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Optimization of thin film-microextraction (TF-SPME) method in order to determine musts volatile compounds

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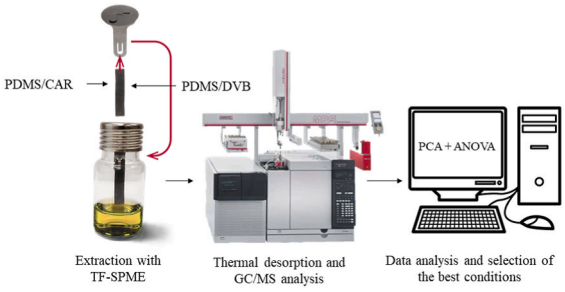
HIGHLIGHTS

- TF-SPME was used for the first time to determine volatile compounds in must.
- The novel thin-film microextraction (TF-SPME) technique were optimized.
- PDMS/CAR and PDMS/DVB absorbents were optimized and compared.
- Both absorbents provided a good extraction of volatile compounds in must.
- The absorbent that provided highest extraction of volatile compounds was PDMS/CAR.

GRAPHICAL ABSTRACT



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ABSTRACT

It is well known that grape aromatic composition is directly correlated to the final wine quality. To determine this composition, a previous stage of selective extraction is necessary, since the aromatic compounds are found in very low concentrations in the grapes. Therefore, in this work, the thin film microextraction technique (TF-SPME) was optimized, for first time, with the aim to analyze the volatile composition of the grape musts. The results obtained with the two commercially available absorbent materials for TF-SPME, polydimethylsiloxane/carboxene (PDMS/CAR) and PDMS/divinylbenzene (PDMS/DVB), were optimized and compared. To carry out the optimization, a randomized factorial design was performed combining the following factors and levels: extraction mode (headspace (HS), or direct immersion (DI)), stirring speed (500 and 1000 rpm), extraction time (1, 3 and 6 h), and extraction temperature (20, 40 and 60 °C). After performing a principal component analysis (PCA) and an analysis of variance (ANOVA) multifactorial, it was concluded that the best conditions for TF-SPME with PDMS/CAR were: direct immersion (DI), 500 rpm, 6 h, and 20 °C, while for TF-SPME with PDMS/DVB no conditions were found that maximized the extraction of most compounds, therefore compromise conditions were chosen: headspace (HS), 500 rpm, 6 h, and 40 °C. Finally, the comparison between the results obtained with both absorbents indicated that the absorbent that extracted better the volatile compounds from the musts with the TF-SPME technique, was PDMS/CAR, under the conditions: direct immersion (DI), 500 rpm, 6 h, and 20 °C.

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1. Introducción

Grape aroma is one of the most important attributes to determine musts and wines quality [1,2]. Aroma components can be classified according to their origin into: primary aromas, secondary or fermentative aromas, and aging aromas [3]. Primary aromas, also called grape aromas, are composed of varietal and pre-fermentative compounds. Grape aroma depends on several factors, including grape variety, climatic conditions, and viticultural practices [4,5]. Varietal aroma is mainly composed of: monoterpenoids, C_{13} norisoprenoids, benzenoids, esters, thiols and methoxypyrazines. Monoterpenoids and C_{13} norisoprenoids are the main contributors to wine aroma, and the pre-fermentative aroma are the "green leaf volatiles" or C6 compounds [2,3,6].

It is necessary to determine and know the aromatic compounds present in the must in order to understand the chemical nature of the wine aroma. These aromatic compounds are found in very low concentrations in the musts, so an effective pre-concentration method is required prior to their analysis in order to carry out their identification and quantification [7–9]. In recent years, different techniques have been used to extract volatile compounds from grapes and wines. From the most classical ones, such as liquid-liquid extraction (LLE) and solid-phase extraction (SPE), to more modern ones such as solid-phase microextraction (SPME) and stir bar sorptive extraction (SBSE) [9]. The latter emerged in order to solve some of the drawbacks of LLE and SPE, such as low sensitivity, difficulty of automation, high sample and solvent consumption, etc. [8]. In SPME and SBSE the absorbents are inserted directly into the thermal desorption system (TD) of the gas chromatograph (GC). SBSE increases the sensitivity by a factor of 50–250 and is much more robust compared to SPME. On the other hand SPME has more absorption materials than SBSE, which improves the polarity range of extractable volatiles and increases the number of applications [8–10]. The fact that there is no technique that greatly increases sensitivity and has multiple absorbers, prompted the birth of the thin film-SPME (TF-SPME).

TF-SPME was first mentioned by Bruheim et al. [11], to improve the sensitivity of previous methods such as SPME or SBSE [12]. TF-SPME consists of a carbon film (20 mm × 4.8 mm) covered with an absorbent material. It can be use by headspace (HS) or by direct immersion (DI). In HS mode, the film is placed on a solid or liquid sample in a closed vial. In DI mode, the film is introduced directly into a liquid sample [9,13]. TF-SPME is more sensitive than SPME because it has a larger phase volume. It also increases the extraction ratio, since it has a larger surface area than SPME [9,12,14,15]. On the other hand, the film is composed of more than one absorbent material, polydimethylsiloxane /divinylbenzene (PDMS/DVB), polydimethylsiloxane/carboxene (PDMS/CAR), and hydrophilic lipophilic balanced/polydimethylsiloxane (HLB/PDMS), thus broadening the range of polarity of the compounds that can extract [15,16]. TF-SPME applications are available for the extraction of organic compounds in water [12,17], in cod liver oil [18], and in olive oil [19], of artificial sweeteners in water [20], and the aroma and flavour profile in food samples (dark chocolate, and cheese) and beverages (coffee, wine, lemon, lime soda, and berry sports drink) [13,15,16]. In these works, TF-SPME provided good extractions of volatile compounds, even improving the extraction with SBSE. Therefore, it has been demonstrated that TF-SPME has several advantages over SBSE and SPME in the matrices mentioned above, and then, it is expected to present the same advantages in other matrices, such as grape must. However, this extraction technique has not yet been used for the analysis of volatile compounds in grape musts.

For all these reasons, in this work, the TF-SPME technique has been optimized for the first time for the extraction of volatile compounds in musts. For this purpose, two types of TF currently available on the market have been used: polydimethylsiloxane/divinylbenzene (PDMS/DVB) and polydimethylsiloxane/carboxene (PDMS/CAR). The optimization was carried out following a design of experiments (DoE). Once the compounds have been extracted, they were identified and quantified by gas chromatography-mass spectrometry (GC-MS) system.

2. Materials and methods

2.1. Materials and reagents

Chromatographic standards linalool, α-terpineol, geraniol, β-damascenone, β-ionone, 2-phenylethanol, isoamyl acetate, ethyl hexanoate, ethyl octanoate, ethyl decanoate, 2-phenethyl acetate, hexanoic acid, octanoic acid, isoamyl alcohol (3-methyl-1-butanol), amyl alcohol (2-methyl-1-butanol), isobutanol (2-methyl-1-propanol), 2-octanol (internal standard, I.S.), 1-hexanol, hexanal, the reagent NaCl and ethanol (EtOH), were purchased from Merck (Darmstadt, Germany). Water was purified through a Milli-Q system Millipore (Bedfords, MA, USA).

TF-SPME with PDMS and carboxen (PDMS/CAR), with PDMS and divinylbenzene (PDMS/DVB) (carbon fabric film thickness 450 μm), liners packed with Tenax TA™, and borosilicate magnetic stirrers were obtained from GERSTEL GmbH & Co (Mülheim an der Ruhr, Deutschland). BP21 capillary column (50 m length, 0.22 mm i. d., and 0.25 μm film thickness) was purchased from SGE (Ringwood, Australia).

Ultra-Turrax was bought from IKA-Werke GmbH & Co. KG (Staufen, Germany). Gas chromatograph was purchased from Agilent Technologies (Palo Alto, CA, USA). The autosampler system consisted of a multipurpose sampler (MPS), equipped with tube tray, thermal desorption unit (TDU), and cooled injection system (CIS-4) connected to a N_2 ranger. Multi-purpose sampler (MPS) and automated thermal desorption unit (TDU) were provided from GERSTEL.

2.2. Grape paste

A total of 50 Tempranillo vines were marked in a vineyard located in Logroño (La Rioja). The bunches belonging to these vines were hand-harvested at their optimal moment of maturation, during the 2019 season, obtaining a total of 44.4 kg of grapes. The bunches were shelled in a box, and subsequently all the berries were mixed. All the berries were crushed in the Ultra-Turrax until a homogeneous paste was obtained. A total of 450 Falcon tubes of 50 mL were filled and frozen (−20 °C) for later use.

2.3. Standards solution

In order to perform a better optimization, a standard solution was prepared in which all the standards were added. The concentration of the standards was: 0.001 mg/mL of linalool, α-terpineol, geraniol, β-ionone, isoamyl acetate, hexanoic acid, octanoic acid, isobutanol, hexanal, amyl alcohol, isoamyl alcohol, and 1-hexanol; 0.0002 mg/mL of ethyl octanoate, ethyl hexanoate and ethyl decanoate; and $9.99{\cdot}10^{-5}$ mg/mL of β-damascenone. This solution was prepared in 12% EtOH.

2.4. Design of experiments (DoE)

A randomised factorial design of 4 factors of 2, 3, 3 and 2 levels each was performed. The programme used to perform the DoE was Minitab 18 (Minitab Inc, Pennsylvania, USA). The factors were: stirring speed (500, and 1000 rpm), extraction time (1, 3, and 6 h), extraction temperature (20, 40, and 60 °C), and extraction mode (headspace (HS), and direct immersion (DI)). Samples were run in duplicate, giving a total of 108 assays for each type of absorbent (PDMS/DVB and PDMS/CAR).

Initially, also the stirring speed of 1500 rpm was tried, but the stirring magnets broke, so we did not continue working at this stirring speed.

2.5. Conditioning and cleaning of TF

The TF were conditioned before the first use and after each use. TF

Table 1
Maximum and minimum values of the relative area with respect to 2-octanol (I.S.) of each compound within each absorbent (PDMS/CAR and PDMS/DVB).

	PDMS/CAR		PDMS/DVB	
	Maximum value	Minimum value	Maximum value	Minimum value
Terpenoids				
Linalool	2.21 **(HS_1000_6_60)**	0.13 **(HS_500_3_60)**	2.21 **(HS_1000_6_60)**	0.40 **(HS_500_1_20)**
α-Terpineol	0.90 **(HS_1000_6_60)**	0.09 **(HS_1000_3_20)**	2.22 **(HS_1000_6_60)**	0.08 **(HS_500_1_20)**
Geraniol	1.81 **(DI_1000_3_40)**	0.03 **(HS_500_3_60)**	1.51 **(DI_500_3_40)**	0.03 **(HS_500_1_20)**
C_{13} norisoprenoids				
β-Damascenone	4.92 **(DI_500_6_20)**	0.13 **(HS_1000_3_20)**	1.01 **(HS_1000_6_60)**	0.04 **(HS_500_1_20)**
β-Ionone	95.16 **(DI_500_6_20)**	0.18 **(HS_1000_3_20)**	13.20 **(DI_1000_6_60)**	0.03 **(HS_500_1_20)**
Benzenoid compounds				
Benzaldehyde	1.57 **(HS_500_1_20)**	0.036 **(HS_1000_3_20)**	0.15 **(HS_500_1_20)**	0.01 **(HS_500_3_40)**
2-Phenylethanol	6.80 **(DI_500_6_20)**	0.15 **(HS_1000_3_20)**	0.96 **(HS_1000_6_60)**	0.06 **(HS_500_1_20)**
Esters				
Isoamyl acetate	2.08 **(DI_500_3_20)**	0.09 **(HS_1000_3_20)**	4.84 **(HS_1000_1_20)**	0.30 **(DI_500_3_60)**
Ethyl hexanoate	4.25 **(DI_500_3_20)**	0.21 **(HS_1000_3_20)**	2.15 **(HS_1000_1_20)**	0.19 **(DI_500_3_20)**
Ethyl octanoate	13.43 **(DI_500_6_20)**	0.13 **(DI_1000_1_40)**	1.59 **(HS_1000_3_20)**	0.05 **(DI_500_3_20)**
Ethyl decanoate	19.54 **(DI_500_3_60)**	0.007 **(DI_1000_1_40)**	2.25 **(DI_1000_6_60)**	0.003 **(DI_500_3_20)**
Fatty acids				
Hexanoic acid	2.46 **(HS_500_1_20)**	0.20 **(HS_1000_1_40)**	1.74 **(HS_500_3_20)**	0.08 **(HS_500_6_20)**
Octanoic acid	0.89 **(DI_500_3_60)**	0.04 **(HS_1000_3_20)**	0.98 **(HS_1000_6_60)**	0.02 **(HS_500_6_20)**
Higher alcohols				
Isoamyl alcohol	0.15 **(DI_500_3_60)**	0.007 **(HS_1000_3_20)**	0.32 **(HS_500_3_40)**	0.007 **(HS_1000_1_20)**
Amyl alcohol	0.04 **(DI_1000_6_40)**	0.005 **(HS_1000_3_60)**	0.12 **(HS_500_3_20)**	0.002 **(DI_500_1_20)**
2-Ethyl-1-hexanol	0.20 **(HS_500_1_20)**	0.009 **(HS_500_3_20)**	0.55 **(HS_500_3_60)**	0.002 **(DI_1000_6_20)**
C6 compounds				
1-Hexanol	1.46 **(DI_1000_1_60)**	0.44 **(HS_1000_3_20)**	1.94 **(HS_500_1_40)**	0.44 **(DI_500_1_60)**
(Z)-3-Hexen-1-ol	0.26 **(DI_1000_1_60)**	0.04 **(DI_1000_1_40)**	0.28 **(DI_500_6_20)**	0.009 **(HS_1000_6_60)**
(E)-2-Hexen-1-ol	0.62 **(HS_1000_6_40)**	0.05 **(DI_1000_1_40)**	0.28 **(DI_500_1_20)**	0.001 **(DI_500_1_60)**
Hexanal	5.39 **(HS_500_1_20)**	0.12 **(HS_1000_3_20)**	6.27 **(HS_1000_1_20)**	0.48 **(DI_1000_3_60)**
2-Hexenal	4.54 **(HS_1000_6_20)**	0.23 **(HS_1000_3_20)**	0.83 **(HS_500_3_20)**	0.10 **(DI_500_3_40)**
Other compounds				
Heptanal	3.12 **(HS_500_1_20)**	0.009 **(HS_1000_3_60)**	0.06 **(HS_1000_6_40)**	0.002 **(DI_1000_1_40)**
Decanal	0.51 **(HS_1000_1_60)**	0.003 **(DI_1000_1_40)**	0.09 **(HS_1000_6_60)**	0.001 **(DI_1000_1_40)**
Furanmethanol	3.91 **(DI_1000_3_40)**	0.01 **(HS_1000_6_40)**	2.18 **(DI_1000_6_60)**	0.004 **(HS_1000_6_40)**
Acetol	1.41 **(DI_500_3_40)**	0.02 **(DI_1000_1_40)**	0.58 **(DI_1000_6_60)**	0.002 **(DI_500_1_40)**
Methyl jasmonate	10.21 **(HS_1000_6_20)**	0.02 **(HS_500_3_40)**	4.06 **(DI_500_1_60)**	0.002 **(HS_1000_6_40)**

The conditions that gave these values are shown in parentheses. HS: headspace. DI: direct immersion. Stirring speed: 500 or 1000 rpm. Extraction time: 1, 3, or 6 h. Extraction temperature: 20, 40, or 60 °C.

were placed in a desorption tube and thermally desorbed using an autosampler, controlled with GERSTEL MAESTRO software, coupled to the gas chromatography (GC) system. The helium flow was 75 mL/min. Following the manufacturer's recommendations, the TDU temperature programme was set from 40 °C to 250 °C (50 min) at a rate of 100 °C/min. The temperature of the injection system CIS-4 increased from 40 °C to 250 °C (5 min) at a rate of 12 °C/s.

2.6. Optimization of volatile compounds extraction by TF-SPME

An aliquot of 8 mL of centrifuged must sample (15 min, 4500 rpm), 1 mL of the standards solution, and 25 μL of the 2-octanol solution (5 μL 2-octanol/100 mL EtOH) were added in a 10 mL (DI) or 20 mL (HS) screw capped vial. A TF-SPME (PDMS/CAR, or PDMS/DVB) device was suspended in the screw capped vial. A borosilicate magnetic stirrer was added. All samples were stirred at the working speed (500, or 1000 rpm), at the specified time (1, 3, or 6 h) and at the definite temperature (20, 40, or 60 °C). After extraction, the TF-SPME device was removed, dried with a tissue paper, and was placed in an empty TDU tube with a glass wool plug at the base. The TDU tube was sealed with a transport adapter and placed in a 40 position Twister rack on the MPS robotic for automated analysis.

2.7. TF desorption conditions

The volatile analysis was performed using an automated TDU. The method used for the determination of must volatile composition is based on that described by Sánchez-Gómez et al. [21] with some modifications. TF were thermally desorbed in a stream of helium as carrier gas at a flow rate of 75 mL/min in the TDU in splitless desorption mode, increasing the temperature from 40 °C to 250 °C at a rate of 60 °C/min and holding at the final temperature for 5 min. The analytes were focused in a programmed temperature vaporising injector (CIS-4), containing a Tenax TA-packed liner with 20 mg of Tenax, held at −40 °C with liquid N_2 cooling prior to injection. After desorption and focusing, the CIS-4 temperature was programmed from −40 °C to 230 °C at 12 °C/s and held at 240 °C for 5 min to transfer volatiles onto the analytical column. The CIS-4 operated in solvent vent mode (purge flow to split vent of 80 mL/min, vent 75 mL/min and pressure 20.85 psi).

2.8. Chromatographic conditions for TF-SPME

The desorbed volatile compounds were separated in an Agilent 7890A gas chromatograph system (GC) coupled to a triple quadrupole (QqQ) Agilent 7000C electron ionization mass spectrometric detector, operating in simple quadrupole (Q).

The oven temperature of GC was programmed at 40 °C (2 min), raised to 80 °C (5 °C/min, held for 2 min) then to 130 °C (10 °C/min, held 5 min) then to 150 °C (5 °C/min, held for 5 min) and finally to 230 °C (10 °C/min, held 5 min). Transfer line temperature was 230 °C. The MS operated in scan mode (35–300 amu) with ionization energy set at 70 eV. In order to carry out the identification of each compound, the mass spectra obtained were compared with those of the NIST library, and chromatographic retention index of each standard. Compounds for which no standard was added were identified by comparing their mass spectra with NIST. To avoid matrix interferences, it was integrated by ion extraction chromatogram (EIC), isolating the target ion of each compound individually. The target ions were *m/z* 41 for 2-hexenal, *m/z*

Table 2
Variables (compounds) of each principal component (PC) for each of the absorbents (PDMS/CAR and PDMS/DVB).

PC	PDMS/CAR	PDMS/DVB
1	α-Terpineol (0.87) β-Damascenone (0.85) β-Ionone (0.79) Linalool (0.77) Ethyl octanoate (0.75) Ethyl decanoate (0.72) Geraniol (0.64) Decanal (0.60)	Isoamyl acetate (0.91) Hexanal (0.91) Ethyl hexanoate (0.79) Amyl alcohol (0.76) 2-Hexenal (0.73) 1-Hexanol (0.72)
2	Heptanal (0.92) Benzaldehyde (0.90) Hexanoic acid (0.89) 2-Ethyl-1-hexanol (0.77)	Ethyl octanoate (0.91) Ethyl decanoate (0.86) β-Ionone (0.79) β-Damascenone (0.79)
3	(Z)-3-Hexen-1-ol (0.92) (E)-2-Hexen-1-ol (0.91) 1-Hexanol (0.90) Hexanal (0.67)	2-Phenylethanol (0.94) Octanoic acid (0.91) α-Terpineol (0.74) Geraniol (0.67)
4	Isoamyl acetate (0.92) Ethyl hexanoate (0.87)	(E)-2-Hexen-1-ol (0.86) (Z)-3-Hexen-1-ol (0.78) Hexanoic acid (−0.74)
5	Methyl jasmonate (0.90) 2-Hexenal (0.82)	Acetol (0.94) Furanmethanol (0.89)
6	Furanmethanol (0.71) Octanoic acid (0.65) 2-Phenylethanol (0.64)	Heptanal (0.72) Isoamyl alcohol (0.61)
7	–	2-Ethyl-1-hexanol (0.91)

The percentage weight of each variable within each component is shown in parentheses.

43 for isoamyl acetate, decanal, and acetol, *m/z* 45 for 2-octanol (I.S.), *m/z* 55 for isoamyl alcohol, *m/z* 56 for 1-hexanol, and hexanal, *m/z* 57 for amyl alcohol, 2-ethyl-1-hexanol, and (E)-2-hexen-1-ol, *m/z* 59 for α-terpineol, *m/z* 60 for hexanoic acid, and octanoic acid, *m/z* 67 for (Z)-3-hexen-1-ol, *m/z* 69 for β-damascenone, and geraniol, *m/z* 70 for heptanal, *m/z* 71 for linalool, *m/z* 77 for benzaldehyde, *m/z* 83 for methyl jasmonate, *m/z* 88 for ethyl hexanoate, ethyl octanoate, and ethyl decanoate, *m/z* 91 for 2-phenylethanol, *m/z* 98 for furanmethanol, and *m/z* 177 for β-ionone. Finally, a semi-quantification was carried out by comparing the area of each compound with that of 2-octanol (I.S.), thus obtaining the relative area.

2.9. Method validation

Method validation has been carried out for the optimized conditions, i.e., PDMS/CAR thin film, direct immersion, 20 °C, 6 h, and agitation at 500 rpm.

The selectivity of the method is the ability of an analytical method to accurately and specifically measure the analyte without interference from impurities, degradation products or excipients that may be present in the sample. No interference peaks were observed in any of the samples.

The accuracy and precision of the method was validated by the recovery (%) and the RSD (%), respectively. For this purpose, the same sample was measured 8 times. The recovery (%) values obtained were in the range of 79.19–106.84%. The RSD (%) values were in the range of 4.2–17.76%.

The linearity of the method was studied using 4–7 points of different concentrations (μg/L), for each of the standards. Three replicates of each point were performed. A good linearity was observed with determination coefficients (R^2) in the range of 0.964–0.996.

2.10. Statistical analysis

Statistical analysis was proposed in which the values of relative area of all the volatile compounds obtained in each of the described experimental conditions studied simultaneously in order to find which conditions maximize the overall extraction of volatiles compounds. Prior to the analysis, an exhaustive descriptive analysis was performed to detect and eliminate, when necessary, the outliers. This descriptive analysis verified that there was a significant degree of correlation between the volatile compounds, justifying the need to analyze all the volatile compounds together using a multivariate treatment.

First, a principal component analysis was performed to reduce the dimensionality of the problem by synthesizing the volatile compounds into a reduced number of principal components [22]. In order to facilitate the interpretation of the principal components obtained, a varimax rotation was applied. Secondly, and consistent with the experimental design described above, a multifactorial analysis of variance (ANOVA) was used, taking the scores of each principal component as the response variable.

Finally, an ANOVA was performed to compare the best conditions of the two absorbents (PDMS/CAR, and PDMS/DVB). In this case, the volatile compounds were not treated as a set, but the relative area values of each were compared individually.

3. Results and discussion

3.1. Must volatile composition

The maximum and minimum values of relative area obtained for each volatile compound with each of the absorbents can be seen in Table 1. In order to simplify the results description, only the conditions that gave the maximum and minimum value for each compound are shown. The musts compounds that were identified in the chromatograms are those shown in Table 1.

With both, the PDMS/CAR and the PDMS/DVB absorbents, a total of 26 compounds were identified. These compounds were classified into 8 chemical families: terpenoids (linalool, α-terpineol, and geraniol), C_{13} norisoprenoids (β-damascenone, and β-ionone), benzenoid compounds (benzaldehyde, and 2-phenylethanol), esters (isoamyl acetate, ethyl hexanoate, ethyl octanoate, and ethyl decanoate), fatty acids (hexanoic acid, and octanoic acid), higher alcohols (isoamyl alcohol, amyl alcohol, and 2-ethyl-1-hexanol), C6 compounds (1-hexanol, (Z)-3-hexen-1-ol, (E)-2-hexen-1-ol, hexanal, and 2-hexenal), and other compounds (heptanal, decanal, furanmethanol, acetol, and methyl jasmonate). Within these, terpenoids, C_{13} norisoprenoids, and some benzenoid compounds are those that have the lowest perception thresholds, so their contribution to the aroma is important [4,23]. In the case of terpenoids, α-terpineol was best extracted with the PDMS/DVB absorbent, while geraniol was a little better extracted with the PDMS/CAR absorbent. On the other hand, it can be observed that in both absorbents, the best conditions for the extraction of linalool and α-terpineol compounds were headspace, 1000 rpm, 6 h, and 60 °C (HS_1000_6_60) (Table 1). Geraniol also matched in that with both absorbents it was most effectively extracted by direct immersion, for 3 h and at 40 °C. Terpenoids are formed from the precursor mevalonate, a metabolite derived from acetyl-CoA [2]. Linalool, α-terpineol, and geraniol are among the most important terpenoids for grape aroma [24]. Linalool is associated with the aromatic descriptors coriander seed, and rose and geraniol with geranium and rose [3,25].

In the case of C_{13} norisoprenoids, both β-damascenone and β-ionone compounds were most effectively extracted with the PDMS/CAR absorbent. Within this, the best conditions were, for both, direct immersion, 500 rpm, 6 h, and 20 °C (DI_500_6_20) (Table 1). C_{13} norisoprenoids are compounds derived from the breakdown of carotenoids [26–29]. β-Damascenone, and β-ionone are two of the most important C_{13} norisoprenoids due to their low perception thresholds [24,30]. For

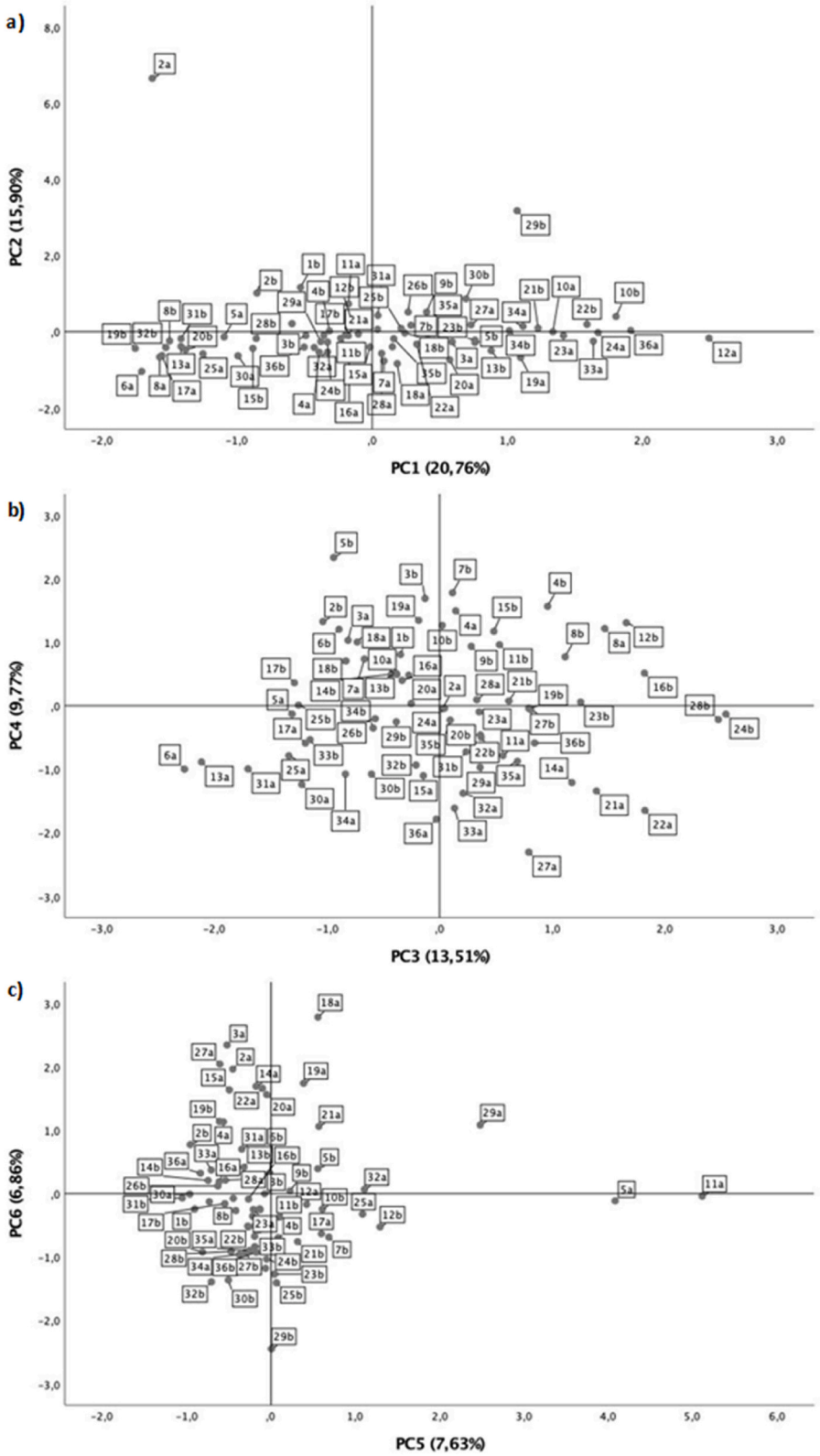

Fig. 1. Principal component analysis plots for PDMS/CAR absorbent. a) PC1 *vs* PC2, b) PC3 *vs* PC4, and c) PC5 *vs* PC6. Each number shown in the graphic corresponds to a combination of conditions. The letters a and b after each number correspond to the two replicates of each combination of conditions.

β-damascenone descriptors of baked apples, honey, and floral fruity aromas have been reported [26,30,31]. The β-ionone contributes violet aroma to red wine [30].

The benzenoid compounds benzaldehyde and 2-phenylethanol were also better extracted with PDMS/CAR absorbent than with PDMS/DVB absorbent. Benzaldehyde was better extracted under the following conditions: headspace, 500 rpm, 1 h, and 20 °C (HS_500_1_20), with both absorbents (Table 1). The most important benzenoid compound is

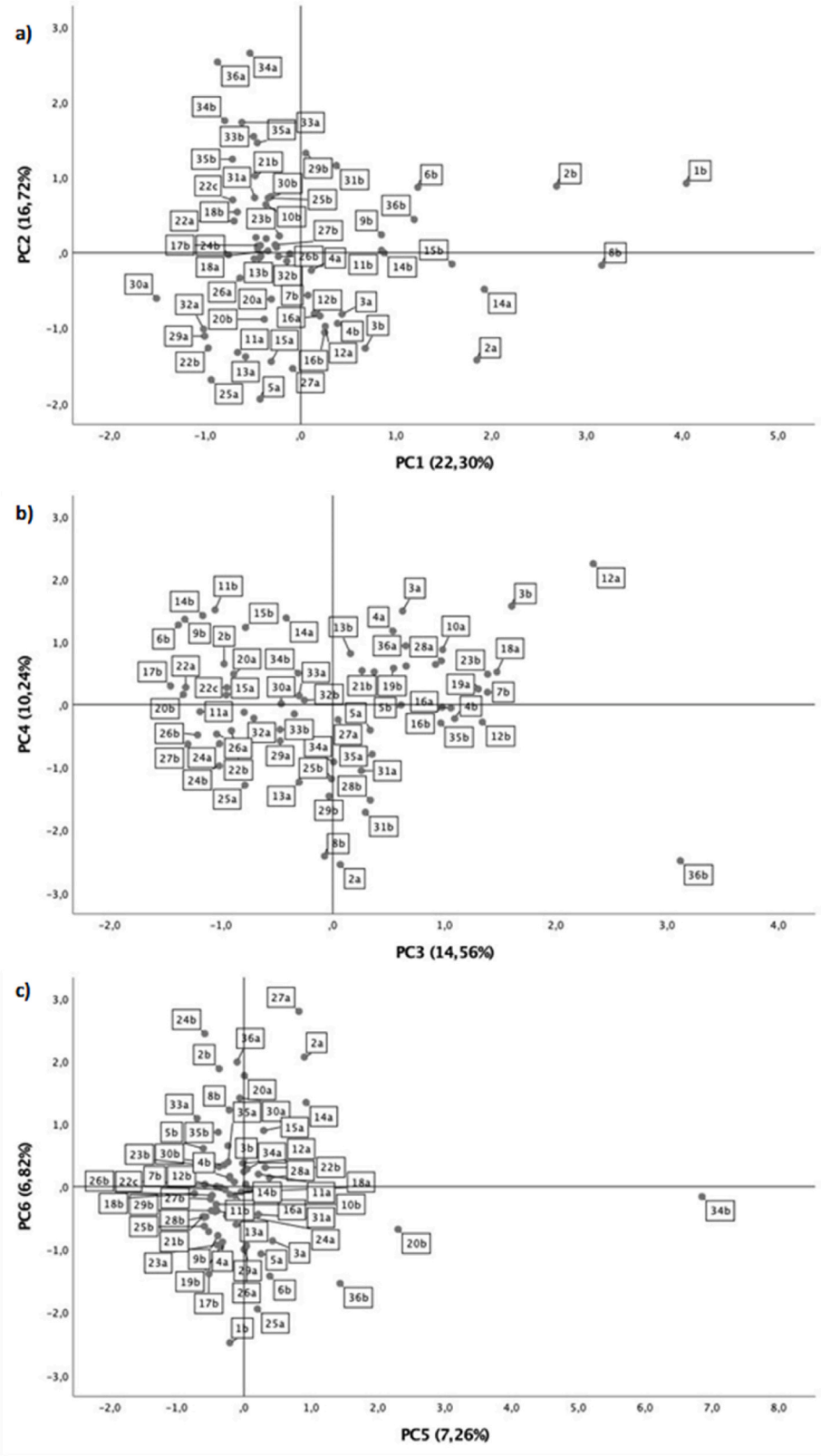

Fig. 2. Principal component analysis plots for PDMS/DVB absorbent. a) PC1 *vs* PC2, b) PC3 *vs* PC4, and c) PC5 *vs* PC6. Each number shown in the graphic corresponds to a combination of conditions. The letters a and b after each number correspond to the two replicates of each combination of conditions.

2-phenylethanol, which provides floral aromas [3].

The esters ethyl hexanoate, ethyl octanoate, and ethyl decanoate were better extracted with the PDMS/CAR absorbent, while isoamyl acetate was better extracted with the PDMS/DVB adsorbent (Table 1). Esters play an important role in wine aroma, but are mainly formed during the alcoholic fermentation [1], and the ethyl esters contribute to the pleasant aroma of wines.

Regarding the fatty acids in musts, hexanoic acid was better

Table 3
Tests of inter-subject effects obtained from multifactorial analysis of variance (ANOVA) for each absorbent (PDMS/CAR and PDMS/DVB).

		PDMS/CAR										PDMS/DVB									
	PC	1		2		3		4		5		1		2		3		4		5	
	Factors	F	**p**	F	**p**	F	**p**	F	**p**	F	**p**	F	**p**	F	**p**	F	**p**	F	p	F	p
Individual	**Mode**	2.465	0.126	1.557	0.221	0.195	0.662	5.456	**0.026**[a]	2.502	0.123	28.979	**0.000**[a]	0.014	0.905	97.065	**0.000**[a]	0.043	0.838	0.000	0.987
	RPM	0.014	0.907	1.137	0.294	0.206	0.653	0.102	0.751	0.981	0.329	0.197	0.660	1.567	0.220	0.139	0.712	0.087	0.770	0.000	0.987
	Time	9.843	**0.000**[a]	1.392	0.263	6.353	**0.005**[a]	1.539	0.230	2.626	0.087	14.600	**0.000**[a]	7.241	**0.003**[a]	1.302	0.287	0.860	0.433	0.000	0.987
	T[a]	0.260	0.773	3.135	0.057	1.335	0.277	19.779	**0.000**[a]	1.813	0.179	38.234	**0.000**[a]	4.653	**0.017**[a]	1.567	0.225	6.865	**0.004**[a]	0.000	0.987
Second-order interactions	**Mode**[a]**RPM**	0.000	0.990	0.099	0.755	0.767	0.387	0.192	0.664	4.751	**0.037**[a]	0.092	0.764	0.200	0.658	0.219	0.643	0.261	0.613	0.000	0.987
	Mode[a]**Time**	0.611	0.549	4.756	**0.015**[a]	0.06	0.942	0.159	0.854	1.330	0.278	6.185	**0.006**[a]	0.869	0.430	0.222	0.802	0.260	0.773	0.000	0.987
	Mode[a]**T**[a]	2.039	0.146	2.227	0.124	0.035	0.966	0.017	0.983	0.101	0.904	15.993	**0.000**[a]	2.592	0.092	15.225	0.000	1.943	0.161	0.000	0.987
	RPM[a]**Time**	0.032	0.968	0.602	0.553	2.831	0.073	0.227	0.798	1.581	0.221	0.469	0.630	0.508	0.607	0.499	0.612	1.356	0.273	0.000	0.987
	RPM[a]**T**[a]	0.169	0.845	0.363	0.698	1.460	0.247	0.341	0.714	0.846	0.438	1.164	0.326	0.760	0.477	4.510	0.019	0.974	0.389	0.000	0.987
	Time[a]**T**[a]	0.068	0.991	2.357	0.074	1.476	0.232	0.709	0.592	1.369	0.266	4.132	**0.009**[a]	2.872	0.040	2.281	0.084	1.283	0.299	0.000	0.987

[a] Indicates significant effect of the factor or second-order interaction ($p \leq 0.05$). Mode: Extraction mode. RPM: Stirring speed. Time: Extraction time. T[a]: Extraction temperature.

extracted with PDMS/CAR, while octanoic acid improved it extraction with the PDMS/DVB absorbent (Table 1). Fatty acids are precursors of a large number of alcohols, aldehydes, ketones, acids, esters, and lactones [1].

In the case of higher alcohols, all are better extracted with the PDMS/DVB absorbent (Table 1). On this absorbent, the three compounds were better extracted with the following conditions: headspace, 500 rpm, and 3 h (Table 1).

Respect to the C6 compounds in the musts, 1-hexanol, (Z)-3-hexen-1-ol, and hexanal, were better extracted with the PDMS/DVB absorbent, while (E)-2-hexen-1-ol, and 2-hexenal, with the PDMS/CAR absorbent. C6 compounds, in high concentrations, can provide negative notes to the wines. C6 compounds mainly consist of alcohols and aldehydes. These compounds are derived from the fatty acids and are responsible for herbaceous and green aromas, therefore they are called "green leaf volatiles" [1,4,32].

Finally, the other compounds that could be identified in the musts samples, were better extracted with PDMS/CAR absorbent. Although there are currently no studies comparing PDMS/CAR TFs *versus* PDMS/DVB TFs for the extraction of volatile compounds in musts by TF-SPME, there are some studies comparing fibers formed by these same absorbents for the extraction of volatile compounds with SPME. Jelen [33] compared the fiber coatings: polydimethylsiloxane (PDMS), polyacrylate (PA), carboxene/divinylbenzene/polydimethylsiloxane (CAR/DVB/PDMS), polydimethylsiloxane/divinylbenzene (PDMS/DVB) and polydimethylsiloxane/carboxene (PDMS/CAR) for the extraction of volatile compounds in white wine by HS-SPME. The author observed that the PDMS/CAR fiber provided the best response in the extraction of carbonyl compounds (furfural and benzaldehyde), while the PDMS/DVB fiber gave better results in the extraction of alcohols, esters and terpenes. Moreover, it can be observed that fibers containing CAR in their structure performed much better than the remaining ones. Sánchez-Palomo et al. [34] reported that HS-SPME using a PDMS/DVB fiber is a rapid and useful method for the quantification of volatile compounds in the grape skins and pulp, allowing rapid screening of aromatic compounds in grapes of different varieties or cultivars. On the other hand, Slaghenaufi et al. [35] suggested that the best compromise to efficiently extract all the different classes of volatile sulfur compounds in wine was obtained using HS-SPME with PDMS/CAR fiber. Petrozziello et al. [36] compared various absorbents for the extraction of norisoprenoids in wine, including both, PDMS/DVB and PDMS/CAR. In this case, they observed that the best extraction of β-damascenone and β-ionone compounds occurred with PDMS/DVB fiber, which is the opposite of what was observed in this study.

In view of the results shown in Table 1, it is not possible to choose either the absorbent material or the conditions that maximize the extraction of most of the compounds, so it is necessary to use a multivariate statistical analysis.

3.2. Principal component analysis (PCA)

A PCA was performed to reduce the total number of compounds (variables) to a reduced number of principal components (PCs). The number of variables used for each of the absorbents was 26 (Table 1). In all cases, it was found that the Kaiser-Meyer-Olkin (KMO) test of sampling adequacy obtained sufficiently large values (between 0 and 1): in all cases, values above 0.8 were obtained [37]. Barlett's test of sphericity was also significant in all cases. Both tests indicated that the underlying PC extraction method was adequate. The values of the communalities of each of the variables were high in all cases. With this analysis, 6 principal components were retained in TF-SPME-PDMS/CAR, and 7 in TF-SPME-PDMS/DVB. This analysis has simplified the study of the relationship between the values obtained in the aromatic profile with the experimental factors, since it has allowed the identification of groups of volatile compounds with correlative performance. Table 2 shows the compounds that make up each of the PCs.

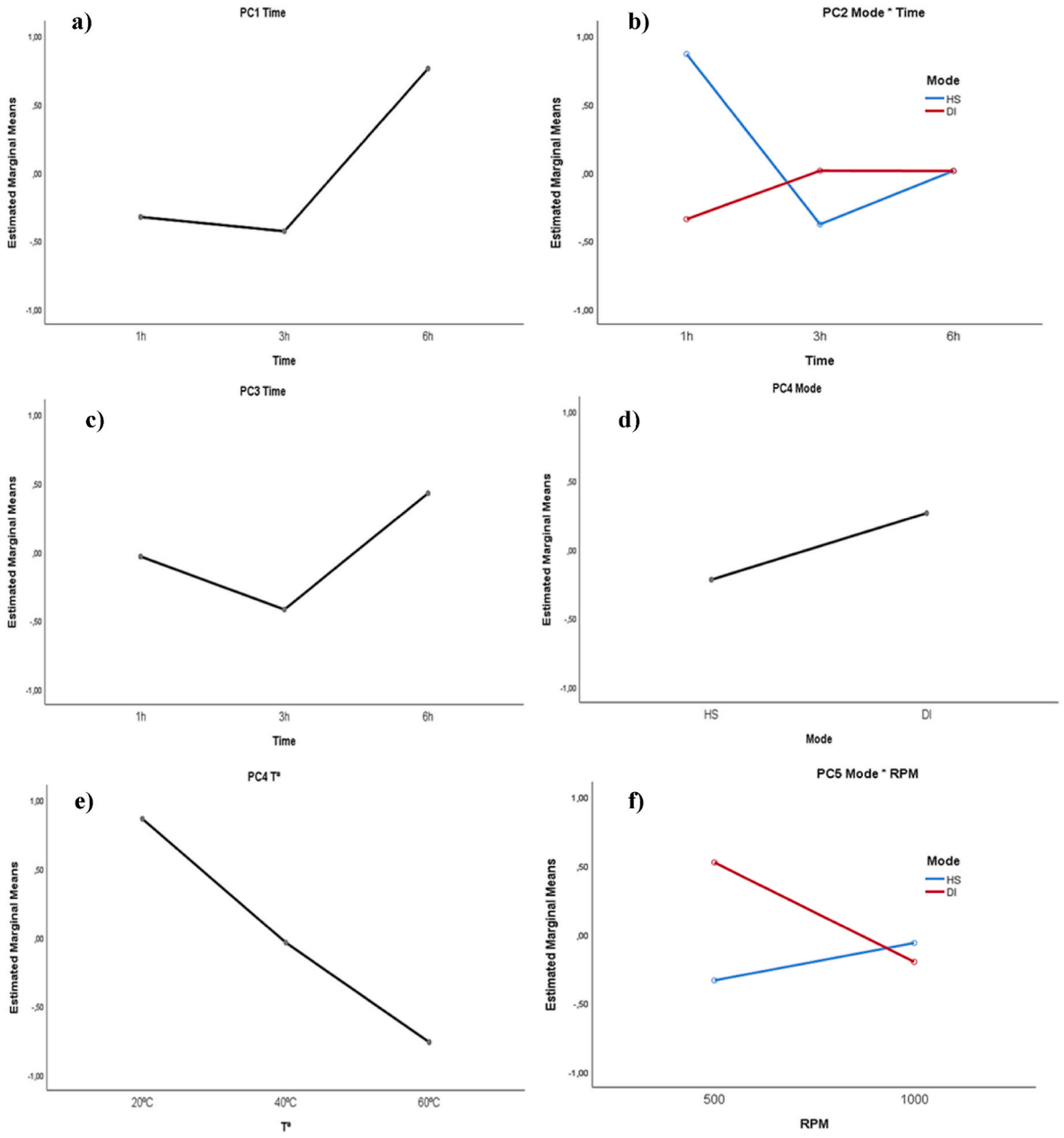

Fig. 3. Plots of estimated marginal means for PDMS/CAR absorbent. a) Time factor for PC1, b) Mode*Time interaction for PC2, c) Time factor for PC3, d) Mode factor for PC4, e) Temperature factor for PC4, and f) Mode*RPM interaction for PC5.

3.2.1. PDMS/CAR

A total of 6 PCs were obtained, explaining an overall variance of 74.43%. PC1 is formed by the three terpenoids (α-terpineol, linalool, and geraniol), the two C_{13} norisoporenoids (β-damascenone, and β-ionone), two esters (ethyl octanoate, and ethyl decanoate), and decanal (Table 2). PC2 is formed by heptanal, the benzenoid compound benzaldehyde, the fatty acid hexanoic acid, and the higher alcohol 2-ethyl-1-hexanol. PC3 is composed of four C6 compounds ((Z)-3-hexen-1-ol, (E)-2-hexen-1-ol, 1-hexanol, and hexanal). PC4 consists of the esters isoamyl acetate and ethyl hexanoate. PC5 is formed by methyl jasmonate, and the C6 compound 2-hexenal. Finally, PC6 is formed by furanmethanol, the fatty acid octanoic acid, and the benzenoid compound 2-phenylethanol (Table 2). For this PDMS/CAR absorbent, the resulting groups were quite ordered according to their chemical family.

Fig. 1 shows the plots of the sample scores on the PCA for the PDMS/CAR absorbent. Fig. 1a shows the PCA for PC1 and PC2. PC1 explains a variance of 20.76%, and PC2 of 15.90%. Fig. 1b shows the PCA for PC3 and PC4. PC3 explains a variance of 13.51%, and PC4 explains a variance of 9.77%. Finally, Fig. 1c shows the PCA of PC5 and PC6. PC5 explains a variance of 7.63%, and PC6 explains a variance of 6.86%. In Fig. 1a and b, there was no sample that deviated excessively from the rest. However, in Fig. 1c, samples 29a, 5a, and 11a, are separated at PC5. Despite this, no conclusion can be made because each sample was run in duplicate, and their pairs are next to the rest of the samples, so it does not indicate that these conditions are the best for the extraction of the compounds from PC5. For the choice of the best conditions, it was necessary to use ANOVA.

3.2.2. PDMS/DVB

A total of 7 PCs have been formed, which explain a total variance of 77.90%. The compounds with the highest weight in PC1 was formed by two esters (isoamyl acetate, and ethyl hexanoate), three C6 compounds (hexanal, 2-hexenal, and 1-hexanol), and one higher alcohol (amyl alcohol) (Table 2). PC2 was formed by two esters (ethyl octanoate, and

Table 4
Best conditions within each principal component chosen from inter-subject tests and plots of estimated marginal means, and global conditions for each absorbent (PDMS/CAR and PDMS/DVB).

	PDMS/CAR			PDMS/DVB			
PC	Compounds	Best conditions	Opt. conditions	PC	Compounds	Best conditions	Opt. conditions
1	α-Terpineol β-Damascenone β-Ionone Linalool Ethyl octanoate Ethyl decanoate Geraniol Decanal	**6 h**	**DI_500_6_20**	**1**	Isoamyl acetate Hexanal Ethyl hexanoate Amyl alcohol 2-Hexenal 1-Hexanol	HS-1 h > HS-3 h > **HS-6 h** HS-20 °C > **HS-40 °C** > HS-60 °C 20 °C-1 h > 20 °C-3 h > 40 °C-1 h	**HS_500_6_40**
2	Heptanal Benzaldehyde Hexanoic acid 2-Ethyl-1-hexanol	HS-1 h > > DI-3 h ≈ **DI-6 h** > > HS-6 h		**2**	Ethyl octanoate Ethyl decanoate β-Ionone β-Damascenone	**6 h** > 3 h > 1 h 60 °C > **40 °C** = 20 °C	
3	(Z)-3-Hexen-1-ol (E)-2-Hexen-1-ol 1-Hexanol Hexanal	**6 h**		**3**	2-Phenylethanol Octanoic acid α-Terpineol Geraniol	**DI > HS**	
4	Isoamyl acetate Ethyl hexanoate	**DI** **20 °C**		**4**	2-Hexen 1-ol (Z)-3-Hexen-1-ol Hexanoic acid	20 °C ≈ **40 °C**	
5	Methyl jasmonate 2-Hexenal	**DI-500**		**5**	Acetol Furanmethanol	No significative	
6	Furanmethanol Octanoic acid 2-Phenylethanol	No significative		**6**	Heptanal Isoamyl alcohol	No significative	
-				**7**	2-Ethyl-1-hexanol	No significative	

HS: headspace. DI: direct immersion. Stirring speed: 500 or 1000 rpm. Extraction time: 1, 3, 6 h. Extraction temperature [a]: 20, 40, 60 °C.

ethyl decanoate), and two C_{13} norisoprenoids (β-ionone, and β-damascenone). PC3 was composed of a benzenoid compound (2-phenylethanol), a fatty acid (octanoic acid), and two terpenoids (α-terpineol, and geraniol). PC4 was formed from two C6 compounds ((E)-2-hexen-1-ol, and (Z)-3-hexen-1-ol), and a fatty acid (hexanoic acid). PC5 was composed from two compounds from the group defined as "others" (acetol, and furanmethanol). PC6 was formed by another compound of the group "others" (heptanal), and a higher alcohol (isoamyl alcohol). Finally, PC7 was formed by the higher alcohol 2-ethyl-1-hexanol.

In the case of the PDMS/DVB sorbent, each of the PCs consisted of several families of compounds, whereas in the PDMS/CAR sorbent, most of the groups consisted mainly of one or two families (Table 2).

Fig. 2 shows the PCA corresponding to the PDMS/DVB absorbent. Fig. 2a shows the PCA of PC1 and PC2. PC1 explained a variance of 22.30%, and PC2 explained a variance of 16.72%. Fig. 2b shows the PCA of PC3 and PC4. PC3 explained a variance of 14.56%, and PC4 explained a variance of 10.24%. Fig. 2c shows the PCA of PC5 and PC6. PC5 explained a variance of 7.26%, and PC6 explained a variance of 6.82%. Samples 2a and 2b correspond to two replicates of the same conditions, but the separation between samples 2a and 2b from the rest of the samples is not significant enough to conclude that these conditions are the best for the extraction of most volatile compounds (Fig. 2a). In Fig. 2b, the samples that separate to a greater extent from the rest are 12a and 36b, but these conditions cannot be chosen, since they do not correspond to two replicates. Finally, in Fig. 2c, sample 34b is the one that maximises the extraction of compounds belonging to PC5, but its replicate is placed next to all the others. Therefore, in order to choose the pair of conditions that maximises the extraction of most of the volatile compounds, ANOVA has to be used.

3.3. Selection of optimal conditions for must volatile compounds extraction

ANOVA was used to choose which conditions optimized the extraction of volatile compounds. To do this, it was checked that all the parametric assumptions necessary for the use of this technique were met. Table 3 shows the results of the test of inter-subject effects, showing the F value as well as the p value for each PC. The factors or interactions that were significant within each PC and each sorbent were studied.

3.3.1. PDMS/CAR

Table 3 shows that for PC1 and PC3, only the Time factor was significant. In PC2, only the Mode*Time interaction was significant. In PC4, the Mode and T^a factors were significant. Finally, in PC5 only the Mode*RPM interaction was significant. In the case of PC6 neither factor or interaction was significant, so it is not shown in Table 3.

Once the factors and interactions that had a significant effect were known, the plots of marginal means of these factors or interactions were studied (Fig. 3). Fig. 3 shows the plots of marginal means of the individual factors and interactions that had a significant effect, resulting from the ANOVA performed with the results obtained by TF-SPME-PDMS/CAR. Fig. 3a shows the plot for the Time factor in PC1. It can be observed that the best time conditions for the extraction of the compounds belonging to PC1 were 6 h (Table 4). Fig. 3b shows the Mode*Time interaction for PC2. In this case the best conditions for the extraction of compounds belonging to PC2 were headspace (HS) for 1 h (Table 4). In Fig. 3c it can be observed that the best conditions for the Time factor for compounds belonging to PC3 are 6 h (Table 4). Fig. 3d and e shows the graphs for the Mode and T^a factors, respectively, in PC4. The mode that obtained the best results for this group of volatile compounds was direct immersion (DI) (Table 4) and the temperature 20 °C (Table 4). Finally, Fig. 3f shows the Mode*RPM interaction for PC5. It can be seen that the combination of conditions that showed the best extraction of compounds belonging to PC5 was working by direct immersion (DI) at 500 rpm (Table 4).

Table 4 shows the best conditions within each PC, as well as the optimal conditions finally chosen for each group of compounds. The selection of the optimal conditions of each absorbent has been carried out considering the experimental conditions that produce the highest values in each of the principal components, and searching among them for an equilibrium solution in order to find the global maximization. In this case, for PDMS/CAR, the optimum conditions chosen were: direct immersion, 500 rpm, 6 h, 20 °C (DI_500_6_20) (Table 4). These conditions only slightly compromised the extraction of compounds belonging to PC2, as they are not the best conditions for that group. Despite this, these conditions were chosen because DI-6 h was the second best option

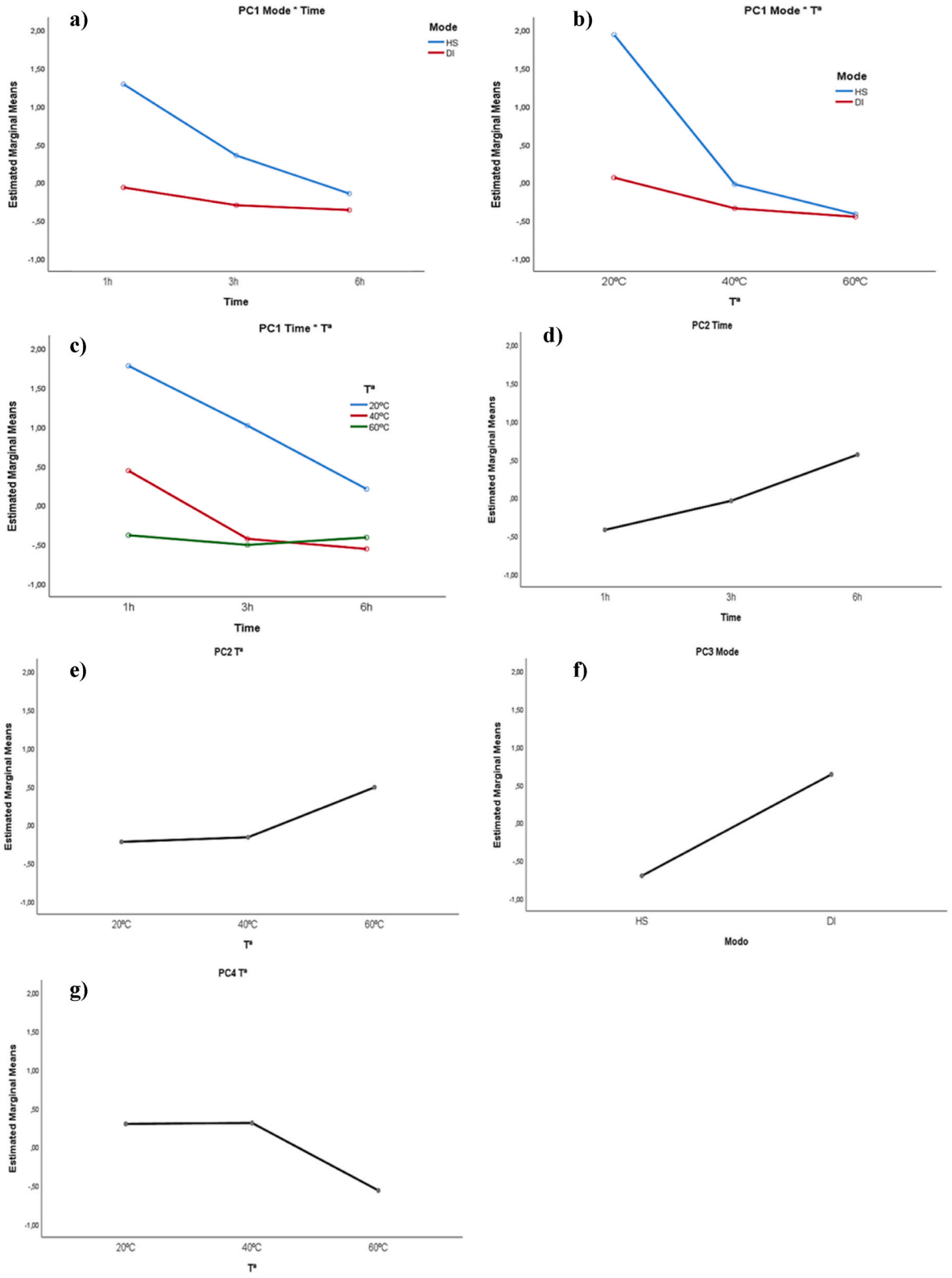

Fig. 4. Plots of estimated marginal means for PDMS/DVB absorbent. a) Mode* Time interaction for PC1, b) Mode*Temperature interaction for PC1, c) Time*-Temperature interaction for PC1, d) Time factor for PC2, e) Temperature factor for PC2, f) Mode factor for PC3, and g) Temperature factor for PC4.

for PC2, so the compromise was not going to be very great.

3.3.2. *PDMS/DVB*

Table 3 shows that for PC1 the Mode, Time, and T^a individual factors and Mode*Time, Mode*T^a, and Time*T^a interactions were significant. If there are significant interactions, the individual factor is not taken into account. For PC2, the factors Time and T^a were significant. For PC3, only the Mode factor was significant. Finally, for PC4, only the T^a factor was significant. In the case of PC5, PC6, and PC7 neither factor or interaction was significant, so they are not shown in Table 3.

Table 5
Values of relative area with respect 2-octanol (I.S.) obtained with the optimal conditions of the two absorbents (PDMS/CAR and PDMS/DVB) for each of the compounds.

	PDMS/CAR	PDMS/DVB	p
Terpenoids			
Linalool	**1.68 ± 0.31**	0.92 ± 0.06	**0.05***
α-Terpineol	0.54 ± 0.47	0.34 ± 0.06	0.60
Geraniol	0.52 ± 0.25	0.11 ± 0.01	0.11
C_{13} norisoprenoids			
β-Damascenone	**4.59 ± 1.10**	0.18 ± 0.03	**0.01***
β-Ionone	**69.61 ± 21.16**	1.33 ± 0.13	**0.02***
Benzenoid compounds			
Benzaldehyde	**1.37 ± 0.46**	0.04 ± 0.01	**0.03***
2-Phenylethanol	**0.77 ± 0.03**	0.11 ± 0.02	**0.00***
Esters			
Isoamyl acetate	0.98 ± 0.16	1.17 ± 0.03	0.21
Ethyl hexanoate	**2.72 ± 0.29**	0.51 ± 0.06	**0.00***
Ethyl octanoate	**8.89 ± 3.30**	0.43 ± 0.03	**0.04***
Ethyl decanoate	4.37 ± 2.97	0.10 ± 0.01	0.15
Fatty acids			
Hexanoic acid	3.06 ± 1.21	0.64 ± 0.52	0.08
Octanoic acid	1.12 ± 0.64	0.18 ± 0.15	0.15
Higher alcohols			
Isoamyl alcohol	0.00 ± 0.00	**0.04 ± 0.02**	**0.01***
Amyl alcohol	0.00 ± 0.00	**0.03 ± 0.00**	**0.00***
2-Ethyl-1-hexanol	**0.18 ± 0.02**	0.01 ± 0.00	**0.00***
C6 compounds			
1-Hexanol	0.87 ± 0.11	0.73 ± 0.06	0.20
(Z)-3-hexen-1-ol	**0.09 ± 0.02**	0.02 ± 0.02	**0.04***
(E)-2-Hexen-1-ol	0.33 ± 0.21	0.01 ± 0.01	0.14
Hexanal	**6.25 ± 1.08**	0.92 ± 0.08	**0.01***
2-Hexenal	**1.01 ± 0.07**	0.13 ± 0.01	**0.00***
Other compounds			
Heptanal	3.32 ± 1.57	0.01 ± 0.00	0.07
Decanal	**0.60 ± 0.16**	0.02 ± 0.01	**0.02***
Furanmethanol	0.96 ± 0.45	0.06 ± 0.08	0.08
Acetol	1.67 ± 0.96	0.02 ± 0.02	0.10
Methyl jasmonate	0.18 ± 0.18	0.01 ± 0.01	0.29

Data are shown as mean ± standard deviation (n = 3 and n = 2). *indicate significant differences between absorbents ($p \leq 0.05$). Bold indicates the highest value of the compound showing significant differences.

As in the previous case, once the significant factors and interactions in each PC are known, the marginal mean plots are used to select the best conditions for these factors or interactions (Fig. 4). Fig. 4 shows the plots of marginal means of the factors and interactions that have a significant effect, resulting from the ANOVA performed with the results obtained by TF-SPME-PDMS/DVB. Fig. 4a, b, and c show the plots of the significant interactions for PC1. The best conditions for the extraction of these compounds were headspace (HS) for 1 h, headspace (HS) at 20 °C, and 1 h at 20 °C (Table 4). In Fig. 4d and e, it can be seen that the best conditions for PC2 were 6 h for the Time factor and 60 °C for the T^a factor (Table 4). Fig. 4f shows that the best extraction mode for PC3 compounds is by direct immersion (DI) (Table 4). Finally, Fig. 4g shows the best conditions for the T^a factor for PC4, in this case 20 °C and 40 °C (Table 4).

Table 4 shows the best conditions within each PC, as well as the optimal conditions finally chosen. In the case of PDMS/DVB, it was very difficult to choose optimal conditions, as they were different for each PC. Nevertheless, the conditions 500 rpm, 6 h, and 40 °C were chosen, as it was an intermediate for all PCs, which did not compromise too much the extraction of any group of compounds. Thus, they were tested by headspace and direct immersion (HS/DI_500_6_40). 500 rpm was chosen because it was not a significant factor and it is less aggressive for the magnets.

In order to choose between HS and DI, 2 replicates of HS_500_6_40 conditions and 2 replicates of DI_500_6_40 conditions were made. It was finally obtained that HS_500_6_40 conditions provided a better extraction than DI_500_6_40, providing a higher amount of extraction in most of the compounds.

3.4. Comparison of PDMS/CAR and PDMS/DVB optimal conditions

Finally, the optimum conditions for each sorbent were compared. To carry out the comparison, 3 replicates were prepared with PDMS/CAR, by DI, at 500 rpm, for 6 h, at 20 °C, and 2 replicates with PDMS/DVB, by HS, at 500 rpm, for 6 h, at 40 °C (those selected in the previous section).

The results of this comparison are shown in Table 5, which lists the means of the relative area results obtained for each much volatile compound, as well as their standard deviation (n = 3 and n = 2). In the work of Kfoury et al. [16], they compare the three types of absorbents available for TF-SPME, including PDMS/CAR and PDMS/DVB, in beverage extractions. In this work, the results obtained show that the PDMS/CAR sorbent provides better results for low polarity compounds and very volatile compounds (VVOCs). However, the PDMS/DVB sorbent shows better results with volatile compounds (VOCs) and semi-volatile compounds (SVOCs). However, in our work, it can be seen that, in most cases where there were significant differences, the value was higher for the PDMS/CAR absorbent. Linalool, β-damascenone, β-ionone, benzaldehyde, 2-phenylethanol, ethyl hexanoate, ethyl octanoate, 2-ethyl-1-hexanol, (Z)-3-hexen-1-ol, hexanal, 2-hexenal, and decanal, were better extracted with the PDMS/CAR sorbent. Nevertheless, isoamyl alcohol, and amyl alcohol were better extracted with the PDMS/CAR sorbent. Therefore, it can be concluded that the sorbent that provides a higher extraction of volatile compounds for the TF-SPME technique is PDMS/CAR, under the conditions: direct immersion (DI), agitation at 500 rpm for 6 h at 20 °C (DI_500_6_20).

4. Conclusions

This is the first time that this extraction technique, thin film-microextraction (TF-SPME), has been used to determine the must grape volatile composition. Firstly, it can be concluded that the both sorbents compared, PDMS/CAR and PDMS/DVB are a good choice for the extraction of volatile compounds in must. After studying the principal component analysis (PCA) and the multifactorial analysis of variance (ANOVA), it was concluded that the optimum conditions for the PDMS/CAR sorbent were: direct immersion (DI), agitation at 500 rpm for 6 h at 20 °C (DI_500_6_20). In the case of the PDMS/DVB sorbent, the results were not so conclusive, so two intermediate conditions were tested, finally choosing the conditions: headspace (HS), agitation at 500 rpm for 6 h at 40 °C (HS_500_6_40). Finally, the comparison between the conditions chosen for each sorbent confirmed that the best sorbent for the TF-SPME method in order to extract volatile compounds in must was PDMS/CAR. Therefore, the best absorbent in order to analyze the grape volatile compounds was PDMS/CAR and the optimal conditions were carried out the extraction at 20 °C, during 6 h, by direct immersion (DI), with agitation at 500 rpm.

CRediT authorship contribution statement

Sandra Marín-San Román: Investigation, Methodology, Formal analysis, Writing – original draft. **José Miguel Carot:** Formal analysis, Writing – review & editing. **Itziar Sáenz de Urturi:** Investigation, Writing – review & editing. **Pilar Rubio-Bretón:** Investigation, Writing – review & editing. **Eva P. Pérez-Álvarez:** Investigation, Writing – review & editing. **Teresa Garde-Cerdán:** Supervision, Funding acquisition, Investigation, Methodology, Writing – review & editing.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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