{b} ± 4.3	68.8 ^{c} ± 6.9	34.0 ^{a} ± 3.4
Ketones					
2-Heptanone	15.9 ^{c} ± 0.8	8.1 ^{b} ± 2.1	3.7 ^{a} ± 0.9	2.9 ^{a} ± 0.7	5.5 ^{ab} ± 1.0
4-Nonanone	1.7 ± 0.4	1.7 ± 0.5	1.4 ± 0.2	1.1 ± 0.1	1.3 ± 0.1
6-Methyl-5-hepten-2-one	19.7 ± 3.3	12.3 ± 1.4	18.4 ± 3.2	19.2 ± 7.7	15.0 ± 0.5
Benzenic compounds					
Dimethyl benzene	11.9 ^{a} ± 4.2	12.0 ^{a} ± 2.9	16.4 ^{a} ± 0.9	11.5 ^{a} ± 2.3	55.3 ^{b} ± 18.0
1,3,5-Trimethyl benzene	5.9 ± 2.5	4.8 ± 0.9	5.3 ± 0.1	4.0 ± 0.4	5.9 ± 2.1
2-Methyl-(2-propenyl)-benzene	14.8 ± 7.1	10.3 ± 3.9	7.7 ± 0.8	13.8 ± 3.7	8.1 ± 0.9
Benzaldehyde	81.9 ^{a} ± 7.4	89.1 ^{a} ± 9.6	76.7 ^{a} ± 8.5	50.9 ^{a} ± 6.4	407.7 ^{b} ± 88.9
Acetophenone	21.7 ^{b} ± 5.6	17.3 ^{ab} ± 7.0	11.0 ^{a} ± 2.0	9.3 ^{a} ± 1.2	16.8 ^{ab} ± 3.9
Guaiacol	8.4 ^{a} ± 1.4	14.0 ^{a} ± 6.1	24.6 ^{b} ± 5.0	23.5 ^{b} ± 6.1	10.0 ^{a} ± 2.0
Benzyl alcohol	27.2 ^{a} ± 14.8	17.1 ^{a} ± 3.3	12.9 ^{a} ± 6.2	8.5 ^{a} ± 1.3	742.6 ^{b} ± 102.6
Phenylethanol	9.6 ^{a} ± 2.1	6.2 ^{a} ± 0.3	8.2 ^{a} ± 0.9	8.7 ^{a} ± 2.4	14.3 ^{b} ± 3.1
Phenol	21.1 ± 10.2	11.1 ± 1.4	10.8 ± 0.9	11.5 ± 4.7	10.7 ± 1.2
Vainillin	118.4 ^{b} ± 61.5	98.0 ^{ab} ± 20.0	77.9 ^{ab} ± 14.9	63.7 ^{ab} ± 13.1	49.1 ^{a} ± 9.1
Esters					
Ethyl hexanoate	1.1 ^{a} ± 0.2	0.7 ^{a} ± 0.2	0.5 ^{a} ± 0.1	0.6 ^{a} ± 0.1	2.7 ^{b} ± 0.8
2-Ethylhexyl acetate	3.6 ^{a} ± 0.3	3.3 ^{a} ± 0.3	4.3 ^{a} ± 0.4	3.8 ^{a} ± 0.4	17.4 ^{b} ± 0.6
Ethyl octanoate	4.8 ^{a} ± 2.9	4.8 ^{a} ± 3.3	4.2 ^{a} ± 0.5	4.5 ^{a} ± 1.7	28.1 ^{b} ± 12.3
Ethyl decanoate	11.5 ^{a} ± 2.2	12.9 ^{a} ± 2.2	15.7 ^{a} ± 1.4	12.6 ^{a} ± 1.0	47.4 ^{b} ± 20.2
Isopropyl miristate	20.2 ^{c} ± 6.8	18.0 ^{bc} ± 3.1	9.2 ^{a} ± 3.0	9.0 ^{a} ± 1.2	12.4 ^{ab} ± 3.5
Isopropyl palmitate	50.1 ± 41.4	61.4 ± 33.8	39.0 ± 17.1	54.3 ± 31.5	60.0 ± 15.7
Ethyl hexadecanoate	5.6 ^{ab} ± 1.3	6.6 ^{ab} ± 1.1	4.3 ^{a} ± 0.4	6.7 ^{ab} ± 3.3	8.2 ^{b} ± 0.8
Alcohols					
2-Ethyl-1-hexanol	35.5 ^{b} ± 0.7	40.8 ^{b} ± 10.6	13.3 ^{a} ± 2.9	9.6 ^{a} ± 1.6	148.8 ^{c} ± 20.6
1-Phenoxy-2-propanol	15.0 ^{a} ± 3.2	34.9 ^{b} ± 16.0	23.6 ^{ab} ± 6.7	14.4 ^{a} ± 3.1	7.6 ^{a} ± 0.8
2-Phenoxy-1-propanol	4.9 ^{ab} ± 1.5	6.7 ^{b} ± 1.1	6.3 ^{b} ± 2.7	3.8 ^{ab} ± 1.3	2.6 ^{a} ± 1.6
2-Phenoxyethanol	50.1 ± 14.3	63.8 ± 18.7	73.3 ± 35.8	70.0 ± 36.1	46.1 ± 20.3
Furanic and pyranic compounds					
Furfural	225.9 ^{b} ± 21.4	328.3 ^{c} ± 3.8	191.0 ^{b} ± 33.4	520.1 ^{d} ± 68.0	97.7 ^{a} ± 23.8
2-Pentylfuran	53.7 ^{b} ± 8.8	31.5 ^{a} ± 12.4	39.1 ^{a} ± 5.9	55.9 ^{b} ± 8.1	24.9 ^{a} ± 2.5
2,3-Dimethylpyrazine	2.5 ^{c} ± 0.3	3.5 ^{d} ± 0.3	0.1 ^{a} ± 0.0	0.2 ^{a} ± 0.0	0.6 ^{b} ± 0.0
5-Methylfurfural	46.1 ^{b} ± 17.6	48.3 ^{b} ± 16.1	15.8 ^{a} ± 1.4	36.6 ^{b} ± 6.6	8.3 ^{a} ± 1.2
1H-Pyrrole-2-carboxaldehyde	38.5 ^{b} ± 13.8	47.5 ^{b} ± 6.6	3.8 ^{a} ± 0.2	14.8 ^{a} ± 4.7	4.9 ^{a} ± 1.2

Different letters (a, b, c, and d) indicate significant statistical differences between samples. Volatile compounds were identified by comparing them with authentic standards from Sigma-Aldrich, except those marked with (*), which were tentatively identified.

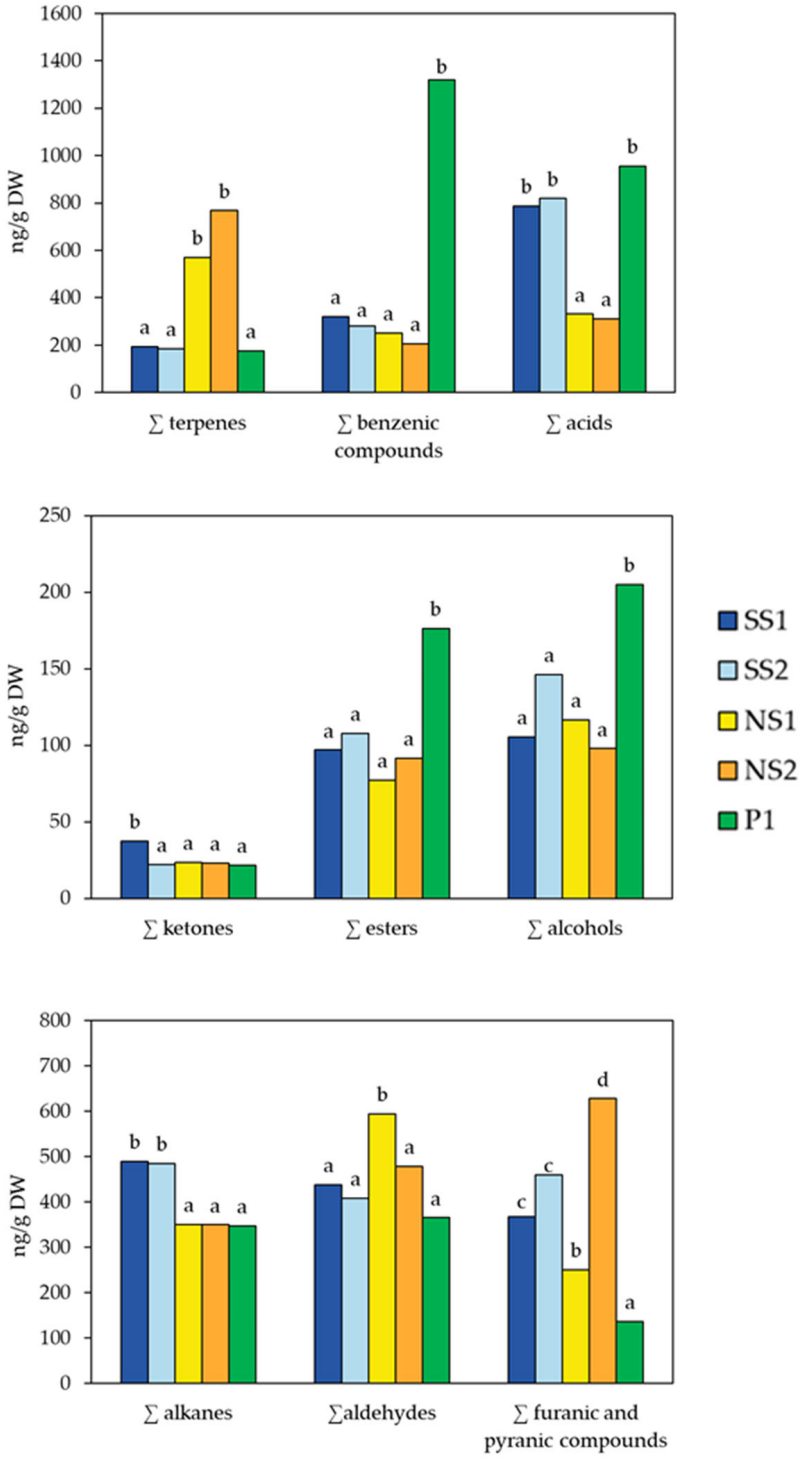

Figure 1. Chemical families of volatile compounds identified in natural corks via HS-SPME-GC-MS. Different letters (a, b, c, and d) indicate significant statistical differences between samples.

These compounds come from the degradation of suberin hydrocarbon chains [21], so the presence and concentration of alkanes in corks could be influenced by factors such as the age of the cork oak, more than their geographical origin.

A significant cluster of compounds identified was composed of terpenes, with a total of 15 compounds. These compounds are synthesized in plants in response to stress and play a vital role in communicating with their environment [22,23]. Terpenes are well known for their unique aromas, such as floral, sweet, citrus, camphor, herbal, and spicy, and are

characterized by their low olfactory detection thresholds [24]. While all samples contained the identified terpenes, noticeable differences were observed in their concentrations. The NS1 and NS2 samples, obtained from the same geographic location, exhibited a remarkable concentration of total terpenes that differed significantly from the other samples. This differentiation was mainly attributed to their high levels of α-copaene and limonene. α-Copaene is a sesquiterpene that imparts woody and spicy notes and is present in various plant species [25,26]. Limonene, on the other hand, is a monoterpene with a sweet and citrus aroma that occurs in high amounts in the peels of citrus fruits [27]. Earlier studies have identified both terpenes in wine model solution extracts of corks, implying their possible migration to wine during aging and/or storage [13].

While no significant differences in the overall terpene content was noted between the NS1 and NS2 samples, the latter, with lower visual quality, exhibited notably higher concentrations of several terpenes, including α-pinene, camphene, eucalyptol, camphor, α-terpineol and isothymol methyl ether. These terpenes, except isothymol methyl ether, have previously been found in natural and conglomerate cork from different origins [11,13,14], and some of them have been identified as important odorants in natural cork stoppers using gas chromatography–olfactometry [11].

In addition to isothymol methyl ether and its isomer, thymol methyl ether, other terpenes such as α-muurolene, cadalene and methyl dihydrojasmonate were detected in natural cork stoppers for the first time. Although these terpenes have not been previously described in cork stoppers, it is well known that plants, including *Quercus suber*, emit several floral and woody volatile terpenes as defense agents [28].

In contrast, the NS1 and NS2 samples from northeast Spain showed a lower content of acids compared to the SS1 and SS2 samples from south Spain and P1 from Portugal. Out of the seven identified acids, notable ones included acetic acid in SS1, SS2 and P1 corks, hexanoic and octanoic acids in SS1 and SS2 corks, and 2-ethylhexanoic acid in P1 corks. These acids are typically present in the volatile fraction of wines, but due to their high thresholds, their negative olfactory notes do not significantly contribute to wine aroma [29]. Acetic acid was the major acid identified, which could have originated from thermal procedures used during the processing of cork planks and stoppers. This is because its formation from wood constituents, mainly from acetate groups of hemicelluloses, has been reported at high temperatures [30].

Aldehydes and ketones were among the other volatile carbonyl compounds identified in the cork samples. These compounds are produced through the lipid oxidation of free fatty acids found in the waxlike and suberin fractions of the corks [21]. The total aldehyde content was higher in the NS1 samples, primarily due to the greater concentration of nonanal resulting from the oxidation of oleic acid. However, the NS2 corks, which shared the same geographical origin, did not show significant differences from the rest of the samples. Some of the aldehydes identified, such as (E)-2-octenal, decanal and (E)-2-nonenal, have been associated with the "sawdust" aroma of wines aged in new oak wood barrels [31]. Regarding ketones, SS1 corks with a higher 2-heptanone content stood out, although no significant differences were found with the SS2 corks, which were also from southern Spain.

Among the benzene compounds identified, several alcohols, aldehydes, ketones, and esters were detected. The P1 samples from Portugal were distinguished by their elevated concentration of total benzene compounds, primarily because of their greater content of dimethyl benzene, benzaldehyde, benzyl alcohol and phenylethanol. However, no significant differences were observed among the cork samples from Spain.

Benzaldehyde, known for its bitter almond-like odor, was prominent in all the samples, particularly in the P1 corks. Benzaldehyde in corks can originate from different sources, including the natural degradation of lignin and the use of certain processing techniques during cork manufacturing. The presence of benzaldehyde in corks has been studied, and its impact on the sensory properties of wine has been evaluated [14,16].

On the other hand, vanillin and guaiacol, both identified in all natural cork samples, are well-known compounds associated with the aroma of vanilla and spices found in wines

aged in oak barrels [32]. Both compounds originate from the natural degradation of lignin, which occurs during the production of barrels through the storage and heating of oak wood [33]. The presence of these compounds in cork may increase during the processing of cork planks and stoppers. In addition, higher concentrations of these compounds have been found in corks contaminated by microorganisms capable of attacking lignin, similar to benzaldehyde and benzyl alcohol [21]. The transfer of these compounds to hydroalcoholic solutions, such as wine, has also been demonstrated [11,34], so their presence in corks must be considered, particularly in the case of guaiacol, which is associated with the musty aroma of "cork taint" [34].

In addition to their high content of benzene compounds, the P1 cork samples from Portugal also showed higher levels of esters and total alcohols. Among the esters, three medium-chain fatty acid ethyl esters, ethyl hexanoate, ethyl octanoate, and ethyl decanoate, were particularly prominent. These compounds are known for their fruity notes in young white wines [35]. Previous studies have identified ethyl hexanoate in corks [13], and research has shown that other ethyl esters and acetates, such as ethyl butyrate or butyl acetate, can migrate from cork to wine solutions, potentially enhancing wine aroma [11]. Therefore, the presence of these esters in corks could have a positive impact on wine quality.

Regarding alcohols, the P1 corks stood out for their significant content of 2-ethyl-1-hexanol, which differentiated them from the other samples in terms of alcohol composition. Although this compound is naturally present in various plants and has been identified in corks from different geographical origins in previous studies [12,16,21], its widespread use as a fragrance ingredient in plastics and pesticides raises concerns about possible contamination in cork production [36].

Finally, the analysis revealed the presence of five furanic and pyranic compounds, with furfural being the major compound, especially in the Spanish samples, followed by 2-pentylfuran, 5-methylfurfural, 1H-pyrrole-2-carboxaldehyde, and 2,3-dimethylpyrazine. These compounds, known for their toasty aroma, are typically produced by the thermal degradation of polysaccharides and are commonly found in woods of the Quercus genus [33]. However, cork sanitization processes that use high temperatures can also increase the levels of these compounds. Rocha et al. [37] described the influence of the heat treatment of corks on their content of furfuraldehydes. More recently, Besalú et al. [38] observed an increase in the content of furfural and 5-methylfurfural in heat-treated corks and related these compounds with their sweet aromatic notes. The second major compound, 2-pentylfuran, is a lipid oxidation product of linoleic acid [39]. Meanwhile, 2,3-dimethylpyrazine is a volatile alkylpyrazine generated via the Maillard reaction [40].

To identify the variables that have the strongest correlation with the differentiation of cork samples, a principal component analysis (PCA) was conducted on the datasets. The first three principal components explained 66.0% of the total variance. The results of the PCA are presented in Figure 2, which shows the samples plotted in a two-dimensional plane defined by the first two principal components (PC1 and PC2). PC1, which explained 29.3% of the total variance, separated the samples from the northeast of Spain from the rest. This differentiation was driven by their higher levels of several terpenes, including thymol methyl ether, α-copaene and limonene, as well as their lower levels of butanoic, hexanoic, octanoic, nonanoic and acetic acids, and other compounds such as isopropyl miristate and acetophenone. On the other hand, PC2, which explained 25.8% of the total variance, clearly differentiated the samples from Portugal from those in Spain. This variable was inversely correlated with benzene compounds including dimethyl benzene, benzaldehyde and phenylethanol, ethyl esters such as ethyl decanoate, ethyl octanoate and ethyl hexanoate, as well as other compounds such as 2-ethylhexanol, ethylhexanoic acid and eicosane, which were more abundant in the Portuguese samples.

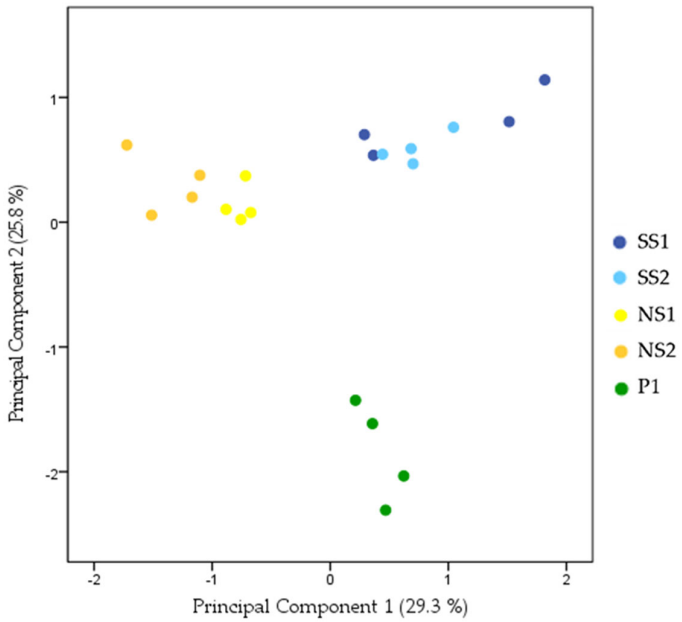

Figure 2. Representation of the cork samples in a two-dimensional plane defined by the first two principal components.

Although the samples analyzed were of different geographical origins and visual qualities, the differentiation was mainly due to their origin. It is reasonable to state that the origin of cork samples has a significant impact on their volatile composition, as the chemical composition of cork is influenced by a range of factors such as soil, climate and the age of the cork oak tree. However, the visual quality is a subjective parameter, which depends mainly on the external appearance of the stoppers.

4. Conclusions

HS-SPME-GC-MS is a fast and simple method that allows the extraction and identification of volatile compounds from natural cork stoppers without prior sample preparation. This method is a very useful tool for the quality assessment of cork, allowing the selection of the most suitable parameters in each stage of the processing, in order to minimize the increase in undesired compounds. The complete characterization of the volatile fraction of corks with a total of 68 compounds identified was carried out, facilitating the correlation of olfactory deviations with specific chemical compounds, such as correlating furanic compounds with sweet aromatic notes, or terpenes with floral or woody notes. This is of great significance for both the cork and wine industries, as it can increase the sensory quality of corks and minimize the risks of unwanted compound migration from the cork to the wine. Additionally, the proposed HS-SPME method allows the traceability of cork stoppers to be monitored, evidencing the relationship between the volatile composition and the geographical origin of the cork planks.

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