



Monitoring the evolution of volatile compounds using gas chromatography during the stages of production of Moscatel sparkling wine



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ABSTRACT

This study reports, for the first time, the main changes that occur with some important aroma compounds of Moscatel sparkling wines during winemaking, measured using headspace solid-phase microextraction, one-dimensional and comprehensive two-dimensional gas chromatography (GC × GC) with mass spectrometry detection (MS). The best conditions of volatile extraction included the use of PDMS/DVB fibre, 2 mL of wine, 30% of NaCl, 40 °C for 30 min. The chromatographic profile of sparkling wines showed decreasing amounts of monoterpenes (limonene, 4-terpineol, terpinolene, citronellol, α-terpineol, linalool, hotrienol, and nerol oxide), increasing amounts of esters (terpenyl esters, ethyl octanoate, ethyl decanoate and hexyl acetate) and alcohols (1-nonanol and 2-phenylethanol). Sixty-nine compounds co-eluted in the first dimension; only six co-eluted in the second dimension. GC × GC/TOFMS allows more detailed study of the volatile profile of sparkling wines.

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1. Introduction

The volatile fraction of wines influences their quality since these compounds are associated to their organoleptic characteristics (Clarke, 2003). The amount of a particular compound or even its absence depends on several factors related to the grape (variety and cultivation conditions), winemaking conditions (condition of grapes, fermentation, yeast strain) and post fermentation treatments (aging and storage) (Ruiz-Bejarano, Castro-Mejías, Rodríguez-Dodero, & García-Barroso, 2013; Zhang, Pan, Yan, & Duan, 2011).

Aroma is a key attribute for consumers and the fruity and floral character of Moscato grapes is responsible for its unique and pleasant flavour. Moscatel sparkling wine is produced from a single alcoholic fermentation of the must of grapes of Moscatel variety. Its production process is similar to the one employed for Asti, the Italian sparkling wine (Clarke, 2003). Originally (1978–1986) Moscatel sparkling wine was termed as “Brazilian Asti” and from

1992 to 2000 as “Asti process Moscatel sparkling”, however because of the denomination of origin of the wines of the Asti region (*Asti Spumante*) and also due to the great commercial potential of the local product, the Brazilian industry decided to leave behind the original name and change the designation of the wines to Moscatel Sparkling (Lona, 2009). According to Brazilian Law, Moscatel sparkling wine results from fermentation of the must of grapes of Moscatel variety in a closed recipient and contains 7–10% (v/v) of alcohol below 20 °C and at least 20 g of residual sugar and should provide 4 atm pressure to the final product below 20 °C (Brasil, 2004).

As previously observed in the literature, the characteristic varietal aroma of Moscatel wine may be attributed to terpenic compounds, including linalool, nerol, geraniol, α-terpineol, hotrienol and rose oxide, which originate from Moscatel grapes. Furthermore, esters, acids, C13-norisoprenoids and alcohols have also been identified as contributing to aroma of these wines (Bordiga, Rinaldi, Locatelli, Piana, & Travaglia, 2013; Ruiz-Bejarano et al., 2013). It is expected that these compounds would persist through the entire process of vinification. The extent to which these compounds remain in the wine is influenced by, for

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example, the practices of winemaking (Robinson et al., 2014). Chemical/biochemical transformations may occur during wine-making and aging processes, resulting in loss of the floral/fruity varietal aroma, which is an important characteristic of Moscatel sparkling wine. Besides that, off flavours may also arise during fermentation. There is one research work about aging of Moscatel sparkling wine, but it does not encompass transformations that may happen during winemaking (Bordiga et al., 2013). Despite the importance of Moscatel sparkling winemaking, nothing is reported about this subject in the scientific literature.

Headspace solid-phase microextraction (HS-SPME) has been extensively used for the study of wine volatiles (Bordiga et al., 2013; Ruiz-Bejarano et al., 2013; Zhang et al., 2011), due to its simplicity, sensitivity, ease of automation and also because it is solvent free and offers the possibility of extraction and concentration integrated in one step. When utilising SPME, method development needs to be conducted efficiently, mainly considering the type of fibre coating, sample volume and other extraction conditions, in order to improve the extraction efficiency (Welke, Zanús, Lazarotto, Schmitt, & Zini, 2012). To the knowledge of the authors there is no SPME method developed to maximise the extraction of terpenes from the headspace of Moscatel sparkling wines, even though a few trials have been made to investigate appropriate conditions to extract volatiles from Cava sparkling wines (Riu-Aumatell, Bosch-Fusté, López-Tamames, & Buxaderas, 2006).

The determination of volatile compounds is commonly performed by one-dimensional gas chromatography (1D-GC). A closer look at these 1D-GC results of wine analysis shows that there are some unresolved peaks, due to the high complexity of the samples. Two or more co-eluting compounds may prevent the achievement of a correct identification of these volatile compounds and this is especially important when other co-eluting compounds hide traces of aroma active compounds (Beens & Brinkman, 2005). Comprehensive two-dimensional gas chromatography (GC \times GC) has emerged as a powerful analytical technique (Welke & Zini, 2011).

Former works of this research group have focused on the use of HS-SPME-GC \times GC/TOFMS for the elucidation of the volatile profile of Brazilian traditional sparkling wines (Nicolli, Welke, Closs, Manfroí, & Zini, 2015; Welke, Zanús, Lazarotto, Pulgati, & Zini, 2014). In those studies, the main volatile components that contributed to the differences among base wines and their respective sparkling wines were: C13-norisoprenoids (TDN, vitispirane and β -damascenone), ethyl esters (laurate, 2-hydroxybutanoate, decanoate, 2-hydroxypropanoate, pentanoate), alcohols (4-butoxy-1-butanol, 1-propanol, methionol), aldehydes (3-phenyl-2-propenal, nonanal, undecanal), acids (acetic, 2-ethylhexanoic, butanoic), ketones (acetoin, diacetyl), and phenols (4-vinylguaiaicol, 4-ethylphenol) (Welke, Zanús, Lazarotto, Pulgati et al., 2014). In another study, a preliminary characterisation of Brazilian Moscatel sparkling wines identified as major compounds: 3-methyl-1-butanol, hexanoic acid, ethyl hexanoate, linalool, hotrienol, 2-phenylethyl alcohol, nerol oxide, diethyl succinate, α -terpineol, ethyl octanoate, octanoic acid, decanoic acid and ethyl decanoate (Nicolli et al., 2015). GC \times GC was useful to identify some compounds that co-eluted in the first dimension and contribute to aroma such as diethyl succinate (floral), α -terpineol (rose), ethyl octanoate (fruity) and octanoic acid (cheesy) (Nicolli et al., 2015; Welke, Zanús, Lazarotto, Pulgati et al., 2014).

The objective of this study is, for the first time, to investigate the main changes that occur with some important aroma compounds of Moscatel sparkling wines during vinification, using Brazilian wines and an optimised HS-SPME method. The potential of GC \times GC was also investigated, especially with regards to separation of important aroma contributors in the second chromatographic dimension.

2. Material and methods

2.1. Wine making process and samples

Three winemaking processes were carried out by Perini winery, located in Garibaldi city, in Rio Grande do Sul state, Brazil, using three different grape musts for each one of them, during the years 2010/2011 (Oct/Nov 2010, May/Jun 2011; Sep/Oct 2011). Rio Grande do Sul is well known for its wineries and is responsible for more than 90% of Brazilian wine production. Perini produces 750 L of Moscatel sparkling wine per year (per harvest) using one ton of grapes during 2010/2011. During these years, their total production of wines was approximately 300,000 L and it has subsequently grown to 500,000 L.

The harvest of the grapes (Moscato Bianco and Moscato R2, clone of Moscato variety) was performed during the day and the grape berries were separated from the rachides to improve the quality of the final wine, avoiding bitterness. Part of the grape harvest was cultivated by Perini winery and another part was purchased from third parties.

Grape berries passed through a pneumatic press and the resulting must was filtered with diatomaceous earth and was cooled to avoid fermentation. Also, in the beginning of the fermentation, bentonite and potassium caseinate were added to avoid oxidation and promote stabilisation. Fermentation process may take from one to more than 30 days to start and the yeast employed was *Saccharomyces cerevisiae bayanus*. During the first filtration potassium bisulfite was added to produce SO₂. Beta-glucanase and a beta-glycolytic enzyme were also added to the must to lower its viscosity. In the first 15 days, temperature varied from 10 to 20 °C. When the pressure reached 5 atm and the alcoholic content was around 7–9% (v/v), the tank was cooled to –3 °C to stop fermentation and provide tartaric stabilisation. At this moment, the tank was closed to take advantage of carbon dioxide. Sugar content was corrected at this point through the addition of sucrose, as it should be around 80–90 g/L. After two days in the tank at –3 °C, the second filtration took place and diatomaceous earth was employed again for this purpose. After two more days in the tank, under the same temperature conditions, the third filtration took place in cellulose plates (cellulose pores vary from 1 to 2 μ m). A fourth filtration followed, also using cellulose plates with pore size of 1 μ m and 0.45 μ m or diatomaceous earth. Automatic bottling was performed below 0 °C and samples were stored at the same temperature until extraction and analyses were performed (Lona, 2009; Rizzon, Meneguzzo, & Gasparin, 2005).

The samples were collected at six different stages of Moscatel sparkling winemaking process and were analysed using 1D-GC/MS: (1) grape must, (2) first filtration (filtered must after yeast addition) (3) second filtration (filtered must after 6 days of fermentation), (4) third filtration (filtered must after 12 days of fermentation), (5) fourth filtration (sparkling wine obtained after 20 days of fermentation) and (6) bottled sparkling wine.

2.2. Analytical reagents, and supplies

The standard compounds ethyl acetate, ethyl butanoate, ethyl isobutanoate, ethyl isovalerate, ethyl hexanoate, ethyl lactate, ethyl octanoate, ethyl decanoate, ethyl dodecanoate, diethyl succinate, ethyl phenylacetate, isoamyl acetate, ethyl 2-phenylacetate, hexyl acetate, ethyl sorbate, 1-propanol, 1-hexanol, 1-dodecanol, 2-phenylethyl alcohol, hexanoic acid, octanoic acid, nonanoic acid, decanoic acid, α -terpineol, limonene, eucalyptol, terpinolene, linalool, 1-menthol, nerol, and β -damascenone were purchased from Aldrich (Steinheim, Germany). Individual stock solutions of each compound (10 mg L^{–1}) were prepared in double-distilled ethanol purchased from Nuclear (São Paulo, Brazil).

Model wine was prepared with (+)-tartaric acid (6 g L^{-1}) supplied by Synth (São Paulo, Brazil) and 10% of ethanol double-distilled (95%, Vetec, Rio de Janeiro, Brazil) in MilliQ deionised water. The pH was adjusted to 3.5 with sodium hydroxide (Nuclear, São Paulo, SP, Brazil). Ultra-pure water was prepared using a Milli-Q water purification system (Millipore, Bedford, MA). In order to obtain a sample as close to the real wine matrix as possible, the stock standard solutions were diluted in model wine to perform the extraction of each standard compound by SPME to proceed with their identification in the real samples. Sodium chloride of analytical grade, purchased from Nuclear was oven dried at 110°C overnight before use. Twenty-millilitre headspace vials with magnetic screw caps sealed with silicone septa were bought from Supelco (Bellefonte, PA).

2.3. Optimisation of SPME

For the first time the extraction conditions of volatile compounds using HS-SPME were optimised for Moscatel sparkling wines. In order to find the most appropriate conditions of extraction, the parameters tested were fibre coating, sample volume, temperature, extraction time and percentage of sodium chloride.

The extraction efficiency of six SPME fibre coatings was tested in order to find which coating would have the highest affinity toward volatiles and semivolatile wine constituents. The SPME fibres were purchased from Supelco (Bellefonte, PA): $7 \mu\text{m}$ polydimethylsiloxane (PDMS), $100 \mu\text{m}$ PDMS, $85 \mu\text{m}$ PA, $85 \mu\text{m}$ Carboxen-polydimethylsiloxane (CAR/PDMS), $65 \mu\text{m}$ polydimethylsiloxane-divinylbenzene (PDMS/DVB) and 50/30 divinylbenzene/Carboxen/polydimethylsiloxane (DVB/CAR/PDMS). All fibres were 1 cm long and were conditioned according to the manufacturer's recommendation prior to their first use. The extraction efficiency of SPME fibre coatings was tested using the following conditions: 2 mL of sample, 30% of NaCl, temperature and time of extraction of 40°C and 10 min, respectively. Three different values were tested for wine sample volumes (1, 2 and 3 mL), temperature of extraction (30 , 40 and 50°C) and extraction times (10, 30 and 50 min). Samples were extracted without NaCl and with 15% and 30% of salt. All experiments were run four times. Some components were chosen to monitor the extraction efficiency due to their well-known importance to the aroma of sparkling wines (Bordiga et al., 2013; Ruiz-Bejarano et al., 2013). They were terpinolene, linalool, hotrienol, α -terpineol, citronellol, geraniol, hexanol, 2-phenylethanol and hexyl acetate.

2.4. Chromatographic analyses

A Shimadzu gas chromatograph coupled to a quadrupole mass spectrometric detector (GC/MS), model QP2010 (Kyoto, Japan) was employed to perform headspace analyses of volatile compounds with the following columns: a 5% diphenyl-95% dimethyl polysiloxane (DB-5, $60 \text{ m} \times 0.25 \text{ mm} \times 0.25 \mu\text{m}$) and a polyethylene glycol (DB-Wax, $30 \text{ m} \times 0.25 \text{ mm} \times 0.25 \mu\text{m}$). The oven was kept at 45°C for 5 min and it was heated up to 180°C at a rate of 3°C min^{-1} , reaching a final temperature of 240°C at $20^\circ\text{C min}^{-1}$. Injector and detector temperature were kept at 250°C , while helium (analytical purity 99.999%, Linde Gases, Canoas, RS, Brazil) was employed as carrier gas. Analyses were performed in splitless mode and flow rate was 1.0 mL min^{-1} . The MS parameters included electron ionisation at 70 eV and a mass range (m/z) of 45–450.

The GC \times GC system consisted of an Agilent 6890N (Agilent Technologies, Palo Alto, CA) equipped with a Pegasus IV time-of-flight mass spectrometric detector (Leco Corporation, St. Joseph, MI). The GC system was equipped with a secondary column oven and a non-moving quadjet dual stage thermal modulator. During

modulation, cold pulses were generated using dry nitrogen gas cooled by liquid nitrogen, whereas heated nitrogen gas was used for hot pulses. The system was also equipped with a CTC CombiPAL autosampler (CTC Analytics, Zwingen, Switzerland) with an agitator and SPME fibre conditioning station. The injector, transfer line and ion source temperature were at 250°C . Oven temperature was kept at 45°C for 0.5 min and was raised to 240°C at 3°C min^{-1} . The secondary oven was kept 10°C above the primary oven throughout the chromatographic run. The first column was a DB-5 ($60 \text{ m} \times 0.25 \text{ mm} \times 0.25 \mu\text{m}$) and the second column was a 50% phenyl 50% methyl-polysiloxane (DB-17 ms, $1.70 \text{ m} \times 0.18 \text{ mm} \times 0.18 \mu\text{m}$). MS parameters and carrier gas employed, as well as flow rate were the same as reported for 1D-GC/MS. The period of modulation was 7 s, detector voltage -1750 V , and acquisition rate $100 \text{ spectra s}^{-1}$.

Chromatographic analyses were always run in triplicate.

2.5. Compounds identification and data processing

Thirty-one compounds (listed in Section 2.2) were positively identified through comparison of retention time and mass spectral data of unknown compounds with those of authentic standards. Tentative identification of wine aroma compounds in 1D-GC as well as with GC \times GC analyses was achieved comparing experimental linear temperature programmed retention index (LTPRI) with retention indices reported in the scientific literature (Adams, 2007; NIST library, version 2005). Retention data of a series of *n*-alkanes (C9–C24), obtained under the same experimental conditions employed for the chromatographic analysis of wine volatiles were used for experimental LTPRI calculation. Whenever a compound was tentatively identified, differences between experimental and reported LTPRI were not higher than 15. Mass spectrometric information of each chromatographic peak was compared to NIST mass spectral library, considering a minimum similarity value of 75%. Mass spectral similarity (usually called “similarity”, “S”) is a mass spectral match factor reported in the range of 0–99, with a higher value corresponding to a better fit. “Similarity” describes how well the library hit matches the peak when using all masses. It does not carry a unit (Dallüge et al., 2002). Whenever an LTPRI had not been found in the scientific literature to match with the experimentally determined LTPRI, only the chemical class of the wine volatile compound was assigned, based only on mass spectral information.

Volatile compound behaviour during the optimisation of extraction process and during winemaking process was monitored through normalised chromatographic area. A normalised area of a volatile compound was calculated dividing its chromatographic area after a certain period of time in the process by the maximum chromatographic area achieved for this same compound during the whole process. Area percentage of each volatile compound of a Moscatel sparkling wine was calculated considering the sum of chromatographic areas of all detected peaks as 100%. A semiquantitative approach was employed for determination of area percentage, as response factors of the different compounds were considered equal for the sake of simplicity.

3. Results and discussion

3.1. Optimisation of SPME

3.1.1. Fibre coating screening

Fig. 1 shows the comparison of the extraction efficiency of volatile compounds of Moscatel sparkling wine by HS-SPME with different fibre coatings. For each fibre coating, extraction performance was measured by total chromatographic area and number

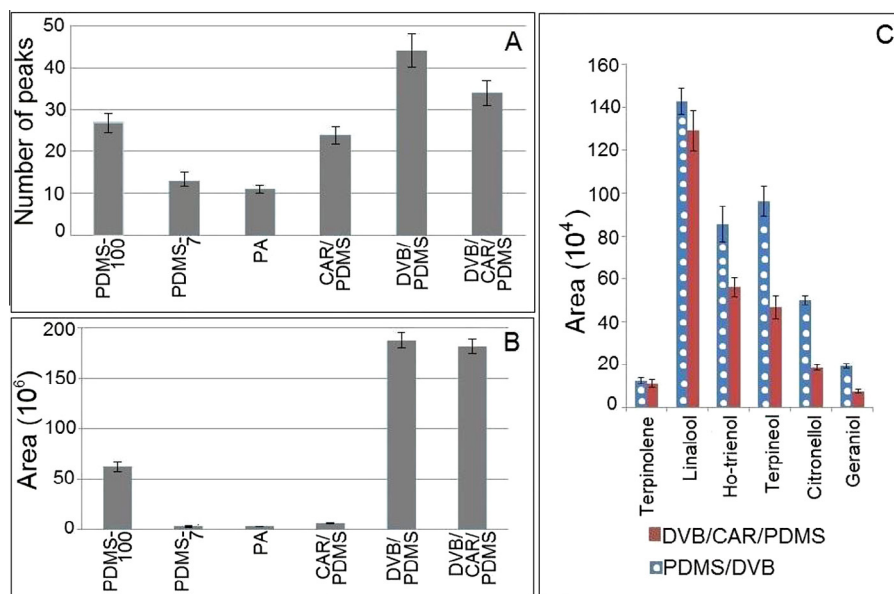


Fig. 1. Comparison of the extraction efficiency of volatile compounds of Moscatel sparkling wine using HS-SPME with different fibre coatings: [100 μ m polydimethylsiloxane (PDMS), 7 μ m PDMS, 85 μ m polyacrylate (PA), 85 μ m Carboxen-polydimethylsiloxane (CAR/PDMS), 65 μ m polydimethylsiloxane-divinylbenzene (PDMS/DVB) and 50/30 divinylbenzene/Carboxen/polydimethylsiloxane (DVB/CAR/PDMS)]. All experiments were performed in triplicate. Chromatographic conditions are described in item Section 2.4.

of extracted compounds. The PA fibre has polar characteristics and allowed the extraction of a lower number of compounds (Fig. 1). This fact is explained by the low capacity of PA coating for extracting relatively nonpolar compounds, such as terpenes. These compounds are typical of Asti Spumante and Moscato d'Asti wines, which are similar to Moscatel sparkling wines evaluated in this work, and terpenes represented approximate 30% of tentatively identified compounds in these wines (Bordiga et al., 2013). The PDMS fibre, which represents a non-polar coating, also showed low performance for the extraction of volatiles. The use of 100 μ m PDMS coating allowed extraction of 27 volatile compounds, while using 7 μ m PDMS only 13 compounds were extracted. Such a decrease could be expected since a thinner coating results in a smaller volume available for analyte sorption. Similarly, CAR combined with PDMS presented extraction efficiency as low as the one seen for PA and PDMS, when compared to other bi-polar fibre coatings tested in this work (PDMS/DVB and DVB/CAR/PDMS) considering total chromatographic area and number of extracted compounds. CAR/PDMS is suggested for extraction of very volatile compounds. However, some volatiles extracted using this fibre include nerol oxide, linalool oxide, and geraniol, which are characteristic of Moscatel wine, have 10 or more carbons in their structure and are considered to be of intermediate volatility (Ruiz-Bejarano et al., 2013).

PDMS/DVB and DVB/CAR/PDMS coatings showed the best performance and PDMS/DVB allowed achieving the highest number of chromatographic peaks and chromatographic area (Fig. 1A and B). Bonino et al. (2003) evaluated the influence of PDMS/DVB, CAR/PDMS and PDMS coatings for the extraction of volatiles of Italian Ruché wines and have found PDMS/DVB as the best option to determine volatile compounds. The authors tentatively identified 59 volatile compounds, including terpenes, ketones, alcohols, C13-norisoprenoids, esters and acids (Bonino et al., 2003). The combination of DVB with other coatings increases both the porosity and the polarity of the fibre, improving the retention of the analytes on the fibre as compared to a coating that consists only of PDMS. A more significant fact is the better efficiency of PDMS/DVB in comparison to DVB/CAR/PDMS for the extraction of some terpenic compounds, including terpinolene,

linalool, hotrienol, terpineol, citronellol and geraniol, as shown in Fig. 1C. One possible reason for the better performance of the DVB-containing fibres may be the occurrence of π - π interactions between unsaturations of terpenic compounds and benzene rings of the DVB polymer (Carrillo, Garrido-Lopez, & Tena, 2006). Due to results obtained, PDMS/DVB fibre was utilised for all experiments that followed these preliminary trials.

3.1.2. Influence of extraction volume

After fibre selection (DVB/PDMS), the influence of sample volume in volatile extraction efficiency was verified and the conditions used for volatiles extraction were: 30% of NaCl, temperature and time of extraction of 40 $^{\circ}$ C and 10 min, respectively. The influence of the sample volume on the extraction of volatile compounds is shown in Fig. 2A. The areas of 2-phenylethanol, linalool, hexyl acetate and α -terpineol increased when 2 mL of sample were used during extraction compared to the use of 1 mL. The extraction done with 3 mL of wine resulted in an expressive decreasing of chromatographic area of two compounds (2-phenylethanol and linalool). For this reason, the volume used in the following experiments was 2 mL. Mestres, Busto, and Guash (1998) found that extraction was favoured when the sample volume was increased (lower headspace/wine volume ratio); however, the repeatability was lower. On the other side, in case of extraction performance of volatiles of Chardonnay base wine, sample volume has not been considered as a significant parameter (Welke et al., 2012).

3.1.3. Influence of extraction temperature

The third parameter evaluated in order to find the best conditions of volatiles extraction was temperature. The best fibre (DVB/PDMS) and the most adequate volume of sample (2 mL) already defined were used in this step along with 30% of NaCl and 10 min extraction. Fig. 2B shows that 40 $^{\circ}$ C as extraction temperature achieved higher chromatographic areas for most compounds, with the exception of α -terpineol. This terpene presented a higher chromatographic area at 50 $^{\circ}$ C. A possible explanation for this fact is that the degradation of linalool may occur with higher extraction temperatures and may result in the formation of α -terpineol (Haleva-Toledo, Naim, Zehavi, & Rouseff, 1999). The

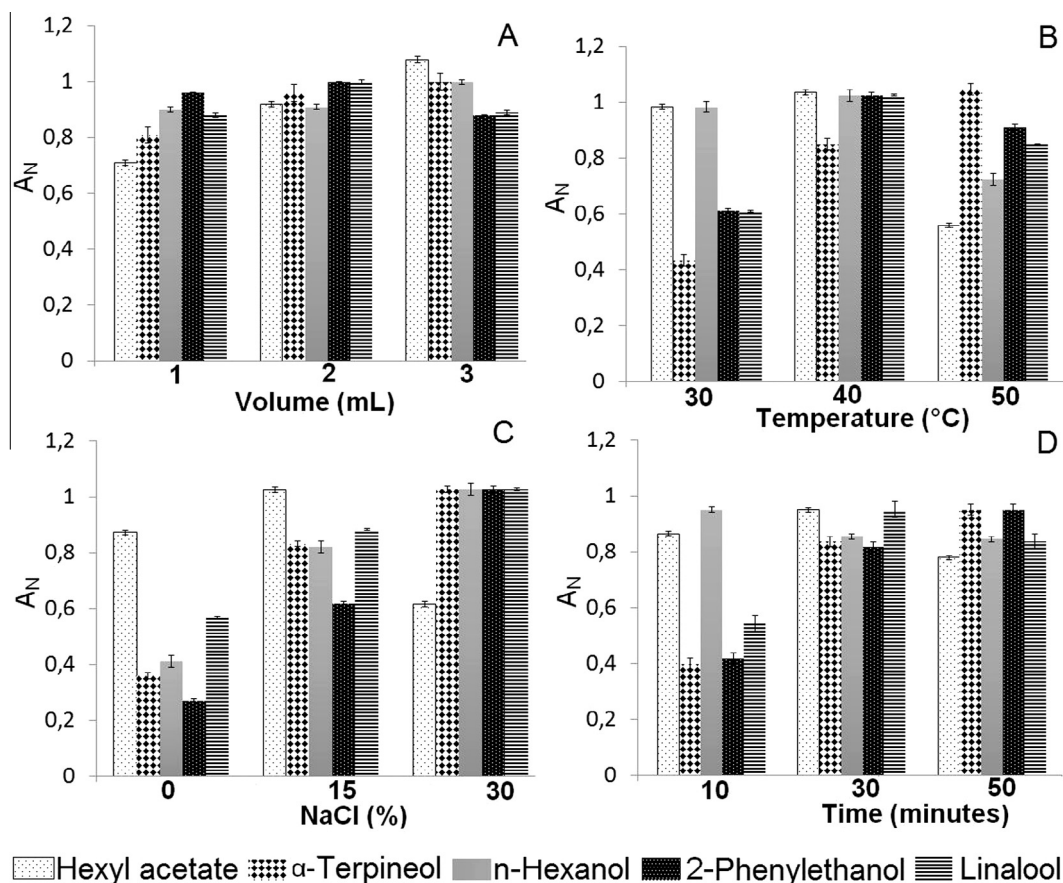


Fig. 2. Extraction efficiency for some representative compounds present in the volatile fraction of Moscatel sparkling wine according to (A) volume of sample, (B) extraction temperature, (C) ionic strength and (D) extraction time. All extraction was performed using 65 μ m polydimethylsiloxane-divinylbenzene (PDMS/DVB) fibre. Chromatographic conditions were described in Section 2.4. All experiments were performed in triplicate.

temperature selected for the extraction of volatiles was 40 °C in order to prevent degradation reactions. Similar results were found by Setkova, Risticvic, and Pawliszyn (2007), who tested different temperatures of extraction from 30 to 80 °C to extract volatile compounds from Canadian ice wines. The optimal temperature was different for the various volatile compounds found in wines, but the authors observed that at temperatures higher than 45 °C the chromatographic area of some compounds decreased. Two opposite phenomena contribute to these results: (i) high temperatures are supposed to release more analytes into the headspace, allowing better extraction with increasing temperature due to the enhanced mass transfer (Mestres et al., 1998) and (ii) high temperature can adversely affect the sorption of analytes in/on the coating due to thermodynamic reasons (decrease of partition coefficients) and consequently the extraction efficiency of the coating decreases as the temperature rises (Setkova et al., 2007).

3.1.4. Influence of extraction time

The best SPME coating fibre (DVB/PDMS), the most adequate sample volume (2 mL) and extraction temperature (40 °C) were used to test the influence of time in volatiles extraction. The salt concentration used in this step was 30%. The chromatographic area of hexyl acetate, 2-phenylethanol, linalool and α -terpineol increased when the extraction occurred for 30 min in comparison to 10 min. Furthermore, the performance of extraction decreased for some analytes when extraction time was higher (50 min, Fig. 2D). This behaviour may be related to competition of other components of the sample for the active sites of the fibre, which may also cause displacement of volatiles during the adsorption

step (Rodrigues, Caldeira, & Câmara, 2008). Thirty minutes was chosen as the most suitable extraction time.

3.1.5. Influence of ionic strength

The last parameter evaluated in the optimisation of conditions of volatiles extraction was the salt concentration. All other previously optimised parameters were used (DVB/PDMS fibre coating, 2 mL of sample, 40 °C and 30 min, respectively), were used to test the influence of ionic strength. Normalised peak areas increased with increasing salt concentration for the majority of volatile compounds tested (Fig. 2C). For this reason, a concentration of 30% of NaCl concentration was employed to improve extraction conditions. Other published research works have also considered this amount of salt as appropriate for SPME for different wines, including Chardonnay (Welke et al., 2012), Verdelho, Arnusburger, Boal and Malvazia (Rodrigues et al., 2008), and Cabernet Sauvignon (Robinson, Boss, Heymann, Solomon, & Trengove, 2011a, 2011b).

The most suitable conditions to obtain high efficiency in HS-SPME of volatile compounds in sparkling Moscatel wines were: PDMS/DVB coating, sample volume of 2 mL, 30% of NaCl, extraction time and temperature of 30 min and 40 °C.

3.2. Evolution of volatile compounds during the elaboration of Moscatel sparkling wines

The optimised HS-SPME method was applied to the extraction of volatile compounds of samples from six stages of the Moscatel sparkling winemaking process. The nineteen volatile compounds monitored during the winemaking were terpenic compounds (limonene, 4-terpineol, terpinolene, citronellol, α -terpineol,

linalool, hotrienol, geraniol, nerol oxide, citronellyl acetate, neryl acetate, geranyl acetate), alcohols (1-nonanol, 1-hexanol, 2-phenylethanol) and esters (hexyl acetate, ethyl hexanoate, ethyl octanoate, ethyl decanoate). These compounds were chosen considering their importance to flavour of Moscatel wines according to scientific reports (Bordiga et al., 2013; Clarke, 2003; Ruiz-Bejarano et al., 2013). Even though the quantitative and sensorial analyses provide important information for a precise definition of the influence of each volatile compound to wine aroma, a general discussion regarding the possible contribution of several important volatile compounds is worth presenting and such an approach has already been adopted in other scientific papers (Bordiga et al., 2013; Rodrigues et al., 2008; Zhang et al., 2011). Comments on aroma contribution were included in this discussion and are detailed in Table 1 for each volatile compound positively or tentatively identified in Moscatel sparkling wines.

The box plots of Fig. 3 shows the range of normalised areas for each volatile compound in each processing step and, consequently, provides information on the behaviour of volatile compounds during winemaking. The median is represented by the blue triangle inside the box; minimum and maximum values are designated by the bar hedges outside the box and the black line indicates the average normalised area of a compound at each stage of wine making. The top and bottom rows of the box indicate that 75% (top) and 25% (bottom) of the samples have lower and higher chromatographic areas, respectively, than the value expressed by these bars. Fig. 3S shows the normalised areas for the same compounds shown in Fig. 3, but in separate curves, each one corresponding to one industrial process.

Monoterpenes such as limonene, 4-terpineol, terpinolene, citronellol, α -terpineol, linalool, hotrienol, nerol, and nerol oxide decreased their chromatographic area during the stages of winemaking, as shown in Fig. 3. Terpenes are found in grape skins and are transferred to wine during maceration, which is the first step of winemaking. These compounds generally remain unchanged after the fermentation process (Clarke, 2003; Zhang et al., 2011). Terpenes and their derivatives tend to possess floral aromas, which are characteristic of Moscatel grapes and, consequently, responsible for varietal aroma of Moscatel wines (Bordiga et al., 2013; Ruiz-Bejarano et al., 2013). Because of this special role of terpenes in Moscatel sparkling wines, decrease in their normalised areas during winemaking deserves further attention, including investigation of the influence of the stages of vinification process, such as the several steps of filtration, including possible evaporation, incorporation in yeast membranes, and acetylation that may also occur. Steyer et al. (2013) have shown strong evidence that enzymatic acetylation is one of the main reasons for terpenols disappearance from musts during fermentation of white wines. Their comments point to the importance of terpenyl acetates (sweet floral aroma for geranyl acetate and fresh citrus for citronellyl acetate) and the lack of investigation on this subject, although odour thresholds are considered to be higher for terpenyl acetates than for terpenols.

1-Nonanol and 2-phenylethanol chromatographic areas increased during the stages of winemaking, while the amount of 1-hexanol decreased. 2-Phenylethanol is formed during fermentation and is influenced by the amount of nitrogen in the must, since it is synthesised from a keto acid resulting from the oxidative deamination of phenylalanine (Mauricio, Moreno, Zea, Ortega, & Medina, 1997).

1-Nonanol (as well as 1-hexanol) may be formed due to the oxidation of fatty acids (Shetty, Paliyath, Pometto, & Levin, 2014). After grape crushing, lipoxigenase catalyses the transformation of lipids to alcohols, so that their concentrations increase during grape maceration (Salinas, Garijo, Pardo, Zalacain, & Alonso, 2003; Shetty et al., 2014). The reduction of 1-hexanol content

during the winemaking may have occurred due to the formation of the corresponding ester, hexyl acetate which is a product of yeast metabolism (Ribéreau, Dubourdieu, Donèche, & Lonvoud, 2006). The behaviour of ethyl hexanoate is different and deserves further investigation. Patrianakou and Roussis (2013) have reported that ethyl hexanoate decreased during vinification due to a hydrolysis reaction that forms hexanoic acid. Hexanol decrease (aroma described as herbaceous and grassy (Peinado, Moreno, Muñoz, Medina, & Moreno, 2004) and consequent increase of hexyl acetate might contribute to the intensification of fruity characteristics of Moscatel sparkling wines, although cheese notes might be observed in sparkling wines if an increase in hexanoic acid concentration occurs.

Esters (ethyl octanoate, ethyl decanoate and hexyl acetate) increased their chromatographic area during the production of Moscatel sparkling wines. Esters are secondary aromas, arising during fermentation and result from the combination of alcohols and acids (Clarke, 2003). These compounds are important components of wine aroma, due to their fruity and floral notes (Welke, Zanús, Lazarotto, & Zini, 2014). Zhang et al. (2011) studied the evolution of esters during the fermentation of Syrah wines and observed two peak levels for esters during alcoholic fermentation, probably as a result of their hydrolysis under the action of cellular esterases, the activity of which increased at the end of fermentation. The action of these enzymes was not observed in Moscatel sparkling wines evaluated in this work, since the concentration of ethyl octanoate and ethyl decanoate increased throughout winemaking.

The present study takes into consideration only the general trends of volatile profile during the winemaking process. However a next step of investigation should also employ the use of internal standards for each one of the volatile compound classes and should also consider the ethanol percentage of every sample extracted and analysed, as these improvements would give a more precise and quantitative view of the volatile profile during vinification. Although, data obtained in this present simpler approach were not to the detriment of the results and discussion, as several trends surpassed contrary possible ethanol effects, such as in the case of ethyl octanoate and decanoate, 2-phenylethanol, nonanol and hexanol, and acetate esters (Zhang et al., 2011).

3.3. Volatile profile of Moscatel sparkling wines

The 1D-GC/MS chromatogram and GC \times GC/TOFMS plots of the HS-SPME of a Moscatel sparkling wine are shown in Fig. 4. Table 1 presents the names of the volatile compounds positively or tentatively identified using both chromatographic techniques, LTPRI values, mass spectral similarity (S), chromatographic area percentage and the possible contributions to aroma of each volatile compound. The superior performance of GC \times GC can be observed through the higher number of tentatively identified compounds (172) against only 42 compounds elucidated by 1D-GC.

Compounds of eleven different chemical classes were detected in Moscatel sparkling wines including esters that represent 39.5% of the total of identified compounds, followed by terpenes (22.1%), alcohols (11%), ketones (7%), acids (5.7%), ether (4.1%), aldehydes (3%), sulphur compounds (3%), lactones (1.7%), phenols (1.7%) and C13-norisoprenoids (1.2%). These characteristics are important to the aromatic quality of sparkling wines, since esters and terpenes may positively contribute to the aroma. Bordiga et al. (2013) also found esters and terpenes as the predominant classes of compounds in still Moscato wines produced in Italy.

The compounds that presented higher chromatographic area percentages (>3%) in Moscatel sparkling wines were hexyl acetate (# 52, 12.9% of Table 1), 3-methyl-1-butanol (# 14, 9.9%), 2,3-butanediol (# 17, 7.5%), *cis*-3-hexenyl acetate (# 51, 4.0%),

Table 1

Volatile compounds identified or tentatively identified using HS-SPME-1D-GC/MS and HS-SPME-GC × GC/TOFMS in the stages of Moscatel sparkling wine elaboration and their possible contribution to aroma. Chromatographic conditions are described in item Section 2.3.

# Compound ^k	CASRN ^j	S ^c	LTPRI _{exp} ^d 1D-GC	LTPRI _{exp} ^e GC × GC	LTPRI _{lit} ^f	% Area	Odour ^g
<i>Acids</i>							
1 Acid ^a (1)	–	928	nd ^h	764	nf ⁱ	0.027	–
2 Butanoic acid	107-92-6	946	nd ^h	799	789	0.150	cheese ¹
3 2-Methyl butanoic acid (4)	116-53-0	829	nd ^h	857	853	0.067	cheese ¹
4 Hexanoic acid ^b	112-37-8	908	989	996	981	1.625	fatty ¹
5 Heptanoic acid	616-62-6	882	nd ^h	1068	1058	0.030	cheese ¹
6 2-Ethyl hexanoic acid (18)	149-57-5	834	nd ^h	1127	1123	0.047	nf ⁱ
7 Octanoic acid ^b	124-07-2	766	1180	1199	1182	0.334	fatty ¹
8 Nonanoic acid ^b	112-05-0	887	1283	1274	1273	0.231	fatty ¹
9 Decanoic acid ^b (27)	334-48-5	924	1372	1380	1373	3.305	rancid ¹
10 Undecanoic acid	112-37-8	838	nd ^h	1569	1561	0.054	oil ¹
<i>Alcohols</i>							
11 Propanol ^b	71-23-8	854	nd ^h	551	536	0.004	ripe fruit ¹
12 Alcohol ^a	–	950	nd ^h	710	nf ⁱ	1.527	–
13 Alcohol ^a	–	888	nd ^h	722	nf ⁱ	0.360	–
14 3-Methyl-1-butanol (1)	123-51-3	874	750	765	773	9.957	solvent ¹
15 Diol ^a	–	893	nd ^h	776	nf ⁱ	0.167	–
16 1-Pentanol	71-41-0	841	nd ^h	784	771	0.038	balsamic ¹
17 2,3-Butanediol	19132-06-0	953	795	795	779	7.492	fruity ¹
18 3-Hexen-1-ol	928-96-1	951	883	860	856	0.639	green, bitter ¹
19 1-Hexanol ^b	–	895	nd ^h	872	870	1.731	grass ¹
20 2-Heptanol (6)	6033-23-4	873	nd ^h	905	900	0.075	orange ¹
21 Alcohol ^a (7)	–	890	nd ^h	928	nf ⁱ	0.033	–
22 1-Heptanol	111-70-6	903	nd ^h	973	969	0.039	lemon ²
23 1-Octen-3-ol	3391-86-4	876	nd ^h	982	981	0.073	mushroom ¹
24 2-Ethyl hexanol (12)	104-76-7	883	1031	1032	1033	0.127	floral, fruity ¹
25 2-Nonanol (16)	628-99-9	813	nd ^h	1104	1100	0.373	fruity ¹
26 2-Phenylethyl alcohol ^b	60-12-8	948	1119	1117	1106	2.442	rose, honey ¹
27 1-Nonanol (20)	143-08-8	811	1175	1174	1169	0.007	raspberry ²
28 2,2-Butoxyethoxy ethanol (22)	112-34-5	907	nd ^h	1194	1192	0.716	nf ⁱ
29 Dodecanol ^b	112-53-8	912	nd ^h	1419	1409	0.342	unpleasant, flowery ¹
<i>Aldehydes</i>							
30 Benzaldehyde	100-52-7	903	nd ^h	965	958	0.028	almond ¹
31 Phenyl acetaldehyde (13)	122-78-1	833	nd ^h	1047	1043	0.011	rose ¹
32 Decanal	112-31-2	912	nd ^h	1207	1209	0.017	orange ¹
33 Undecanal	112-44-7	884	nd ^h	1310	1310	0.003	floral ¹
34 Dodecanal	112-54-9	884	nd ^h	1391	1398	0.060	floral ¹
<i>Esters</i>							
35 Ester (2)	–	885	nd ^h	753	765	0.668	–
36 Ethyl acetate ^b	141-78-6	897	nd ^h	794	807	2.409	fruity, solvent ¹
37 Ethyl 2-methyl propionate ^b	97-62-1	862	nd ^h	780	761	0.286	fruity ¹
38 Isobutyl acetate	110-19-0	935	nd ^h	787	788	1.348	banana ³
39 Ethyl butanoate ^b	105-54-4	938	807	807	799	1.700	apple ¹
40 Ethyl lactate	97-64-3	907	nd ^h	818	813	0.090	fruity ¹
41 Ethyl 3-methyl butanoate ^b (4)	108-64-5	891	nd ^h	857	851	0.038	apple ¹
42 2-Methylbutyl acetate (5)	624-41-9	857	nd ^h	880	885	0.494	pear ⁵
43 Isoamyl acetate ^b (5)	123-92-2	906	886	880	880	3.631	banana ¹
44 Ethyl pentanoate (6)	539-82-2	847	nd ^h	905	901	0.010	apple ¹
45 Amyl acetate	628-63-7	892	nd ^h	916	926	0.018	banana ¹
46 Methyl hexanoate (7)	106-70-7	883	nd ^h	928	934	0.064	floral, fruity ⁸
47 Propyl 2-methyl butanoate	539-90-2	877	nd ^h	956	955	0.022	nf ⁱ
48 Butyl 3-methyl propanoate	105-68-0	836	nd ^h	971	969	0.050	nf ⁱ
49 2-Ethylbutyl acetate	10031-87-5	844	973	973	986	0.024	banana ¹
50 Ethyl hexanoate ^b	123-66-0	893	1003	1002	1003	3.336	apple ¹
51 Hexenol acetate (10)	3681-71-8	956	1010	1007	1002	4.084	nf ⁱ
52 Hexyl acetate ^b (11)	142-92-7	892	1016	1018	1009	12.90	pear ¹
53 Ethyl 3-methyl pentanoate	5870-68-8	834	nd ^h	1023	1004	0.070	nf ⁱ
54 1-Methylhexyl acetate	5921-82-4	863	nd ^h	1044	1047	0.052	nf ⁱ
55 Ethyl 2-hexenoate (13)	1552-67-6	917	nd ^h	1047	1046	0.058	pineapple ¹
56 Ester ^a (14)	–	936	nd ^h	1057	nf ⁱ	0.069	–
57 Ethyl furoate (14)	614-99-3	829	nd ^h	1057	1047	0.028	balsamic ¹
58 Ethyl 2-hydroxy-4-methyl pentanoate	10348-47-7	794	nd ^h	1060	1078	0.024	lemon ¹
59 Propyl hexanoate	626-77-7	913	nd ^h	1097	1091	3.200	fruity ¹
60 Ethyl sorbate ^b (15)	2396-84-1	832	nd ^h	1102	1089	0.004	celery ⁴
61 Ethyl heptanoate (15)	106-30-9	808	nd ^h	1102	1083	0.031	fruity ¹
62 Hexyl propanoate	2445-76-3	875	nd ^h	1109	1108	0.051	nf ⁱ
63 Heptyl acetate (17)	112-06-1	886	nd ^h	1114	1110	0.090	pungent ⁶
64 Ester ^a (18)	–	814	nd ^h	1127	nf ⁱ	0.067	–
65 Methyl octanoate (18)	111-11-5	914	1126	1127	1125	0.248	citrus ¹
66 2-Octyl acetate	2051-50-5	754	nd ^h	1142	1147	0.004	nf ⁱ

(continued on next page)

Table 1 (continued)

#	Compound ^k	CASRN ^j	S ^c	LTPRI ^d _{exp} 1D-GC	LTPRI ^e _{exp} GC × GC	LTPRI ^f _{lit}	% Area	Odour ^g
67	2-Ethylhexyl acetate	103-09-3	842	nd ^h	1152	1144	0.044	nf ⁱ
68	Ethyl benzoate (20)	93-89-0	838	nd ^h	1174	1170	0.016	fruity ⁷
69	Diethyl succinate ^b	123-25-1	959	1187	1184	1179	0.475	fruity ¹
70	cis-3-Hexenyl butyrate	16491-36-4	941	nd ^h	1187	1184	0.940	nf ⁱ
71	Hexyl butanoate (22)	2639-63-6	906	nd ^h	1194	1190	0.139	fruity, floral ⁸
72	Ethyl octanoate ^b (23)	106-32-1	883	1200	1197	1197	2.012	fruity ¹
73	Methyl salicylate (23)	119-36-8	844	nd ^h	1197	1188	0.012	cooked fruit ⁵
74	Octyl acetate	112-14-1	904	1215	1212	1214	1.011	fruity ¹
75	Ester ^a	–	803	nd ^h	1227	nf ⁱ	0.025	–
76	Phenylethyl acetate ^b	101-97-3	941	nd ^h	1244	1260	4.400	rose ⁵
77	Isopentyl hexanoate	2198-61-0	902	nd ^h	1249	1254	0.047	fruity ¹
78	Ethyl 2-phenyl acetate ^b	103-45-7	939	1260	1257	1260	3.673	rose ¹
79	Ester ^a	–	893	nd ^h	1259	nf ⁱ	0.062	–
80	Propyl octanoate	624-13-5	886	nd ^h	1289	1277	0.119	fruity ⁸
81	Ethyl nonanoate	123-29-5	909	1299	1294	1294	1.710	floral, fruity ¹
82	Methyl decanoate	110-42-9	882	1328	1326	1328	0.146	nf ⁱ
83	Isobutyl octanoate	1300784	846	nd ^h	1351	1334	0.009	nf ⁱ
84	3-Hexenyl hexanoate (27)	31501-11-8	903	1393	1380	1379	0.085	nf ⁱ
85	Ethyl decenoate (28)	67233-91-4	825	nd ^h	1388	1389	0.202	fruity ¹
86	Ethyl decanoate ^b	110-38-3	908	1401	1399	1398	3.989	fruity ¹
87	Phenylethyl butanoate	103-52-6	916	nd ^h	1445	1439	0.121	musty ¹
88	3-Methylbutyl octanoate	2035-99-6	886	nd ^h	1448	1450	0.067	pineapple ⁸
89	Propyl decanoate	30673-60-0	836	nd ^h	1491	1493	0.015	nf ⁱ
90	Ethyl undecanoate	627-90-7	812	nd ^h	1496	1477	0.004	nf ⁱ
91	2-Methylpropyl decanoate (30)	30673-38-2	808	nd ^h	1548	1545	0.004	nf ⁱ
92	Ester ^a	–	805	nd ^h	1584	nf ⁱ	0.058	–
93	Ethyl dodecanoate ^b	106-33-2	893	1593	1596	1597	0.353	floral, fruity ¹
94	Ester ^a (31)	–	915	nd ^h	1599	–	0.863	–
95	1-Methylphenyl decanoate	10233-13-3	899	nd ^h	1628	1624	0.035	nf ⁱ
96	2-Phenylethyl hexanoate (32)	6290-37-5	878	nd ^h	1647	1650	0.031	fruity ⁸
97	3-Methylbutyl pentadecanoate (32)	2306-91-4	848	nd ^h	1647	1644	0.019	nf ⁱ
98	2-Ethylhexyl benzoate	5444-75-7	805	nd ^h	1716	1735	0.005	nf ⁱ
99	Ethyl tetradecanoate	124-06-1	879	nd ^h	1796	1790	0.071	nf ⁱ
100	1-Methylethyl tetradecanoate	110-27-0	814	nd ^h	1828	1827	0.006	nf ⁱ
101	Methyl hexadecanoate	112-39-0	845	nd ^h	1929	1929	0.016	nf ⁱ
102	Ethyl hexadecanoate	628-97-7	849	nd ^h	1999	1997	0.106	fatty, fruity ¹
Ethers								
103	Ether ^a (3)	–	877	nd ^h	849	nf ⁱ	0.099	–
104	Ether ^a (5)	–	966	nd ^h	880 ^h	nf ⁱ	0.263	–
105	Ether ^a	–	800	nd ^h	899	nf ⁱ	0.024	–
106	Ether ^a	–	816	nd ^h	911	nf ⁱ	0.718	–
107	1,3-Dimethoxybenzene	151-10-0	863	nd ^h	1169	1158	0.005	nf ⁱ
108	2-(2-Butoxyethoxy)ethanol (22)	112-34-5	806	nd ^h	1194	1196	1.102	nf ⁱ
109	Geranyl ethyl ether	40267-72-9	839	nd ^h	1284	1297	0.411	green ¹⁴
Ketones								
110	Ketone ^a	–	873	nd ^h	741	nf ⁱ	0.128	–
111	Ketone ^a (2)	–	957	nd ^h	753	nf ⁱ	0.470	–
112	2-Heptanone	110-43-0	894	nd ^h	895	888	0.055	nf ⁱ
113	3-Octanone (8)	106-68-3	899	nd ^h	988	984	0.011	herbal ¹
114	6-Methyl 5-hepten-2-one (8)	110-93-0	881	nd ^h	988	984	0.021	fruity ¹
115	Acetophenone	98-86-2	828	nd ^h	1070	1058	0.009	floral ⁷
116	2-Nonanone	821-55-6	905	nd ^h	1094	1094	0.354	fruity ¹
117	Ketone ^a (21)	–	803	nd ^h	1177	nf ⁱ	0.101	–
118	Ketone ^a	–	923	nd ^h	1269	nf ⁱ	0.052	–
119	Ketone ^a	–	800	nd ^h	1279	nf ⁱ	0.057	–
120	2-Undecanone	112-12-9	906	nd ^h	1292	1298	0.140	nf ⁱ
121	2-Tetradecanone (31)	2345-27-9	816	nd ^h	1599	1597	0.036	nf ⁱ
Lactones								
122	Butyrolactone	96-48-0	942	nd ^h	925	918	0.076	sweet, buttery ⁹
123	γ-Nonalactone (26)	104-61-0	831	nd ^h	1367	1372	0.036	coconut ⁷
124	γ-Decalactone	706-14-9	825	nd ^h	1473	1463	0.009	coconut ¹⁰
Phenols								
125	Phenol (9)	108-95-2	815	nd ^h	991	982	0.009	medicinal ⁷
126	Butylhydroxytoluene (BHT) (29)	128-37-0	800	nd ^h	1514	1513	0.017	nf ⁱ
127	2,4-Bis(1,1-dimethylethyl)phenol (29)	96-76-4	892	1509	1514	1519	0.118	nf ⁱ
Sulphur compounds								
128	Sulphur compound ^a	–	855	nd ^h	691	nf ⁱ	0.035	–
129	Sulphur compound ^a	–	804	nd ^h	707	nf ⁱ	0.069	–
130	2-(Methylthio)ethanol (3)	5271-38-5	810	nd ^h	850	838	0.008	nf ⁱ
131	Ethyl 3-methylthio propanoate (16)	13327-56-5	807	nd ^h	1104	1098	0.008	pineapple ¹
132	Dihydro-2-methyl-3-thiophenone (9)	13679-85-1	897	nd ^h	991	1011	0.056	nf ⁱ

Table 1 (continued)

# Compound ^k	CASRN ⁱ	S ^c	LTPRI _{exp} ^d 1D-GC	LTPRI _{exp} ^e GC × GC	LTPRI _{lit} ^f	% Area	Odour ^g
<i>Terpenes</i>							
133 α-Myrcene	1686-30-2	854	nd ^h	979	981	0.004	nf ⁱ
134 β-Myrcene (9)	123-35-3	886	nd ^h	991	990	0.371	geranium, medicine ¹¹
135 α-Phellandrene	99-83-2	869	nd ^h	1005	1002	0.047	fruity, minty ¹²
136 1,3,8- <i>p</i> -Menthatriene (10)	21195-59-5	808	nd ^h	1007	1111	0.032	pine, plastic ¹³
137 δ-Terpinene (11)	99-85-4	830	1058	1018	1014	0.058	nf ⁱ
138 β-Cymene	535-77-3	826	nd ^h	1028	1026	0.007	lemon ¹³
139 Limonene ^b (12)	5989-54-8	939	1029	1031	1029	0.436	licorice ¹²
140 1,8-Cineole (eucalyptol) ^b	470-82-6	872	nd ^h	1033	1029	0.008	minty ¹²
141 <i>cis</i> -Ocimene	3779-61-1	932	1038	1039	1050	0.137	nf ⁱ
142 <i>trans</i> -Ocimene	3338-55-4	914	nd ^h	1049	1047	0.245	nf ⁱ
143 <i>cis</i> -Linalool oxide	34995-77-2	896	1073	1076	1073	0.206	flower ¹⁴
144 Hotrienol	29957-43-5	853	1108	1089	1101	0.023	hyacinth ¹⁴
145 Terpinolene ^b	586-62-9	919	1090	1091	1089	0.110	citrusy ¹¹
146 Linalool ^b (15)	78-70-6	910	1105	1102	1097	3.042	citrus, floral, grape-like ¹
147 Rose oxide (17)	16409-43-1	900	nd ^h	1114	1112	0.085	rose ¹⁵
148 Myrcenol	543-39-5	801	nd ^h	1124	1119	0.006	floral ¹
149 <i>allo</i> -Ocimen (19)	7216-56-0	873	nd ^h	1132	1130	0.030	nf ⁱ
150 2,6-Dimethyl 1,3,5,7-octatetraene (19)	460-01-5	927	nd ^h	1132	1134	0.091	nf ⁱ
151 Camphor	21368-68-3	896	nd ^h	1149	1147	0.005	nf ⁱ
152 α-Terpineol ^b	5986-38-9	813	nd ^h	1154	1150	0.005	floral ⁷
153 Nerol oxide	1786-08-9	920	1155	1157	1158	0.642	flower ¹⁴
154 Epoxylinalol	14049-11-7	863	nd ^h	1172	1163	0.063	nf ⁱ
155 1-Menthol ^b (21)	23283-97-8	829	nd ^h	1177	1188	0.006	nf ⁱ
156 4-Terpineol	20126-76-5	800	1187	1179	1177	0.061	sweet, herbaceous ¹
157 <i>p</i> -Menth-1-en-9-al	29548-14-9	839	nd ^h	1219	1232	0.017	nf ⁱ
158 Citronellol (24)	1117-61-9	938	1234	1229	1226	2.121	citrus ¹
159 Nerol ^b (24)	106-25-2	889	nd ^h	1229	1229	0.109	rose, lime ¹
160 3,7-Dimethyl-1,5-octadien-3,7-diol	13741-21-4	804	1237	1244	1237	0.008	nf ⁱ
161 Geraniol	106-24-1	899	1259	1254	1253	0.156	geranium ¹²
162 Geranial	5392-40-5	831	nd ^h	1272	1277	0.020	lemon, minty ¹²
163 Citronellyl acetate (25)	150-84-5	888	1358	1356	1354	0.245	rose, dust ¹³
164 Neryl acetate (26)	141-12-8	888	1368	1366	1362	0.077	nf ⁱ
165 Geranyl acetate	16409-44-2	905	1389	1386	1379	0.053	citrus, pine ¹⁴
166 Geranyl acetone	3796-70-1	874	nd ^h	1456	1452	0.014	floral ⁷
167 β-Farnesene	18794-84-8	893	nd ^h	1459	1457	0.014	nf ⁱ
168 <i>cis</i> -Bisabolene	6753-98-6	821	nd ^h	1545	1533	0.003	nf ⁱ
169 α-Calacorene (30)	21391-99-1	811	nd ^h	1548	1548	0.003	nf ⁱ
170 Nerolidol	40716-66-3	905	nd ^h	1566	1564	0.018	dry wood, hay ⁵
<i>c13-Norisoprenoids</i>							
171 β-Damascenone ^b (28)	23726-93-4	886	1386	1388	1385	0.393	honey, sweet ¹
172 1,2-Dihydro-1,1,6-trimethylnaphthalene (TDN) (25)	30364-38-6	886	nd ^h	1356	1354	0.050	kerosene ¹⁴

^a Whenever a LTPRI had not been found in the scientific literature to match with the experimentally determined LTPRI, only the chemical class of the volatile compound was assigned.

^b Mass spectrum and LTPRI consistent with those of an authentic standard. These compounds were positively identified;

^c S: spectral similarity (for more explanation, about this parameter, see text).

^d LTPRI_{exp} 1D-GC: Experimental linear temperature programmed retention index (LTPRI) obtained in 1D-GC analyses of wine volatiles and *n*-alkanes (C9–C24) determined in apolar column (DB-5, 5% diphenyl-95% dimethyl polysiloxane); LTPRI for compounds with LTPRI <900 and >2400 were extrapolated.

^e LTPRI_{exp} GC × GC: experimental LTPRI obtained in GC × GC analyses of wine volatiles and *n*-alkanes (C9–C24) determined in apolar (DB-5) × medium polar (DB-17 ms, (50%-phenyl)-methylpolysiloxane) column set and using ¹D retention times. LTPRI for compounds with LTPRI <900 and >2400 were extrapolated;

^f LTPRI_{lit}: values of LTPRI found in scientific literature 1D-GC for apolar column (Adams (2007); Library, NIST/EPA/NIH mass spectral. Software NIST MS Search 2.0. 2005.

^g Typical odour described in literature: odour reported in previous work.

^h nd: compound that had not been detected in 1D-GC.

ⁱ nf: not found in the scientific literature. This applies to columns: “LTPRI_{lit}” and “Odour”.

^j CASRN: chemical abstract service registry number.

^k Co-elutions were numbered from 1 to 32 and compounds that co-elute are followed by their co-elution number between parentheses, after the compound's name. Whenever compounds are followed by the same number, they co-elute.

¹ Welke, Zanusi, Lazarotto, and Zini (2014).

² Tao and Li (2009).

³ Samappito and Butkhup (2010).

⁴ Rosa, Margheri, Moret, Scarponi, and Versini (1983).

⁵ Ferrari et al. (2004).

⁶ Cometto-Muñiz and Cain (1991).

⁷ Fan, Xu, Jiang, and Li (2010).

⁸ Fan and Qian (2005).

⁹ Rocha, Rodrigues, Coutinho, Delgadillo, and Coimbra (2004).

¹⁰ Fan and Qian (2006).

¹¹ Rega, Fournier, and Guichard (2003).

¹² Hognadóttir and Rouseff (2003).

¹³ Benjamin, Tietel, and Porat (2013).

¹⁴ Bordiga et al. (2013).

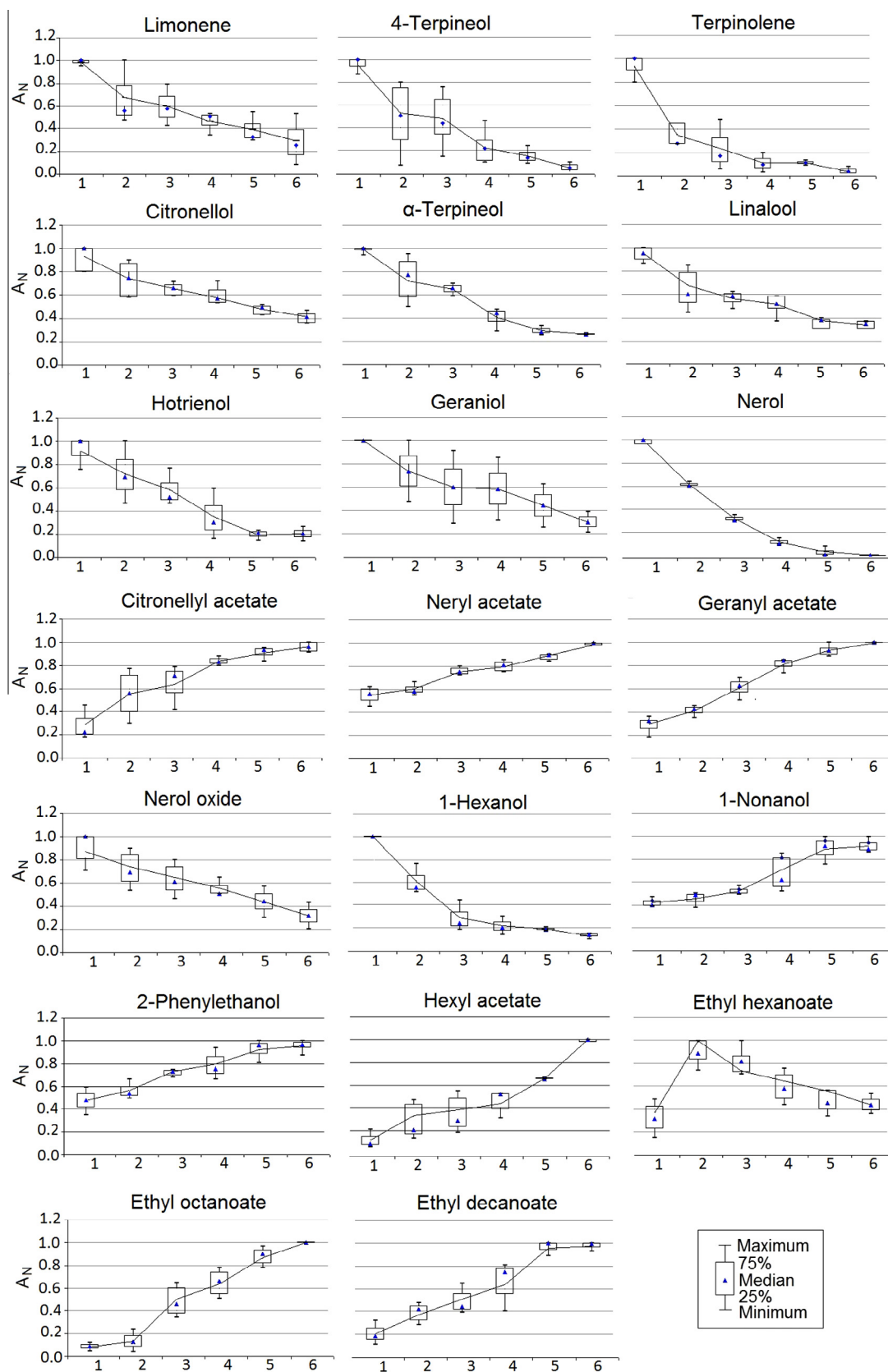


Fig. 3. Normalised area trends for volatile compounds monitored during six steps of winemaking of Moscatel sparkling wines. The steps of winemaking were numbered from 1 to 6 including (1) grape must; (2) first filtration: filtrated must after yeast addition; (3) second filtration: filtrated must after 6 days of fermentation; (4) third filtration: filtrated must after 12 days of fermentation; (5) fourth filtration: sparkling wine obtained after 20 days of fermentation and (6) bottled sparkling wine. Each box corresponds to the inter-quartile range of the normalised area of a compound in each processing step. The median is represented by the blue triangle; minimum and maximum values are designated by the bar hedges outside the box; the black line indicates the average normalised area of a compound at each stage of the winemaking process.

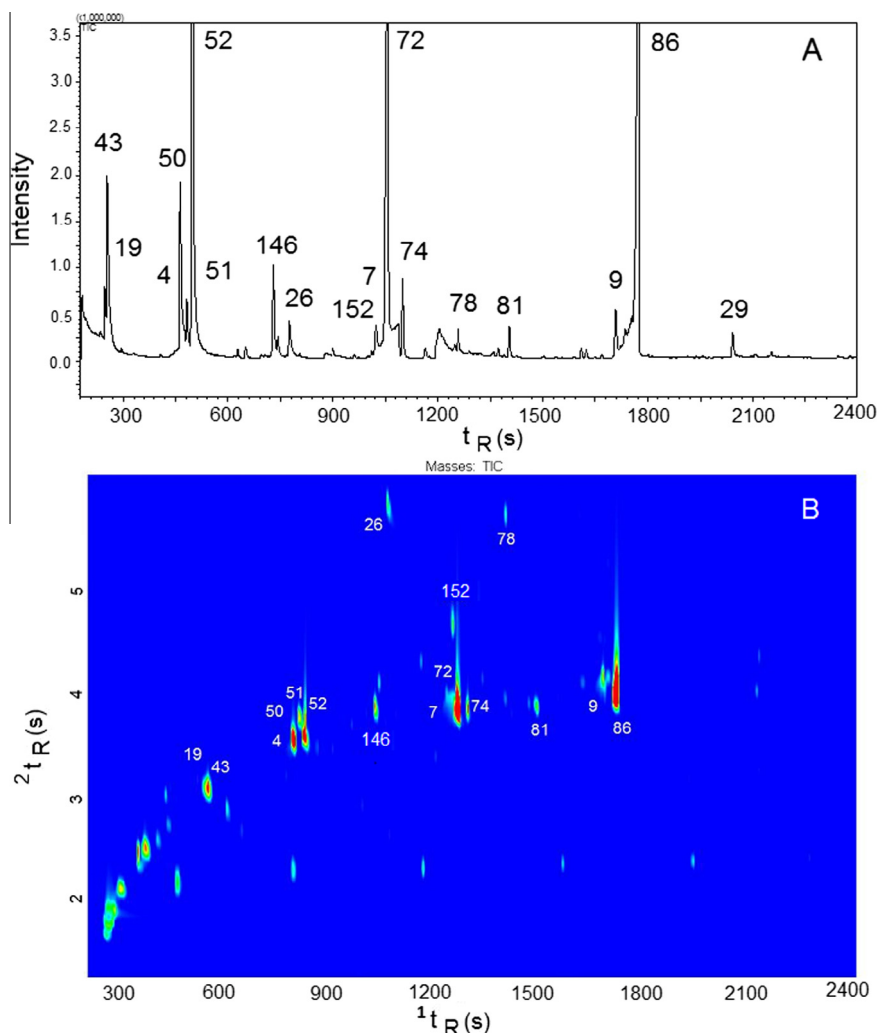


Fig. 4. Chromatograms obtained from the analysis of Moscatel sparkling wine headspace. (A) 1D-GC total ion current chromatogram and (B) GC \times GC colour plot in which the colour gradient reflects the intensity of the TOFMS signal (Z-axis) from low (blue) to high (red). Some compounds were indicated in both figures (A and B) and correspond to those of Table 1. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

ethyl decanoate (# 86, 3.9%), 2-phenylethyl acetate (# 76, 4.4%), ethyl hexanoate (# 59, 3.2%) and decanoic acid (# 9, 3.3%). Two of these major compounds may negatively affect wine aroma quality: 3-methyl-1-butanol [odour of solvent (Peinado et al., 2004)] and decanoic acid [rancid odour (Peinado et al., 2004)].

Some compounds may contribute to aroma even if they are present in small amounts. The intensity of an olfactory sensation does not depend only on the concentration of a volatile compound, but it is also related to volatility and vapour pressure, which define the odour threshold of a particular compound (Clarke, 2003). Furthermore, the CO₂ discharging and bubble bursting on the liquid surface also affects aroma perception of sparkling wines (Liger-Belair, 2005). To avoid the influence of CO₂, samples were degassed under low temperature (Experimental section).

Linalool (#146 of Table 1) was the terpene with highest area percentage (3%), followed by citronellol (2.1%; # 158) and nerol oxide (0.6%; # 153). All these terpenes are reported as positive contributions to wine aroma with sweet and floral notes. One of the compounds positively identified in the Moscatel sparkling wine that deserves to be highlighted is eucalyptol (1,8-cineole, 0.008%, # 140 of Table 1), as it is not a typical compound of wines (Capone, Jeffery, & Sefton, 2012; Kalua & Boss, 2010; Rocha, Coelho, Zrostlíková, Delgadillo, & Coimbra, 2007). However, eucalyptol has been identified in grapes and wines including

Riesling, Cabernet Sauvignon (Kalua & Boss, 2010) and Shiraz from Australia (Capone et al., 2012) and Fernão-Pires produced in Portugal (Rocha et al., 2007). Capone et al. (2012) verified that the proximity of *Eucalyptus* trees to grapevines could directly influence the concentration of eucalyptol present in the corresponding red wines. The highest concentration of this terpene was found in the grapevine leaves, followed by grape stems and then grapes. For this reason, the presence of grape leaves and grape stems in maceration step can considerably enhance the concentration of eucalyptol, which may give minty characteristic to the wine (Capone et al., 2012).

BHT (# 126) and 2,4-bis(1,1-dimethylethyl)phenol (2,4-di-*tert*-butylphenol, # 127) are isomers and are probably volatile contaminants arising from tris-(2,4-di-*tert*-butylphenyl) phosphite, a common plastic stabiliser (Carlsson, Krzymien, Deschenes, Mercier, & Vachon, 2001; Krzymien, Carlsson, Deschenes, & Mercier, 2001). Both compounds have already been found as volatiles in some food matrices as a possible contaminant, such as for example truffles (Davoli, Bellesia, & Pinetti, 2003) and *Evodia* species fruits (Pellati, Benvenuti, Yoshizaki, Bertelli, & Rossi, 2005).

Research articles have reported the presence of BHT in wines: in Cabernet Sauvignon Wines from Western Australia (Robinson et al., 2011a, 2011b), in Pinotage wines from South Africa (Weldegergis et al., 2011) and in sherry wines obtained from

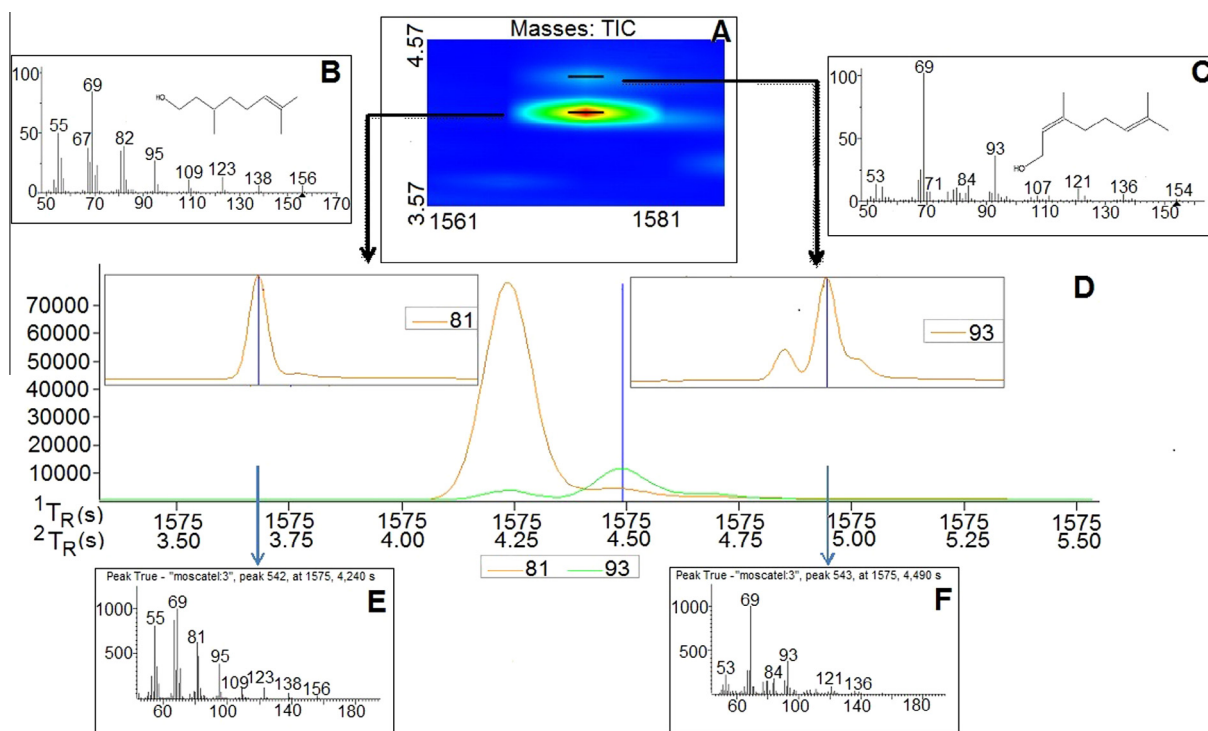


Fig. 5. Co-elution of citronellol and nerol in the first dimension ($^1t_R = 1575$ s) of GC \times GC: (A) separation of these compounds in second dimension ($^2t_R = 4.24$ for citronellol and $^2t_R = 4.49$ for nerol); mass spectra of the library (NIST 2005) for (B) citronellol and (C) nerol; (D) modulated peaks of citronellol (orange line, compound # 158 in Table 1) and nerol (green line, compound # 159 in Table 1); Deconvoluted mass spectra of (E) citronellol and (F) nerol found in Moscatel sparkling wine. Chromatographic conditions are described in item Section 2.4. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

grapes cv. Muscat from Spain (Ruiz-Bejarano et al., 2013). 2,4-Di-*tert*-butylphenol was also found in commercial Cabernet Sauvignon (Robinson et al., 2011a, 2011b) and in Madeira wines in heated and non-heated conditions (Pereira, Cacho, & Marques, 2014).

BHT (# 126) and 2,4-di-*tert*-butylphenol (# 127) could have been easily taken out of the list, as they may be considered only as contaminants coming from plastic caps and this hypothesis has already been raised by Demittenaere et al. (2003) in their study about Greek white wine. However, BHT is also a widespread synthetic antioxidant that is commonly added to foodstuffs (Belitz, Grosch, & Schieberle, 2009) and it is important to mention that its presence in wine volatiles deserves further attention with the aim of verifying its concentration and accurate origin, as wines are supposed to be free of any type of synthetic compound. 2,4-Di-*tert*-butylphenol has been reported as an androgen antagonist in CHO-K1 cells and rainbow trout (Tollefsen, Eikvar, Finne, Fogelberg, & Gregersen, 2008) and has been found in PET-bottled waters (Real et al., 2015). The authors attributed this finding to the plastic material in the bottle caps as a large number of caps are made of polyethylene (PE) and polypropylene (PP) plastics which can be a source of endocrine disruptor compounds (EDC) (ILSI, 2003; Yang, Yaniger, Jordan, Klein, & Bittner, 2011).

Thirty-two co-elutions (69 compounds) were observed using GC \times GC, which included 69 compounds that co-eluted in the first dimension (1D). Among them, six co-elutions were also observed in the second dimension (2D): (i) 2-methylbutanoic acid (# 3 of Table 1) and ethyl 3-methylbutanoate (# 41); (ii) 2-heptanol (# 20) and ethyl pentanoate (# 44); (iii) 3-octanone (# 113) and 6-methyl-5-hepten-2-one (# 114); (iv) phenylacetaldehyde (# 31) and ethyl 2-hexenoate (# 55); (v) heptyl acetate (# 63) and rose oxide (# 147); (vi) decanoic acid (# 9) and 3-hexenyl hexanoate (# 84). Co-elutions were numbered from 1 to 32 and

these numbers are written between parentheses after the compound's name in Table 1. The majority (74%) of co-eluting compounds were not detected in the 1D-GC.

An interesting example of co-elution of volatile compounds of Moscatel sparkling wines in the first dimension ($^1t_R = 1575$ s) included citronellol ($^2t_R = 4.24$ s, # 158 in Table 1) and nerol ($^2t_R = 4.49$ s, # 159 of Table 1). The separation of these compounds was achieved in the second dimension (Fig. 5). Nerol was not detected by 1D-GC and citronellol was one of the compounds reported in the scientific literature (Bordiga et al., 2013; Clarke, 2003; Ruiz-Bejarano et al., 2013) as important to the aroma of Moscatel wines. The concentration of citronellol was monitored during the stages of winemaking by 1D-GC and the results were discussed in Section 3.2. GC \times GC analysis showed that chromatographic areas of both terpenes decreased during winemaking. Comments on possible enzymatic transformations of nerol and citronellol have already been made in the same section, besides other possible hypotheses for the decrease of terpenols.

Seven other compounds (limonene, linalool, citronellyl acetate, neryl acetate, 1-nonanol, hexyl acetate and ethyl octanoate) monitored in Moscatel sparkling wines, using 1D-GC, co-eluted with other components and these co-elutions are listed as follows: limonene (# 139, Table 1) and 2-ethylhexanol (# 24); linalool (#146), ethyl sorbate (# 60) and ethyl heptanoate (# 61); citronellyl acetate (#163) and TDN (#172); neryl acetate (#164) and nonalactone (#123); 1-nonanol (# 27) and ethyl benzoate (# 68); hexyl acetate (# 52) and δ -terpinene (#137); ethyl octanoate (# 72) and methyl salicylate (# 73). They coeluted in 1D and were separated in 2D , using GC \times GC. A preliminary view of GC \times GC results has shown that compounds that co-eluted showed similar behaviour; in other words, if the chromatographic area of one decreased during the winemaking process, the co-eluting partner also showed a decreasing trend.

Co-elution of citronellyl acetate (#163 in Table 1) and TDN (#172 in Table 1) is also worth mentioning as both compounds are reported as important to Moscatel aroma, present antagonistic aroma contributions and could be separated only by GC \times GC. Citronellyl acetate contributes to rose aroma and TDN is reported to contribute with typical “kerosene” or “petrol” aroma of wines (Bordiga et al., 2013). These examples suggest that GC \times GC may be a necessary tool to quantitatively monitor some important aroma compounds of Moscatel sparkling wines. An alternative would be monitoring only some compounds considered as key to sparkling wine aroma, choosing a stationary phase that would render their complete separation in 1D-GC.

4. Conclusion

The optimised HS-SPME method proved to be suitable to monitor trends in the chromatographic profile of volatile compounds during several stages of the industrial production of Moscatel sparkling wines. Changes of the chromatographic profile, such as increasing normalised areas of terpenyl acetates and decreasing areas of monoterpenes (limonene, 4-terpineol, terpinolene, citronellol, α -terpineol, linalool, hotrienol, and nerol oxide) show up as interesting findings, as monoterpene floral aroma is characteristic of Moscatel sparkling wine grapes and consequently, responsible for varietal aroma of Moscatel sparkling wines. Furthermore, there are only a few studies about the contribution of terpenyl acetates to wine aroma and their increasing presence during vinification calls attention to their importance and the need for further investigation about their role in the aroma of Moscatel sparkling wines. Other components increased their participation in the volatile mixture during vinification, such as ethyl octanoate, ethyl decanoate, hexyl acetate and 1-nonanol, which are known to impart fruity notes to these sparkling wines.

GC \times GC/TOFMS has provided a better characterisation of the volatile profile of Moscatel sparkling wines considering the higher number of tentatively identified compounds (172) obtained in comparison with only 42 compounds provided by 1D-GC. Moreover, some co-eluting compounds were correctly identified only with the use of GC \times GC. Thirty-two co-elutions were observed in ¹D and among them some important aroma compounds were found, which were resolved only in the second dimension. These results open perspectives for a more detailed characterisation of volatiles of Moscatel sparkling wines by GC \times GC/TOFMS. A detailed knowledge about volatiles of Moscatel sparkling wine may help the search for markers of wine-making quality and may also aid in the denomination of origin of this specific varietal sparkling wine. The strategy employed in this manuscript may also be used to investigate other aromatic beverages and drinks in relation to the same issues and challenges.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.foodchem.2015.03.013>.

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