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# Differentiation of wines according to grape variety using multivariate analysis of comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometric detection data

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## ABSTRACT

Headspace solid-phase microextraction (HS-SPME) combined with comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry detection (GC×GC/TOFMS) was used to analyse the volatiles in five types of wines elaborated with grapes of Cabernet Sauvignon, Merlot, Chardonnay, Sauvignon Blanc and Pinot Noir varieties. Fisher ratio, principal component analysis (PCA) and stepwise linear discriminant analysis (SLDA) were used to develop a discriminant model and, as a result, 12 volatile compounds enabled differentiation and classification of wines according to grape cultivars. A detailed examination of GC×GC/TOFMS data showed that the use of one-dimensional gas chromatography with a mass spectrometric detector (1D-GC/MS) would probably result in misidentification of some of these 12 compounds, as they showed partial coelution with other components in the first chromatographic dimension.

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## 1. Introduction

Brazil is part of a new group of wine-producing countries. Wines produced in the Serra Gaúcha region, located in the state of Rio Grande do Sul in the South part of Brazil represent 90% of the Brazilian wine production. The cultivation of grapevines and wine production has considerable social and economic impact in this region. The main grape varieties grown in Brazil are: (i) red varieties: Cabernet Franc, Cabernet Sauvignon, Merlot, Pinotage, Pinot Noir and Tannat and (ii) white varieties: Chardonnay, Malvasia Bianca, Muscat White Prosecco and Riesling Italic (Mello, 2012).

Aroma is one of the most important factors in determining wine character and quality (Clarke & Bakker, 2004). The aroma characteristics are the result of complex interactions among several factors: vineyard geographical location (Koundouras, Marinos, Gkouliti, Kotseridis, & van Leeuwen, 2006), which is related to soil and climate characteristics (Sabon, de Revel, Kotseridis, & Bertrand, 2002), grape variety (Armanino, Casolino, Casale, & Forina, 2008), yeast strain (Torrens, Riu-Aumatell, Lopez-Tamames, & Buxaderas, 2004), and technical conditions of wine-making, such as

temperature used in grape maceration, frequency and intensity of maceration procedures (Esti & Tamborra, 2006).

There is evidence that it is possible to establish clear relationships among the volatile fraction of foods or beverage and the following aspects: the raw material employed (Rocha, Coelho, Zrostlikova, Delgadillo, & Coimbra, 2007), the place where material was originated (Green, Parr, Breitmeyer, Valentin, & Sherlock, 2011) and the process of production followed (Cardeal, Souza, Gomes da Silva, & Marriott, 2008). Characterisation of foods and beverages based on volatile content may also be used as a tool for authentication, in order to protect the consumer and/or industry from fraud (Krist, Stuebiger, Bail, & Unterweger, 2006). In addition, volatile composition may be useful for characterisation and differentiation of wines from distinct varieties and for establishing criteria to improve the quality of the wines and guarantee their origin (Mildner-Szkudlarz & Jelen, 2008). In fact, knowledge about wine volatile profile may contribute to the achievement of a geographical indication, such as designation of origin, which serves as a benchmark and guarantees product consistency, defining a product that is characteristic of a certain region (Addor & Grazioli, 2002).

The volatile profile of wines, obtained with one-dimensional gas chromatography with a mass spectrometric detector (1D-GC/MS) has been already used for differentiation and classification of wines according to their geographical origin (Green et al., 2011) or grape cultivar (Zhang et al., 2010), using different multivariate

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techniques. However, very little is reported having multidimensional chromatographic data as a basis (Robinson, Boss, Heymann, Solomon, & Trengove, 2011a).

Comprehensive two-dimensional gas chromatography (GC  $\times$  GC) emerged as a powerful analytical technique that is an excellent choice to unravel the composition of complex samples. This technique is based on the application of two GC columns coated with different stationary phases connected in series through a special interface called a modulator. The modulator is the heart of the instrument because it ensures that separation is both comprehensive (the entire sample is subjected to both separation dimensions) and multidimensional (the sample undergoes two different separation processes and the separation accomplished in one dimension is not lost in the other dimension) (Beens, Janssen, Adahchour, & Brinkman, 2005). The modulator (i) accumulates and traps, (ii) refocuses and (iii) rapidly releases the adjacent fractions of the first dimension column effluent (Semard, Gouin, Bourdet, Bord, & Livadaris, 2011). GC  $\times$  GC is an established technique, offering superior separation capabilities afforded by high peak capacity, selectivity, structural chromatographic peak organisation, and sensitivity enhancement compared to 1D-GC. Considerably more information about constituents of complex samples may be provided, while the time of the analysis remains the same as in 1D-GC (Marriott & Shellie, 2002).

The application of GC  $\times$  GC to wine volatiles and other beverages has recently been reviewed (Welke & Zini, 2011) and examples of investigations of wine compounds may be cited. These include determination of methoxypyrazines in Sauvignon Blanc wines (Ryan, Watkins, Smith, Allen, & Marriott, 2005); methoxypyrazines in Cabernet Franc berries and the resulting wines (Ryona, Pan, & Sacks, 2009); furans, lactones, volatile phenols, and acetals in Madeira wines (Perestrelo, Barros, Camara, & Rocha, 2011); volatiles in Cabernet Sauvignon wine (Robinson, Boss, Heymann, Solomon, & Trengove, 2011b), Pinotage wines (Weldegergis et al., 2011) and Fernão-Pires grapes (Rocha et al., 2007). Former work of this research group on Merlot volatiles has been recently published, where the advantages of GC  $\times$  GC/TOFMS have been highlighted, through a detailed characterisation of Merlot volatiles and also with a preliminary approach of the use of multivariate analysis for discrimination of 24 wine samples according to grape variety (Welke, Manfroi, Zanús, Lazarotto, & Zini, 2012a).

The main purpose of this study is to determine which components may be potential markers of grape variety of different wines made of Cabernet Sauvignon, Merlot, Chardonnay, Sauvignon Blanc and 50% Chardonnay/50% Pinot Noir grape varieties, using HS-SPME-GC  $\times$  GC/TOFMS and chemometric analysis. The analysis of possible coelutions of volatile compounds in the first chromatographic dimension and the separation of formerly coeluted compounds by GC  $\times$  GC are also discussed.

## 2. Material and methods

### 2.1. Samples, analytical reagents, and supplies

All wines investigated ( $\sim$ 13% ethanol, v/v) were of 2009 vintage and were produced in Serra Gaúcha region (latitude 29°S, longitude 51°W, altitude 600–800 m). These samples were provided by Empresa Brasileira de Pesquisa Agropecuária Uva e Vinho (EMBRAPA) in sealed 750-mL bottles and were chosen as the best wine samples in the “National Evaluation of Wines of 2010” event promoted by the Brazilian Association of Enology. Fifty-four samples produced from grapes of *Vitis vinifera* cultivars were analysed. Each one of them was from different production batches. The wine samples of each cultivar were 12 samples of Chardonnay, 11 samples of

50% Chardonnay/50% Pinot Noir, 11 samples of Sauvignon Blanc, 9 samples of Cabernet Sauvignon and 11 samples of Merlot grapes.

Model wine was prepared with (+)-tartaric acid (6 g L<sup>-1</sup>) supplied by Synth (São Paulo, Brazil) and 10% of ethanol in MilliQ deionised water. Twenty-two standard compounds were purchased from Aldrich (Steinheim, Germany) and individual stock solutions of each component were prepared in double distilled ethanol purchased from Nuclear (São Paulo, Brazil). The final concentrations of each one of the 22 standard compounds in the model wine solution are listed between parentheses, as follows: ethyl acetate, ethyl butanoate, ethyl hexanoate, ethyl decanoate, diethyl succinate and propanol (1000  $\mu$ g/L of each standard compound), ethyl propanoate, ethyl 2-methylpropanoate, ethyl lactate, ethyl octanoate, ethyl 3-hydroxybutanoate, hexanol, isoamyl acetate, terpinen-4-ol and eugenol (100  $\mu$ g/L of each standard compound); ethyl 2-methylbutanoate and 2-phenylethyl acetate (50  $\mu$ g/L of each standard compound); 2-phenylethanol, hexanoic acid, octanoic acid, decanoic acid and dodecanoic acid (5000  $\mu$ g/L of each standard compound). The pH was adjusted to 3.5 with sodium hydroxide (Nuclear, São Paulo, Brazil). Ultra-pure water was prepared using a Milli-Q water purification system (Millipore, Bedford, MA).

The SPME fibre (50/30 divinylbenzene/Carboxen/polydimethylsiloxane (DVB/CAR/PDMS) StableFlex) was purchased from Supelco (Bellefonte, PA). The fibre was conditioned according to the manufacturer's recommendation prior to its first use. Sodium chloride (NaCl) of analytical grade was purchased from Nuclear and was oven dried at 110 °C overnight before use. Twenty microlitre headspace vials with magnetic screw caps sealed with silicone septa were purchased from Supelco.

### 2.2. Instrumentation

A CTC CombiPAL autosampler (CTC Analytics, Zwingen, Switzerland) with an agitator and SPME fibre was used to extract the volatiles from the sample vial headspace. The GC  $\times$  GC system consisted of an Agilent 6890N (Agilent Technologies, Santa Clara, CA) equipped with a Pegasus IV time-of-flight mass spectrometer (Leco Corporation, St. Joseph, MI). A DB-Wax column (100% polyethylene glycol; 30 m  $\times$  0.25  $\mu$ m, J&W Scientific Inc., Folsom, CA) was used as first-dimension (<sup>1</sup>D) column, and a DB-17 ms column (50% phenyl-50% methylpolysiloxane; 1.70 m  $\times$  0.18 mm  $\times$  0.18  $\mu$ m, J&W Scientific Inc.) was used as a second-dimension (<sup>2</sup>D) column.

The GC system was equipped with a secondary column oven and non-moving quadjet dual-stage thermal modulator. During modulation, cold pulses were generated using dry nitrogen gas cooled by liquid nitrogen (Linde, Canoas, RS, Brazil), whereas heated dry air was used for hot pulses. The injector, transfer line and ion source temperature were at 250 °C. Oven temperature program conditions were as follows: initial temperature of 35 °C for 5 min, programmed at 3 °C min<sup>-1</sup> to 250 °C, where it remained for 5 min. The secondary oven was kept 10 °C above the primary oven throughout the chromatographic run. The modulator was offset by +25 °C in relation to the primary oven. Helium (99.9999% purity, White Martins, Porto Alegre, RS, Brazil) was used as carrier gas at a constant flow of 1 mL min<sup>-1</sup>. The MS parameters included electron ionisation at 70 eV with ion source temperature at 250 °C, detector voltage of 1750 V, mass range of *m/z* 45–450, and acquisition rate of 100 spectra s<sup>-1</sup>.

### 2.3. Conditions for the extraction of volatiles

The SPME extraction was performed according to previous work: 1 mL of wine in 20-mL glass headspace vials, 30% of NaCl (m/v), without sample agitation, extraction time of 45 min and

extraction temperature of 45 °C (Welke, Zanus, Lazarotto, Schmitt, & Zini, 2012b). The wine samples (10 mL) were spiked with 10 µL of alcoholic solution of 3-octanol at 1.25 mg L<sup>-1</sup> used as internal standard. All samples were kept at 45 °C for 10 min prior to extraction. The headspace was sampled using a 2-cm DVB/CAR/PDMS 50/30 µm fibre. The volatile and semi-volatile compounds were desorbed in the GC inlet at 250 °C for 5 min in splitless mode and the fibre was reconditioned for 5 min at 260 °C prior to each analysis. All samples were analysed in triplicate.

#### 2.4. Data processing

LECO ChromaTOF Version 4.22 software was used for all acquisition control, data processing and Fisher ratio calculations. Automated peak find and spectral deconvolution with a baseline offset of 0.5 and signal-to-noise of three were used during data treatment. Twenty-two compounds (listed in Section 2.1) were positively identified through comparison of retention time and mass spectral data of unknown compounds with those of authentic standards.

Tentative identification of wine volatile compounds was achieved by comparing experimental linear temperature programmed retention index (LTPRI) with retention indices reported in the literature for 1D-GC; a description of this procedure has already been reported elsewhere (von Muhlen, Zini, Caramao, & Marriott, 2008). Retention data of a series of *n*-alkanes (C<sub>9</sub>–C<sub>24</sub>), under the same experimental conditions employed for the chromatographic analysis of wine volatiles were used for experimental LTPRI calculation. Mass spectrometric information of each chromatographic peak was compared to NIST 2005 mass spectral library, considering a minimum similarity value of 80%. Whenever a LTPRI was not found in the scientific literature to match with the experimentally determined LTPRI, only the chemical class of the wine volatile compound was assigned.

#### 2.5. Statistical Analysis

The chemometric analysis was done with Statistica 7.1 software (StatSoft, Inc., Tulsa, OK). The statistical analyses were performed with the normalised peak area of volatile compounds (peak area of each compound divided by internal standard peak area). Calculation of Fisher ratios to determine the features which best describe the data in terms of discriminative power between predefined classes was used for data reduction before PCA (Pierce, Hoggard, Mohler, & Synovec, 2008; Pierce et al., 2006). Fisher ratio is calculated by the square of the difference of the average areas of the analyte present in different classes divided by the sum of the variance of the analyte area inside the same class (Fisher, 1936). Fisher ratios were calculated to determine which analytes are responsible for the main differences between wines produced from grapes of different varieties.

The data from GC × GC/TOFMS of 480 analytes of 54 samples were organised in a 480 (columns) × 54 (lines) matrix, and the chemical variables were normalised before statistical analysis. Considering that the number of wines was relatively small compared to the number of variables (volatile compounds), a reduction in the number of variables was necessary to perform useful multivariate statistical analysis (PCA and linear discriminant analysis – LDA). Variable reduction of the data set was carried out by calculation of the Fisher ratios.

The volatile compounds with the highest Fisher ratios were used in PCA, which is an unsupervised technique that reduces the dimensionality of the data set retaining the maximum amount of variability (Jolliffe, 2002). PCA was used to visualise the different wines in a two-dimensional space and identify the directions in which most of the information is retained; it was applied with

mean-centring data. Furthermore, PCA determines which variable contributes to the differences observed between wine samples.

The significant principal components were used in stepwise linear discriminant analysis (SLDA) that is a supervised method applied for classification purposes. LDA classification was developed by applying a stepwise variable selection algorithm, using Wilks' Lambda as a selection criterion and an *F*-statistic factor to determine the significance of the changes in Lambda when the influence of a new variable is evaluated (*F*-value to enter = 1 and *F*-value to remove = 0.5). Therefore, only the most discriminant variables involved in sample differentiation were selected. The prediction capacity of the discriminant models was studied by cross validation.

### 3. Results and discussion

A colour plot obtained of the Chardonnay wine analysis by HS-SPME-GC × GC/TOFMS is shown in Fig. 1. It provides a clear view of the high number of co-elutions that would have happened with the use of one-dimensional GC. Similar GC × GC profiles were observed for wines produced from other grape varieties.

The normalised data from GC × GC/TOFMS of 480 analytes of 54 wine samples were organised in a 480 (columns) × 54 (lines) matrix and the Fisher ratios were calculated for wines distributed in five classes (C: Chardonnay, C + PN: 50% Chardonnay + 50% Pinot Noir, CS: Cabernet Sauvignon, M: Merlot and SB: Sauvignon Blanc), according to the grape variety used in wine production. The higher the Fisher ratio numerical value the greater the variance among classes of samples for a particular compound. Compounds with Fisher ratio value above a threshold of 970 were used in this work, because the volatile compounds contribution for wine class differentiation was small below this value.

The new matrix, resulting from the use of Fisher ratio, included 77 analytes of 54 samples and was submitted to mean centering treatment before PCA. PCA was used to reduce the complex data set by projection of the original number of variables to a reduced number of variables in order to extract relevant information. It was applied to obtain a more simplified view of the relationship between the samples and volatile compounds. The compounds used in PCA are shown in Table 1. Fourteen principal components with eigenvalues higher than 1 (Kraiser's rule) accounted for 85.8% of the total variance. Principal component 1 (PC1) and PC2 explains 24.2% and 19.6% of the variance (Fig. 2), respectively. The score plot shows five differentiated groups.

The red wines, Cabernet Sauvignon and Merlot, are located in the same quadrant. Chardonnay and Sauvignon Blanc wines were separated by PC2, while Merlot, Cabernet Sauvignon and 50% Chardonnay/50% Pinot Noir wines were most influenced by variables related with PC1. The numbers used in Fig. 2B correspond to those

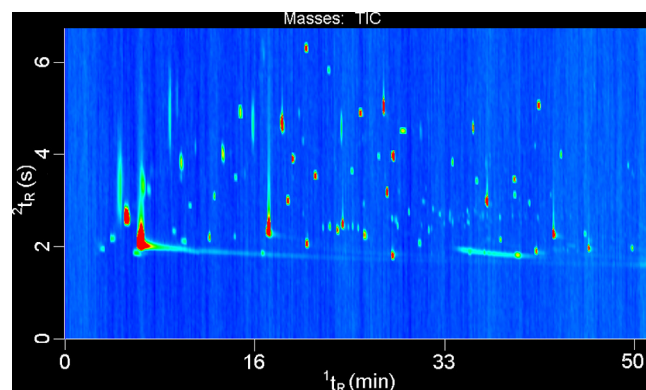


Fig. 1. Colour plot obtained for a Chardonnay wine using HS-SPME-GC × GC/TOFMS.

**Table 1**  
PCA loadings of tentatively identified and unknown compounds of wines by GC × GC/TOFMS, which showed the highest Fisher ratios.

Compound	CAS	LTPRI <sub>exp</sub> <sup>a</sup>	LTPRI <sub>lit</sub> <sup>b</sup>	PCA cluster <sup>c</sup>	PC1	PC2
<i>Alcohols</i>						
2-Methyl-1-propanol	75-65-0	1098	1090 <sup>1</sup>	32/C + PN	-0.03310	-0.71419
2-Methyl-1-butanol	137-32-6	1191	11962	35/C	0.37817	-0.53154
3-Methyl-1-butanol	123-51-3	1200	1209 <sup>1</sup>	70/C + PN	0.02778	0.28934
1-Hexanol <sup>d</sup>	111-27-3	1375	1392 <sup>3</sup>	15/M	-0.08091	0.01705
1-Octanol	111-87-5	1554	1561 <sup>4</sup>	66/C	0.09373	-0.28618
2,3-Butanediol	513-89-3	1563	1580 <sup>1</sup>	52/SB	0.68344	0.41470
C4-Diol <sup>f</sup>		1578	*	62/M	-0.22321	0.222879
(E)-2-Octen-1-ol	18409-17-1	1649	1639 <sup>3</sup>	28/C + PN	0.86085	0.38348
C9-Alcohol <sup>f</sup>		1862	*	4/M	-0.23551	0.01163
2-Phenylethanol	60-12-8	1900	1906 <sup>5</sup>	58/C	0.71045	-0.11502
<i>Acids</i>						
2-Methylpropanoic acid	79-31-2	1568	1566 <sup>6</sup>	2/C	0.11310	-0.72517
Octanoic acid	124-07-2	2096	2086 <sup>1</sup>	53/C	0.34299	-0.39165
<i>Aldehydes</i>						
Acetaldehyde	75-07-0	715 <sup>e</sup>	735 <sup>7</sup>	40/C	0.19962	-0.11672
Hexanal	66-25-1	1107	1092 <sup>8</sup>	59/C	0.40794	-0.06231
Octanal	124-13-0	1272	1287 <sup>8</sup>	50/C + PN	-0.64745	0.550896
Nonanal	124-19-6	1388	1392 <sup>8</sup>	34/M	-0.36449	0.142035
Decanal	112-31-2	1500	1497 <sup>8</sup>	48/C	0.65520	-0.57387
Dodecanal	112-54-9	1733	1720 <sup>9</sup>	64/C + PN	0.327958	0.514836
3-Phenyl-2-propenal (Cinnamaldehyde)	104-55-2	1971	1970 <sup>10</sup>	47/C	0.593404	-0.57756
Pentadecanal	2765-11-9	2051	2034 <sup>11</sup>	75/CS	-0.54517	0.532369
<i>Esters</i>						
Ethyl acetate <sup>d</sup>	141-78-6	870 <sup>e</sup>	885 <sup>3</sup>	55/C + PN	0.48448	0.25556
Ethyl pentanoate	539-82-2	1116	1128 <sup>5</sup>	22/C + PN	-0.11223	-0.35107
Ethyl hexanoate <sup>d</sup>	123-66-0	1238	1239 <sup>1</sup>	41/C	0.616105	-0.07434
Ethyl 2-hydroxypropanoate (Ethyl lactate) <sup>d</sup>	97-64-3	1339	1339 <sup>15</sup>	25/SB	-0.29787	-0.11957
Ethyl 2-hexenoate	27829-72-7	1357	13603	42/C	0.548963	-0.46340
Ethyl 2-hydroxy-3-methylbutanoate	2441-06-7	1403	1399 <sup>5</sup>	72/C	0.418373	-0.16490
Ethyl octanoate <sup>c</sup>	106-32-1	1430	1430 <sup>12</sup>	1/C + PN	-0.33871	-0.22142
Ethyl diethoxyacetate	6065-82-3	1475	1487 <sup>13</sup>	9/C	0.28883	-0.25463
Ethyl 3-hydroxybutanoate <sup>d</sup>	5405-41-4	1514	1524 <sup>12</sup>	46/C + PN	0.298164	0.267851
Ethyl nonanoate	123-29-5	1520	1526 <sup>14</sup>	24/C	0.125135	-0.50219
Methyl 3-hydroxypentanoate	56009-31-5	1552	1554 <sup>15</sup>	74/C	-0.12488	-0.19891
Diethyl propanedioate (Diethyl malonate)	105-53-3	1571	1572 <sup>14</sup>	7/C	0.818813	0.396271
Isoamyl lactate	19329-89-6	1619	1614 <sup>16</sup>	18/C + PN	-0.22687	-0.06927
Ethyl decanoate <sup>d</sup>	110-38-3	1644	1645 <sup>4</sup>	49/C	0.474401	-0.44519
3-Methylbutyl octanoate	2035-99-6	1668	1670 <sup>1</sup>	26/SB	0.771131	0.30796
Ethyl 3-hydroxyhexanoate	2305-25-1	1674	1675 <sup>15</sup>	71/C + PN	0.211391	0.400302
Diethyl succinate <sup>d</sup>	123-25-1	1686	1690 <sup>12</sup>	56/SB	0.714907	0.46451
Ethyl 9-decenoate	67233-91-4	1708	1711 <sup>3</sup>	57/C	0.230666	-0.56289
C12-Ester <sup>f</sup>	-	1715	*	13/C	0.08225	-0.68283
Diethyl pentanedioate	818-38-2	1780	1768 <sup>14</sup>	27/C + PN	0.703071	0.25396
2-Phenylethyl acetate <sup>d</sup>	103-45-7	1799	1800 <sup>6</sup>	20/CS	-0.48024	0.40208
2-Propanyl tetradecanoate (Isopropyl myristate)	110-27-0	1845	1823 <sup>8</sup>	68/C	0.526461	-0.53401
Ethyl dodecanoate	106-33-2	1856	1856 <sup>1</sup>	10/SB	-0.25918	-0.27991
3-Methylbutyl decanoate	2306-91-4	1868	1864 <sup>13</sup>	77/SB	-0.20530	0.15947
Diethyl 2-hydroxybutanedioate	7554-12-3	2038	2041 <sup>12</sup>	23/C + PN	0.781404	0.17367
C13-Ester <sup>f</sup>	-	2433 <sup>e</sup>	*	12/C + PN	0.35022	0.13167
<i>Ketones</i>						
Acetone	67-64-1	800 <sup>e</sup>	818 <sup>15</sup>	44/M	-0.30526	0.030090
2,3-Butanedione	431-03-8	1000	975 <sup>7</sup>	36/C + PN	0.681704	0.414729
3-Penten-2-one	625-33-2	1117	1128 <sup>17</sup>	33/SB	0.63295	0.25921
Cyclopentanone	120-92-3	1146	1150 <sup>18</sup>	43/C	0.435514	-0.13249
3-Hydroxy-2-butanone	513-86-0	1309	1307 <sup>19</sup>	76/C + PN	0.55950	0.238231
2-Methyl-2-cyclopenten-1-one	1120-73-6	1390	1397 <sup>19</sup>	61/C	0.05752	-0.83938
<i>Terpenes</i>						
4-Carene	29050-33-7	1119	1128 <sup>28</sup>	6/C + PN	0.58117	0.54070
p-Cymene	99-87-6	1301	1282 <sup>23</sup>	11/C + PN	0.818903	0.487373
Hotrienol	53834-70-1	1424	1449 <sup>12</sup>	60/M	-0.02890	0.349324
(Z)-Linalool oxide	5989-33-3	1430	1438 <sup>15</sup>	21/C + PN	0.806425	0.31496
Linalool	78-70-6	1554	1546 <sup>1</sup>	14/C + PN	0.71461	0.31392
Terpinen-4-ol <sup>d</sup>	562-74-3	1608	1608 <sup>4</sup>	17/C + PN	0.853037	0.30484
Nerol	106-25-2	1798	1797 <sup>13</sup>	63/C + PN	0.640133	0.299109
α-Calacorene	21391-99-1	1920	1928 <sup>22</sup>	3/C + PN	0.61936	0.42627
Geraniol	7392-19-0	1864	1862 <sup>23</sup>	65/CS	-0.54930	0.412224

Table 1 (continued)

Compound	CAS	LTPRI <sub>exp</sub> <sup>a</sup>	LTPRI <sub>lit</sub> <sup>b</sup>	PCA cluster <sup>c</sup>	PC1	PC2
Nerol oxide	1786–08-9	2377	2385 <sup>23</sup>	16/C	0.113384	–0.59649
β-Santalol	77–42-9	2430 <sup>e</sup>	2434 <sup>24</sup>	31/C + PN	0.536277	0.461651
<i>C13-Norisoprenoids</i>						
β-Damascenone	23726–93-4	1839	1841 <sup>12</sup>	67/CS	–0.52086	0.517262
<i>Phenols</i>						
4-Ethylguaiaicol	2785–89-9	2030	2033 <sup>13</sup>	19/C	0.09764	–0.75589
4-Ethylphenol	123–07-9	2204	2210 <sup>1</sup>	5/C + PN	0.40460	0.22378
<i>Pyrans</i>						
Pyran	–	2001	*	45/C + PN	0.532071	0.122081
<i>Lactones</i>						
δ-Valerolactone(tetrahydro-2(2H)-pyranone)	542–28-9	1589	1609 <sup>21</sup>	39/CS	–0.20526	0.65223
Butyrolactone(dihydro-2(3H)-furanone)	96–48-0	1680	1673 <sup>19</sup>	54/M	0.176041	–0.57734
3-Methyl-2(5H)-furanone	22122–36-7	1700	1683 <sup>20</sup>	38/CS	0.595810	–0.57784
γ-Decalactone(5-hexyldihydro-2(3H)-furanone)	706–14-9	2145	2138 <sup>18</sup>	69/CS	–0.53807	0.50919
<i>Furans</i>						
Furfural	98–01-1	1465	1460 <sup>13</sup>	8/CS	–0.63169	0.51564
5-(Hydroxymethyl)-2-furfural	67–47-0	2480 <sup>e</sup>	2485 <sup>15</sup>	51/CS	–0.07833	0.22135
Furan	–	2564	*	29/C + PN	0.58823	0.55773
<i>Sulphur compounds</i>						
Ethyl 3-(methylsulphanyl)propanoate	13327–56-5	1580	1574 <sup>25</sup>	30/C + PN	0.73181	0.45969
Dihydro-2(3H)-thiophenone	1003–10-7	1574	1553 <sup>26</sup>	37/CS	–0.41190	0.45813
Thiophene-2-carbaldehyde	98–03-3	1689	1684 <sup>27</sup>	73/C + PN	0.62076	0.45988

<sup>a</sup> linear temperature programmed retention index (LTPRI) calculated using *n*-alkanes (C<sub>9</sub>–C<sub>24</sub>) in polar × (DB-Wax) × medium polar (DB-17 ms) column set.

<sup>b</sup> LTPRI: literature LTPRI on a DB-WAX column or equivalent stationary phase: <sup>1</sup> Gurbuz, Rouseff, and Rouseff (2006); <sup>2</sup> Morales and Duque (2002); <sup>3</sup> Tao, Li, Wang, and Zhang (2008); <sup>4</sup> Dall'Asta et al. (2011); <sup>5</sup> Fan and Qian (2006); <sup>6</sup> Sampaio, Garruti, Franco, Janzantti, and Da Silva (2011); <sup>7</sup> Conduro, Verzera, Romeo, Ziino, and Conte (2008); <sup>8</sup> Selli and Cayhan (2009); <sup>9</sup> Lin and Rouseff (2001); <sup>10</sup> Meret, Brat, Mertz, Lebrun, and Guenata (2011); <sup>11</sup> Shiratsuchi, Shimoda, Imayoshi, Noda, and Osajimat (1994); <sup>12</sup> Selli, Canbas, Cabaroglu, Erten, and Gunata (2006); <sup>13</sup> Zhao, Xu, Li, Fan, and Jiang (2009); <sup>14</sup> Ferrari et al. (2004); <sup>15</sup> Umamo, Hagi, Nakahara, Shoji, and Shibamoto (1992); <sup>16</sup> Boido et al. (2003); <sup>17</sup> Garcia, Quek, Stevenson, and Winz (2011); <sup>18</sup> Frohlich, Duque, and Schreier (1989); <sup>19</sup> Sanz, Ansorena, Bello, and Cid (2001); <sup>20</sup> Simon, Esteruelas, Munoz, Cadahia, and Sanz (2009); <sup>21</sup> Lee and Shibamoto (2000); <sup>22</sup> Ferretti, Maggi, and Tirillini (2005); <sup>23</sup> Choi (2003); <sup>24</sup> Watcharananun, Cadwallader, Huangrak, Kim, and Lorjaroenphon (2009); <sup>25</sup> Du, Song, and Rouseff (2011); <sup>26</sup> Guntert et al. (1990); <sup>27</sup> Ledauphin et al. (2004); <sup>28</sup> Le Quéré and Latrasse (1990).

<sup>c</sup> Number refers to the compounds of Fig. 2b; CS: Chardonnay, C + PN: 50% Chardonnay + 50% Pinot Noir, CS: Cabernet Sauvignon, M: Merlot and SB: Sauvignon Blanc.

<sup>d</sup> Identified with standard compounds.

<sup>e</sup> Extrapolated LTPRI for compounds with LTPRI < 900 and > 2400.

<sup>f</sup> CX indicates that the molecule has X carbons in its structure.

\* LTPRI is not available in the literature.

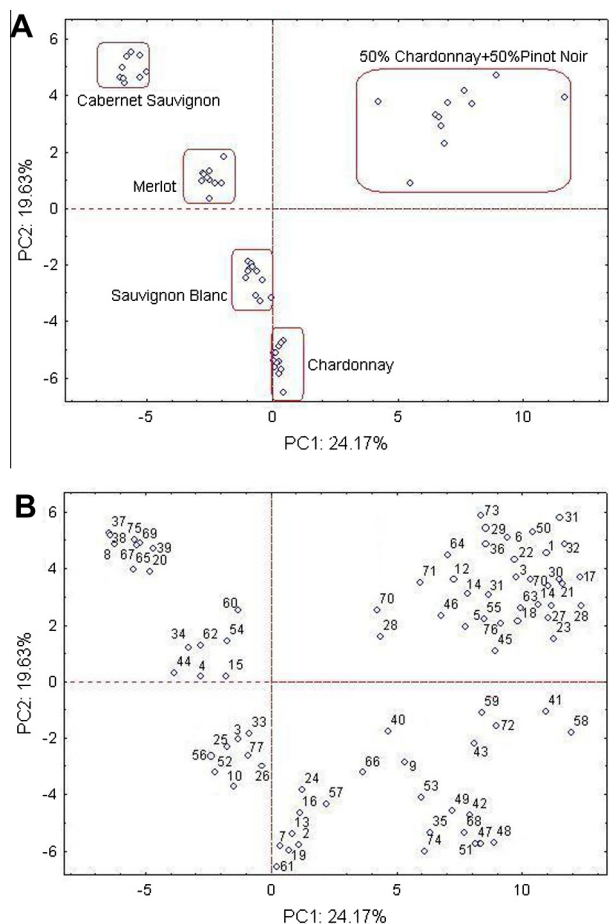
shown in the column corresponding to “PCA cluster” of Table 1. Compounds were arranged in Table 1 according to their chemical classes and in order of increasing LTPRI.

According to Fig. 2, Cabernet Sauvignon wines are characterised by the following tentatively identified compounds: 3-methyl-2(5H)-furanone, tetrahydro-2(2H)-pyranone, furfural, pentadecanal, γ-decalactone, geraniol, β-damascenone, and 2-phenylethylacetate. Merlot wines are associated with an alcohol with nine carbon atoms (C<sub>9</sub> alcohol), a di-alcohol with four carbon atoms (C<sub>4</sub> diol), dihydro-2(3H)-thiophenone, 1-hexanol, 5-(hydroxymethyl)-2-furfural and hotrienol. The compounds related to Sauvignon Blanc wines were ethyl dodecanoate, diethyl succinate, 2,3-butanediol, isoamyl octanoate, 3-methylbutyl decanoate, 3-penten-2-one, ethyl lactate and isoamyl lactate. Chardonnay wines are related to ethyl 9-decenoate, 2-methylcyclopentanone, diethyl malonate, isobutyric acid and nerol oxide. It is interesting to observe that most terpenes (4-carene, *p*-cymene, linalool oxide, β-santalol, terpinen-4-ol, nerol, linalool and α-calacorene) considered important for wine aroma and for differentiation of wine classes are related with 50% Chardonnay/50% Pinot Noir wines. A high dispersion is observed in PC1 for wines from 50% Chardonnay/50% Pinot Noir. Thus, in order to obtain a suitable classification model for assigning volatiles to samples, supervised learning pattern recognition method was applied.

It should be noted that, whereas PCA selects a direction that retains maximal structure among the data in a reduced dimension,

LDA selects a direction that achieves maximum separation between given sample classes (Berrueta, Alonso-Salces, & Heberger, 2007). After PCA, LDA was applied to select the most useful variables in the differentiation between grape varieties used in wine production.

LDA classification model was constructed by applying a stepwise variable selection procedure, so that the most significant variables were selected using Wilks' Lambda as a selection criterion. The selection algorithm Wilks' Lambda is a measure of discrimination between groups. The larger the dispersion among groups the lower the Wilks' Lambda value and the greater the significance of that compound for the classification method (Berrueta et al., 2007). The first variable selected for the discrimination model (Table 2) was ethyl 9-decenoate, because it showed the highest *F*-value (17.63) and, consequently, the lowest Wilks' Lambda value (0.3440). According to this criterion, each selected variable will contribute to a new matrix combination and, as a consequence, *F*-values and the order of selection will be changed. This strategy resulted in a considerable reduction of the dimensionality of the information, because it led to the selection of only 12 variables that are considered most important for the differentiation of wine samples. The 12 volatile compounds selected for LDA were 2,3-butanediol, 4-carene, 3-penten-2-one, diethyl succinate, β-santalol, diethyl malonate, dihydro-2(3H)-thiophenone, tetrahydro-2(2H)-pyranone, alcohol with nine carbon atoms (C<sub>9</sub> alcohol), 3-methyl-2(5H)-furanone, ethyl 9-decenoate and nerol. Each of these



**Fig. 2.** PC1 vs. PC2 scatter plot of the main sources of variability between Chardonnay (C), Merlot (M), CS (Cabernet Sauvignon), SB (Sauvignon Blanc) and C + PN (50% Chardonnay/50% Pinot Noir) wines; (a) distinction between the samples and (b) relation between volatile compounds and the type of wine. The numbers used in b correspond to those shown in the column corresponding to "PCA cluster" of Table 1.

**Table 2**

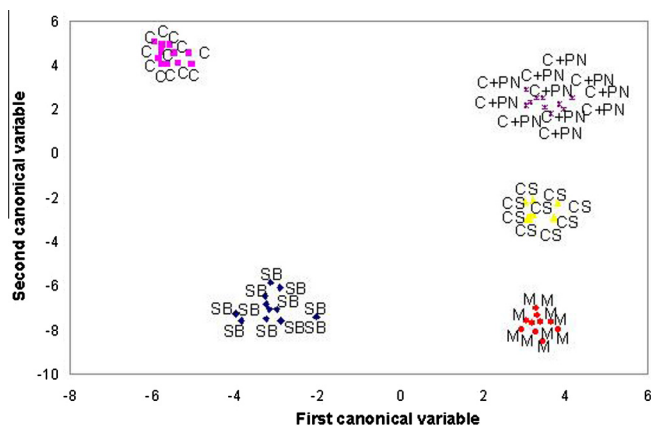
Selection test of discriminating variables between the 14 principal components through the use of Wilks' Lambda and *F*-statistical factor for obtaining a model of differentiation of wines according to grape cultivar used to produce them.

Variable	Wilks' Lambda	<i>F</i>	<i>p</i> <sup>b</sup>
2,3-Butanediol	0.73	3.32	0.02
4-Carene	0.58	8.76	0.00
3-Penten-2-one	0.78	2.52	0.05
Diethyl succinate	0.89	1.03	0.40
β-Santalol	0.36	16.42	0.00
Diethyl malonate	0.45	11.18	0.00
Dihydro-2(3H)-thiophenone	0.93	0.68	0.60
Tetrahydro-2(2H)-pyranone	0.80	2.24	0.08
Furfural	0.99	0.05	0.00
C9-Alcohol <sup>a</sup>	0.60	6.01	0.34
3-Methyl-2(5H)-furanone	0.96	0.29	0.87
Ethyl 9-decenoate	0.34	17.63	0.00
Nerol	0.93	0.66	0.61
Octanal	0.98	0.09	0.30

<sup>a</sup> C9: 9 carbon atoms in the molecule structure.

<sup>b</sup> *p*: Significance level. The variables are discriminant at *p* < 0.05.

discriminant variables represents a canonical variable that turns out to be linear combinations of the original predictors. Each canonical variable represents the direction with maximum separation among classes (Berrueta et al., 2007). The reliability of the obtained classification model was graphically confirmed by the plot



**Fig. 3.** Plot of samples on the plane defined by two canonical discriminant variables after performing LDA. Wine samples are shown by their grape variety: C (Chardonnay), M (Merlot), CS (Cabernet Sauvignon), SB (Sauvignon Blanc) and C + PN (50% Chardonnay/50% Pinot Noir).

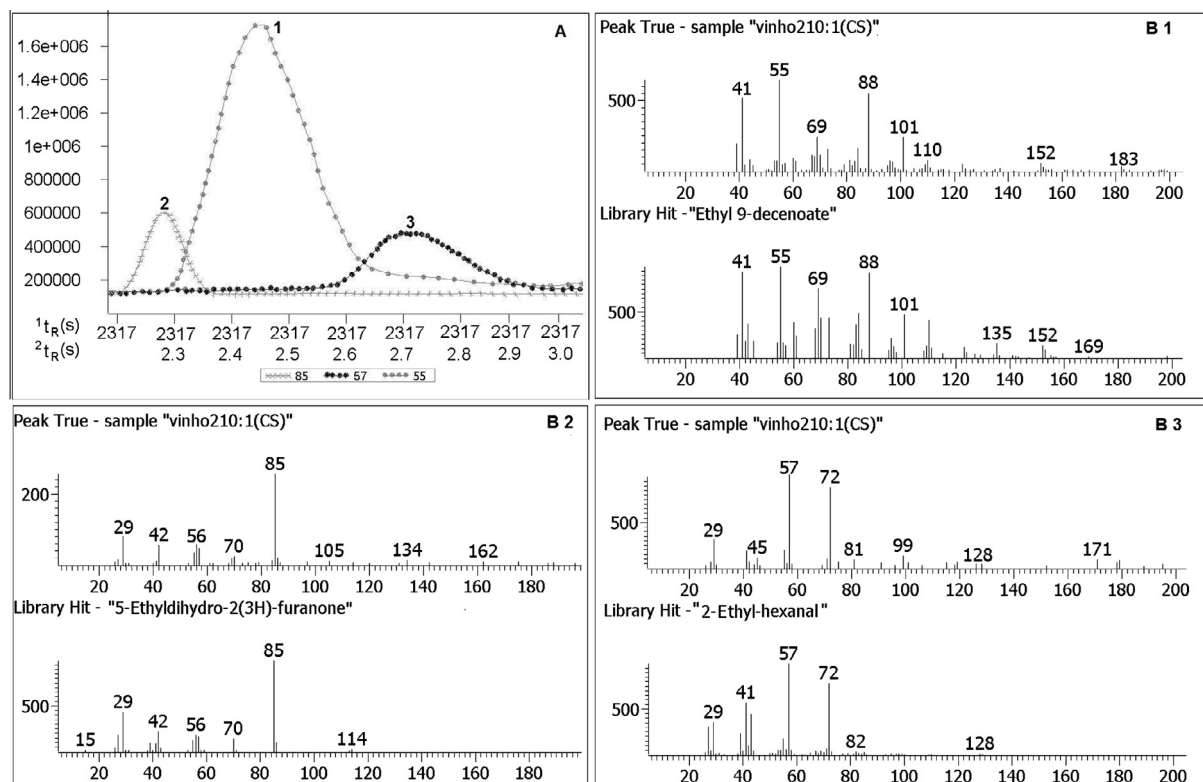
obtained when the samples were projected on the space defined by the first two canonical variables (Fig. 3). A clear separation between the five types of wines was observed. The white wines (Chardonnay and Sauvignon Blanc) were separated from other wines by the first canonical variable, while the red wines (Merlot and Cabernet Sauvignon) and the wine produced with white and red grapes (50% Chardonnay/50% Pinot Noir) were separated from white wines by the second canonical variable.

In order to determine the model stability, the model achieved was validated by cross-validation procedure through a test using samples not used to construct the model. The use of 12 volatile compounds resulted in 100% recognition ability for five wine groups, according to the grape variety used in their elaboration.

Zhang et al. (2010) analysed red Chinese wines from Cabernet Sauvignon, Merlot and Cabernet Gernischt varieties using HS-SPME-GC/MS. ANOVA, PCA and LDA were used to develop a model to discriminate the wines according to the grape variety employed in their elaboration. The model showed 65% recognition ability for the commercial wines. Câmara, Alves and Marques (2006) also used HS-SPME-GC/MS and ANOVA, PCA and LDA to differentiate wines from Boal, Malvazia, Sercial and Verdelho white grape varieties according to their volatile compounds. The prediction ability was 96.4% using nineteen compounds.

The contribution of volatile compounds to the flavour of wines has been investigated in various studies, in order to establish relationships between volatile compounds and sensory attributes associated with both positive and defective perceptions (García-Carpintero, Sanchez-Palomo, Gallego, & Gonzalez-Viñas, 2011a; García-Carpintero, Gallego, Sanchez-Palomo, & Gonzalez-Viñas, 2012; Brenna, Fuganti, & Serra, 2003). Even though quantitative analysis would be necessary for a precise definition of the influence of volatile compounds to wine aroma, this work shows the contribution of 12 volatile compounds to differentiate wines according to the grape variety used for wine elaboration.

The aroma and occurrence of some of these 12 volatile components in wines are not so commonly reported in the scientific literature. The odours of tetrahydro-2(2H)-pyranone and 3-methyl-2(5H)-furanone have been reported as caramel like and they were found in Baga red wine (Rocha, Rodrigues, Coutinho, Delgadillo, & Coimbra, 2004). 3-Methyl-2(5H)-furanone, also known as α-methyl-γ-crotonolactone has been also tentatively identified in Mencía red wine of the Galicia region (Pena, Barciela, Herrero, & Garcia-Martin, 2005). Tetrahydro-2(2H)-pyranone, also known as δ-valerolactone has been reported in noble rotted botrytised Aszú grape berries (Miklosy & Kerenyi, 2004).



**Fig. 4.** Example of spectral deconvolution for identification of volatile compounds extracted by HS-SPME of Cabernet Sauvignon wines analysed by GC × GC/TOFMS. (A) Part of the chromatogram of modulated peaks of three volatile compounds that coeluted in  $^1D$  and partially coeluted in  $^2D$ : (1) blue line: ethyl 9-decanoate,  $m/z$  55, (2) red line: 5-ethylidihydro-2(3H)-furanone,  $m/z$  85, (3) black line, 2-ethylhexanal,  $m/z$  57; (B) mass spectra of the deconvoluted compounds of A and the respective mass spectra of NIST2005 library.

**Table 3**

Information about the 12 volatile compounds considered by the chemometric analyses as the most important for the differentiation of Chardonnay, Merlot, Cabernet Sauvignon, Sauvignon Blanc and 50% Chardonnay/50% Pinot Noir wines.

Compound <sup>a</sup>	Aroma	$^1D$ Coelution	Wine
Diethyl succinate	Vinous <sup>1</sup>	No	Sauvignon Blanc
2,3-Butanediol	Fruity <sup>1</sup>	Ethyl 3-hydroxy pentanoate	Sauvignon Blanc
Nerol	Orange flower <sup>2</sup> , rose <sup>3</sup>	Methyl dodecanoate	Chardonnay/Pinot Noir
3-Penten-2-one	Fruity, spicy <sup>4</sup>	No	Sauvignon Blanc
Diethyl malonate	Ripe fruit, peach, cut grass <sup>1</sup>	5-Methyl-2-furfural	Chardonnay
$\beta$ -Santalol	Woody <sup>5</sup>	(E)-4-Methyl-3-hepten-2-one	Chardonnay/Pinot Noir
Ethyl 9-decanoate	Fruity <sup>6</sup>	5-Ethylidihydro-2(3H)-furanone	
Alcohol-C9	–	2-Ethylhexanal	
4-Carene <sup>b</sup>	–	2-Phenylethyl acetate	Chardonnay
Tetrahydro-2(2H)-pyranone <sup>b</sup>	–	2-Methoxyphenol	Chardonnay/Pinot Noir
Dihydro-2(3H)-thiophenone <sup>b</sup>	–	No	Cabernet Sauvignon
3-Methyl-2(5H)-furanone <sup>b</sup>	–	No	Merlot
			Cabernet Sauvignon

<sup>a</sup> Compounds tentatively identified by comparison of experimental linear temperature programmed retention index (LTPRI) with retention indices reported in the literature for 1D-GC LTPRI (data showed in Table 1) and comparison of experimental mass spectra with NIST 2005 mass spectral library.

<sup>b</sup> No information about aroma was found in the literature. <sup>1</sup> Garcia-Carpintero et al. (2011)a; <sup>2</sup> Genovese, Gambuti, Piombino, and Moio (2007); <sup>3</sup> Ribéreau-Gayon, Glories, and Maujean (2000); <sup>4</sup> Zhang et al. (2010); <sup>5</sup> Brenna et al. (2003); <sup>6</sup> Zhao et al. (2011).

4-Carene had already been tentatively identified in wines produced with Falanghina (*Vitis vinifera* L.) grapes of Campania region (Italy) (Nasi, Ferranti, Amato, & Chianese, 2008). Finally, the odour of dihydro-2(3H)-thiophenone, also known as 2-oxothiolane or 4-thiobutyrolactone was formerly described in an FAO/WHO Compendium as possessing a burnt like aroma (FAO/WHO, 2010), but there is no information of the occurrence of this compound in wines. This is the first time this component is tentatively identified in wine; however, other compounds containing a thiophenone ring have been identified in red

wines (Aznar, Lopez, Cacho, & Ferreira, 2001; Welke et al., 2012a). Further confirmation of its identity, using a standard compound will be necessary.

The presence of the enantiomers of nerol was not investigated, however the contribution of this compound to wine aroma will depend on its chiral form in wine: (+) green and floral or (–) green, spicy, and geranium (Brenna et al., 2003). Considering the 12 volatiles considered as discriminants by the model constructed using Fisher ratio, PCA and LDA, six of them coeluted in  $^1D$  with other compounds and were tentatively identified using GC × GC/TOFMS.

One of the coelutions involved diethyl propanedioate (diethyl malonate,  $^1t_R = 37.92$  min,  $^2t_R = 3.63$  s) and its aroma is described as over-ripe, peach or cut grass (García-Carpintero, Sanchez-Palomo, & Gonzalez-Viñas, 2011b). This compound was separated from 5-methyl-2-furfural ( $^1t_R = 37.92$  min,  $^2t_R = 2.04$  s) only in the second dimension.  $\beta$ -Santalol, that contributes to a woody aroma, ( $^1t_R = 53.10$  min,  $^2t_R = 2.17$  s) also co-eluted with (*E*)-4-methyl-3-hepten-2-one ( $^1t_R = 53.10$  min,  $^2t_R = 3.45$  s), which is similarly associated with woody aroma attributes (Brenna et al., 2003).

Ethyl 9-decenoate contributes to wine aroma with fruity notes (Zhao, Wang, Li, Pei, & Liu, 2011). This compound coeluted in the first dimension and partially coeluted in the second dimension with 5-ethylidihydro-2(3*H*)-furanone and 2-ethylhexanal. Fig. 4A shows the superimposed chromatographic peaks. Spectral deconvolution based on mass spectra differences is quite useful in this case, especially to separate ethyl 9-decenoate from 5-ethylidihydro-2(3*H*)-furanone and 2-ethylhexanal, because they also co-elute in the second dimension. In Fig. 4B, mass spectra of the three compounds are compared with mass spectra from the NIST library.

The discriminant volatile compounds related to wines produced with five different grape cultivars are shown in Table 3. It is interesting to observe that Chardonnay/Pinot Noir wines were differentiated from other wines only by terpenes, including nerol,  $\beta$ -santalol and 4-carene. Two esters (diethyl malonate and ethyl 9-decenoate) were considered discriminants for Chardonnay wines. Cabernet Sauvignon wines were differentiated only by the furanones tetrahydro-2(2*H*)-pyranone and 3-methyl-2(5*H*)-furanone.

#### 4. Conclusions

The use of HS-GC  $\times$  GC/TOFMS associated with multivariate analysis (Fisher ratio, PCA and LDA) to investigate the volatile composition of wines proved to be an interesting approach to differentiate wines according to their original grape cultivars and also to find potential markers of these grape cultivars. These results may help the wine industry to develop more effective quality control methods, in order to produce added value wines. Twelve volatile compounds chosen from a large set of original variables, obtained by GC  $\times$  GC/TOFMS, were enough to discriminate 100% of wines elaborated from five different grapes. Among these 12 compounds, some partially coeluted with other components in the first chromatographic dimension ( $^1D$ ) and were more properly assigned, due to the extra selectivity provided by the second chromatographic dimension and spectral deconvolution.

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