Analytical Methods

Characterization of Muscat wines aroma evolution using comprehensive gas chromatography followed by a post-analytic approach to 2D contour plots comparison

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A B S T R A C T

This study presents the application of a headspace solid-phase microextraction (HS-SPME) method on the analysis of Muscat-based wines volatiles by comprehensive two-dimensional gas chromatography (GC×GC) and Time-Of-Flight mass spectrometry (TOF-MS). The aroma patterns were established for different samples of Asti Spumante and Moscato d’Asti wines, stored in bottles for 6 months at different temperatures. Wines stored at 5 °C for 6 months did not show significant changes in flavor; otherwise, the samples stored at 15 and 25 °C, showed a significant decrease in linalool, β-damascenone, ethyl hexanoate, and ethyl octanoate levels. In these last samples, α-terpineol, hotrienol, nerol oxide, furanic linalool oxides A/B and rose oxide concentrations significantly increased. A mathematical approach was developed and applied to raw data exported after the chromatographic course, in order (i) to normalise different 2D chromatograms, permitting their direct comparison and (ii) to automatically identify and calculate from pixel-to-pixel re-designed 2D chromatograms any differences among key volatile compounds.

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1. Introduction

Volatile compounds play an important role in the organoleptic characteristic of wines. Several hundreds of compounds from very different chemical families account for wine flavor (Gunata, Bayonove, Baumes, & Cordonnier, 1986; Schneider, Razungles, Augier, & Baumes, 2001). Such compounds come from grape (terpenes and terpenoids; C13-norisoprenoids), from fermentation processes (aliphatic alcohols, ethers, acids, aldehydes, etc.) and from ageing (decomposition compounds from carotenoids and carbohydrates, ethyl-esters). Other volatile compounds can be considered imperfection or “off-flavor” of wine (cork tones, oxidise, murine, acetate, hydrogen sulfide) (Lizarraga, Irigoyen, Belsue, & Ezpeleta, 2004). As well known, aroma production in wine is influenced by several factors: environment, grape variety, grape berries ripeness, fermentation conditions, biological factors (firstly the use of a peculiar Saccharomyces cerevisiae strain). In fact, the use of different starter fungal strains allows producing different volatile profiles in wines, even on the same grape variety and exploiting the same technology/process. The volatile aroma is of primary interest in order to assess the quality of a wine, particularly concerning the white dessert wines like Muscat-based ones.

In fact, the “fruity” perception is strictly correlated to the perception of the “freshness” in these wines. Therefore, the oxidation process, or simply the loss in fruity aroma compounds, may significantly affect the perfect balance of a “pleasant wine” flavor.

The characteristic aroma of Muscat wine can be attributed to the presence of different terpenic derivatives: linalool, nerol, geraniol, α-terpineol, citronellol and four derivatives of linalool (Ribereau-Gayon, Boidron, & Terrier, 1975). As previously observed in literature, in one-year aged wine samples, linalool, nerol and geraniol decrease respect to the young Muscat wine to become less important regarding the aroma. On the contrary, α-terpineol, hotrienol, nerol oxide, furanic linalool oxides A/B and rose oxide concentration significantly increase, confirming an oxidative evolution of the aroma (Usseglio-Tomasset, 1983).

Several methods and techniques are reported in scientific literature for the study of the wine volatile aroma: GC, GC–MS, HPLC–MS, sensorial analysis often coupled with multivariate statistical analysis (PCA) (Aznar, Lopez, Cacho, & Ferreira, 2001; Lopez, Ezepeleta, Sanchez, Cacho, & Ferreira, 2004). In all these approaches, the sample preparation is a fundamental step for a representative and significant analysis.

Solid Phase Micro Extraction (SPME) is simple, fast, sensitive, easy-to-automate, solvent-free and can be considered an excellent alternative to all considered methods. The heterogeneous composition of aroma compounds requires different fibre coating phases or...
a multi-phase coated fibre (Liu, Zeng, & Tian, 2005). Therefore, the interpretation of the volatiles aroma is often a complex post-analytical step.

The use of SPME for the study of wines volatiles is well described (Rodriguez-Bencomo, Conde, Garcia-Montelongo, & Perez-Trujillo, 2003), but the simultaneous use of comprehensive GC is not still largely exploited in this field. Comprehensive multidimensional gas chromatography (GC×GC) represents a relatively new technique for the characterization of volatiles complex profiles (Ryan et al., 2004). Since GC×GC technique generates very narrow chromatographic peaks emerging from second column (second dimension), conventional technology such as “old generation quadrupole” (qMS) spectrometers have in some cases too-slow scan rate (up to 25–30 Hz acquisition rate). However, the TOF technology was introduced in order to provide much faster spectral acquisition, up to 500 Hz. Thus, 50–100 spectra per second acquisition rate allows about 10–20 spectra per peak to be obtained, consistent with requirements for accurate reconstruction of the 2D chromatogram, also in the case of the quantitative evaluation (Ryan et al., 2004; Shelle, Marriott, & Morrison, 2001).

The effective limit of this powerful technique is related to the complex post-analysis step: the automatic recognition of such complex peak pattern is not easy to obtain. Some solutions for the direct interpretation of the 2D chromatograms were recently suggested (Mondello et al., 2008). The Computer Vision Image Analysis (CVIA) as well other mathematical strategies, should be make more friendly the post-analysis processing of complex fingerprints (Russ, 2007).

First aim of our work was the conventional application of a head-space analyses (HS-SPME technique) coupled with GC×GC/TOF-MS to the volatiles profiling in different Muscat-based wines from Piedmont (Northern Italy). Secondly, using this chromatographic method, we intended to study the evolution of the volatile aroma during the ageing of “Asti Spumante” and “Moscato d’Asti” wines. For this purpose, twenty-four wine samples 6.5% v/v (twelve of Asti and twelve of Moscato, respectively) were used. All standard chemicals used were purchased from Sigma–Aldrich (Milan, Italy). Water was obtained by Milli-Q instrument (Millipore Corp., Bedford, MA, USA). In this work, the influence of storage temperature on the sensory profile of “Asti Spumante” and “Moscato d’Asti” wines was investigated. For this purpose, twenty-four wine samples 6.5% v/v (twelve of Asti and twelve of Moscato, respectively) were used. Three bottles of each typology, designated “control”, were analysed at time 0 (immediately after the wines were considered ready to be commercialised); then the other wine samples, divided in groups (n = 3), were stored at three different storage temperatures for 6 months (5, 15, and 25 °C, respectively).

The model wines were produced following the standard protocols used for the “Asti Spumante” and “Moscato d’Asti” wines, so using a modified version of the Charmat process. After the grapes were harvested, these were crushed and pressed, with the must filtered and then transferred to large tanks at near-freezing temperatures to prevent fermentation from beginning. The tanks were sealed and pressurized, and then the temperature was raised to allow fermentation to begin. Once the desired alcohol level was reached, the wine was rapidly chilled to stop fermentation, filtered, bottled and corked.

The principal difference between “Asti Spumante” and “Moscato d’Asti” is that fermentation of the latter is stopped sooner and the wine is thus less effervescent because less CO2 is produced and tends to have even lower alcohol. In addition, Asti Spumante is normally sealed with a cork wire cage, while Moscato d’Asti typically has a standard cork, as pressure is less.

2.2. Head-space SPME extraction

A Supelco fibre holder (Bellefonte, PA, USA) and a 50/30-μm divinylbenzene–carboxen–poly(dimethylsiloxane) (DVB–CAR–PDMS) fibre were used. This triphasic fibre has been previously described as the most suitable for adsorbing a large range of volatile compounds from wines and other alcoholic beverages (Rodrigues, Caldeira, & Câmara, 2008). 1 mL of wine was added with 4 mL of water (HPLC-grade) into a 20 mL glass vial, and maintained for 10 min at 35 °C. An exposition of 20 min was applied for the extraction of all samples. After equilibration, the fibre was removed from the sample and the analytes were thermally desorbed in the injector port of the GC.

2.3. GC×GC/TOF-MS

2.3.1. System

All chromatographic analyses were performed using a Trace 2DGC+ modified Tempus TOF mass detector from Thermo Scientific (Rodano, Milan, Italy). The apparatus used was equipped with split/splitless injector. The first column was a DB-5 (30 m × 0.25 mm i.d. × 0.25 μm film thickness); the second column was a DB-225 (1 m × 0.1 mm i.d. × 0.1 μm film thickness). Both columns were from J&W Scientific (Folsom, CA, USA). The columns, both contained in a single oven, were connected using a glass press-fit connector. The cryogenic modulation was obtained with a CO2 dual-jet system, installed at the head of the second dimension column. This device was controlled by a 2DGC on-off Wave Modulator (Thermo Electron).

2.3.2. Chromatographic conditions

The separation was performed using the following temperature program: initial temperature 50 °C, kept for 5 min, ramped at 5 °C/min to 230 °C and held for 15 min. Analysis was performed at 0.8 ml/min constant flow; helium was used as carrier gas. Due to the signal enhancement obtained by modulation of flow eluting from the first column into the second, characterised by a smaller diameter, analysis was performed with 10:1 split ratio, in order to avoid saturation of column 2. Desorption of compounds from fibres was performed in split-injector. A modulation of 2.5 s was applied on the dual-jet modulator. The transfer line temperature was 230 °C and the MS source temperature was 220 °C. The mass spectral data acquisition rate was 25 Hz for all the analyses. The data were collected over a mass range of 35–500 m/z. Total Ion Current chromatograms (TIC) were finally processed using data processing software Xcalibur 1.4 SR1. Peak identification was based on comparison of their mass spectra with those of pure standard from
Sigma–Aldrich and/or with those reported by the MainLib NIST98 commercial library. Mass spectral match factor, similarity >850, was used to decide whether a peak was correctly identified or not. Furthermore, more careful attention was provided by the manual inspection of the mass spectra and/or by the use of additional data, such as the experimentally determined retention index (RI) values and the values reported in the bibliography for chromatographic columns similar to that used as the first dimension column in the present work. For the determination of the Retention Index (RI), calculated on the first dimension (DB5 column), a C₆–C₂₄ n-alkanes series was used. Semi-quantitative data of the aroma compounds were calculated by relating the peak areas of volatiles to the peak area of BHT (Butylated hydroxytoluene) used as internal standard. The concentration of volatiles were expressed as µg/L.

2.3.3. Raw data processing

Mathematica® software (Wolfram Research Inc., Champaign, IL, USA), version 7.0 was used for the processing of 2D chromatograms data. First the data of the 2D contour were exported from ChromCard Thermo software. Secondly, a normalisation procedure was exploited in order to normalise intensity among different chromatograms and obtain comparable images. Finally, a simple procedure was developed in order to automatically compare (pixel-to-pixel approach) the chromatograms.

2.4. Statistical analysis

Results were expressed as mean ± standard deviation (SD) of at least three independent experiments. Differences among samples were estimated by analysis of variance (ANOVA) followed by Tukey’s “Honest Significant Difference”; the statistical significance level was set to 0.05. The principal component analysis (PCA) was also performed, considering both the evolution of wine aroma during ageing, and the differences among Asti and Moscato samples. All statistical analyses were performed using the free statistical software R 2.10.0 version (R Development Core Team, 2008); for the PCA, the FactoMineR package was employed.

3. Results and discussion

3.1. Evolution of Muscat aroma during ageing

A HS-SPME-GC×GC/TOF-MS method was applied for the comprehensive separation of the volatile aroma of two Italian Muscat wines stored in bottles for 6 months at different temperatures (5, 15, and 25 °C). As reported in different researches on wine volatile aroma, SPME technique represent a good “trap” for major and minor volatiles of wine, both polar and non-polar organic compounds, also showing peculiar behaviour in some volatile compounds not detectable in liquid–liquid sample preparation.

Comprehensive 2D-GC coupled with time of flight mass detection is an emerging technology that provides considerably more chemical selectivity than GC/MS, and thus should be ideally suited to the analysis of complex matrices, like food and beverage aromas.

This hyphenated chromatographic approach was firstly evaluated studying 65 commercial Italian Muscat wines, in order to optimise the separation of the principal class of aroma compounds (Bordiga, Coïsson, Piana, Travaglia, & Arlorio, 2009).

The complexity of the volatile profiles of wines analysed did not permit the identification with reasonable certainty (for comparison with databases and by comparison with molecular standards) of all

![Fig. 1. HS-SPME-GC×GC-TOF-MS chromatogram Asti and Moscato wines. The chromatograms show the presence of the main families of compounds typical of the muscat wines; (A) terpenes; (B) alcohols; (C) esters; and (D) acids.](image)
compounds detected and separated using this powerful approach. Despite this, considering series of standard compounds as well as some libraries data, it was possible to identify in the complex fingerprint the presence of the main families of compounds (esters, terpenes, alcohols, acids) typical of white wines. In Fig. 1 is reported the typical bi-dimensional chromatographic profile of Italian muscat-based wines. These data fit with previous works on white wine volatile flavor obtained using GC–MS (Rocha, Coelho, Zrostlikova, Delgadillo, & Coimbra, 2007). Some “off-flavours”, responsible of wine imperfections described as cork tones, plastic, mushroom were also identified; for example, 2,4,6-trichloroanisole (TCA) and 2,4,6-tribromoanisole (TBA) were detected.

The optimised chromatographic method was then applied in this model study to evaluate the aroma profile of muscat-based wines under different storage conditions. Results for quality/quantity characterization of aromatic profile of Asti and Moscato wines are reported in Table 1. In order to summarize the complex dataset, the identified compounds were grouped in chemical subclasses and then analysed by means of multivariate statistical analysis (PCA).

3.1.1. Esters

The content in esters of ageing wines is the result of their esterification/hydrolysis balance, the high concentrations in ethanol particularly favouring the esterification process of ethyl esters, such as ethyl acetate, ethyl hexanoate, and ethyl octanoate. Esters are an important component of wine aroma, especially in muscat-based wines for their fruity and floral notes. The major ester identified in control wines was isoamyl acetate, particularly abundant in Moscato samples. Moscato wines presented high concentrations also of ethyl hexanoate, ethyl octanoate and β-phenethyl acetate.

Ethyl hexanoate, ethyl hydroxybutanoate, ethyl octanoate and β-phenethyl acetate are very sensitive to higher temperature and during ageing generally decrease their contents. This reduction is responsible for the loss of a major aromatic note.

Results obtained from the principal component analysis of esters quantification data are showed in Fig. 2. The first two dimensions resume more than 90% of the total variance of the dataset. The first PC (PC1), which explains 66.7% of the total variance, opposes samples subjected to different storage conditions. Excepting for methyl octanoate, all the compounds were significantly correlated to PC1. Particularly, it can be observed that variables negatively correlated to the first dimension (ethyl acetate, ethyl lactate, diethyl succinate, 2,6-dimethyl-1,7-octadiene-3,6-diol and geranyl ethyl ether) increase in wines stored at the highest temperatures, thereby determining both modification of wine fruity notes and loss of freshness. Conversely, compounds positively correlated to PC1 (among them, the most significant were ethyl butanoate, hexyl acetate and isoamyl acetate) well characterise the “control” wines and those stored at 5 °C.

PC2 (24.5% of the total variance) permits to discriminate Asti and Moscato samples, showing a significant correlation with the qualitative variable “wine typology” (*R*^2^ = 0.823, *p* = 9 × 10^-10^); the highest values on PC2 were observed for Moscato wines. Compounds positively correlated with the second PC are in order of significance ethyl octanoate > ethyl lactate > 2,6-dimethyl-1,7-octadiene-3,6-diol > 2-phenylethyl acetate > ethyl hexanoate, while those negatively correlated were methyl octanoate > 2-phenylethyl butanoate > 2-phenylethyl formate > 2-phenylethyl propionate > ethyl hexanoate.

3.1.2. Terpenes

Terpenic alcohols are volatile compounds, such as, for example, linalool, nerol and geraniol; these compounds are typical of white wines from aromatic grapes (e.g., Muscat, Malvasia), contributing to their characteristic fruity aroma. The storage in bottle can result in natural changes in the content of these aromatic compounds, leading to their conversion in the respective oxides (derivatives of corresponding pyranic and furanic forms).

The main terpenes identified in Asti and Moscato wines are reported in Table 1. Considering the “control” samples, similar concentrations in Muscat wines had been previously reported (Papadopoulou & Roussis, 2001).

Linalool, the major terpenic alcohol identified in control wines, is the compound that principally contributes to the muscat character of wine (Clarke & Bakker, 2004), although the other odour active monoterpenols may contribute to this aroma too (Girard, Fukumoto, Mazza, Delaquis, & Ewert, 2002). Among terpenic compounds, linalool content strongly decreased in wines stored at high temperatures (15 and 25 °C) while it remained essentially unchanged at 5 °C, as previously reported by Di Stefano and Castino (1983). As well established, linalool is very sensitive to high temperature and its molecule undergoes quickly to generate furanic and piranic derivates (linalool oxides), characterised by lower fruity notes. These evidences are confirmed by results obtained applying PCA to the terpenes dataset. Compounds negatively correlated to PC1 (80.8% of the total variance), i.e., in order of significance nerol, β-damascenone, geraniol, 4-terpineol, linalool and citronellol, are associated to control wines and samples stored at 5 °C, while all the oxidised forms of terpenes positively correlate to the first dimension, characterising wines at the highest values of PC1 (principally samples stored at 25 °C) (Fig. 2).

PC2 (11.6% of the total variance) opposes the wine typologies; compounds that showed a positive significant correlation with PC2 were citronellol and (E)-linalool oxide (trans-furan) (prevalent in Asti samples), while (E)-linalool oxide (trans-pyran) and (Z)-linalool oxide (cis-pyran) (more characteristics for Moscato samples) were inversely correlated. Nevertheless, terpenic alcohols were not able to clearly discriminated Asti from Moscato wines; in fact their corresponding 95% confidence ellipses were partially overlapped.

Considering other terpenic alcohols, α-terpineol was constantly increased during ageing to become the quantitatively most important terpenic alcohol in the high temperature storage. α-Terpineol probably originates from an interconversion of terpenes such as linalool and nerol (which are structural and geometric isomers) and a subsequent acid-catalysed cyclization. α-Terpineol (as monoterpenic oxides) has a lower olfactory threshold and its accumulation in the Muscat wines is certainly correlated with the decrease or loss of the typical fruity and floral aroma. As reported in other works (Gunata, Bayonove, Baumes, & Cordonnier, 1985), this phenomenon is observed and occurs during the oxidation-ageing of these wines. It is reasonable to assume that these changes occur even during normal storage in bottle, naturally evolving towards the accumulation of oxidised substances, although in traces.

Hotrienol and nerol oxide were also identified on the 2D contour plot. Their content increased in wines stored at high temperature, confirming the acceleration of oxidation processes in wines. These compounds, identified by classical GC–MS techniques (Di Stefano & Castino, 1983), as well as demonstrated by testing sensory, may be important for the quality definition of muscat product and for the further optimization of its production processes.

Even if present in low amounts, (±) cis-rose oxides have a particularly high score, and play an important role in the aroma perception. According to Di Stefano and Castino (1983) (±) cis-rose oxides increased at higher storage temperatures.

3.1.3. Acids and alcohols

Acids and alcohols contents were significantly affected by temperature during storage. It was possible to highlight a general trend with regard to the organic acids, characterised by notes of rancid notes, which tend to increase their content by making negative notes to wine aroma. On the basis of the principal component analysis,
<table>
<thead>
<tr>
<th>Compound</th>
<th>Odour</th>
<th>RI (DB-5)*</th>
<th>RI Lit*</th>
<th>Identif*</th>
<th>Ester (ASTI 6.5 % v/v)</th>
<th>Alcohols</th>
<th>Terpenes</th>
<th>MOSCATO (6.5 % v/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Control</td>
<td>5 °C</td>
<td>15 °C</td>
<td>25 °C</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>µg/L</td>
<td>µg/L</td>
<td>µg/L</td>
<td>µg/L</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>Pineapple</td>
<td>610</td>
<td>612</td>
<td>M, N, O</td>
<td>469 ± 4.75</td>
<td>500 ± 13.0</td>
<td>623 ± 4.58</td>
<td>703 ± 5.29</td>
</tr>
<tr>
<td>Ethyl lactate</td>
<td>Fruit</td>
<td>787</td>
<td>790</td>
<td>M, N, O</td>
<td>355 ± 2.51</td>
<td>390 ± 8.50</td>
<td>429 ± 7.37</td>
<td>533 ± 1.52</td>
</tr>
<tr>
<td>Ethyl butanoate</td>
<td>Caramel</td>
<td>808</td>
<td>806</td>
<td>N, O</td>
<td>240 ± 3.60</td>
<td>227 ± 7.21</td>
<td>164 ± 8.73</td>
<td>87.1 ± 2.08</td>
</tr>
<tr>
<td>Isomyl acetate</td>
<td>Banana</td>
<td>876</td>
<td>861</td>
<td>M, N, O</td>
<td>564 ± 7.02</td>
<td>534 ± 6.11</td>
<td>319 ± 6.24</td>
<td>118 ± 3.78</td>
</tr>
<tr>
<td>Ethyl hexanoate</td>
<td>Apple</td>
<td>1002</td>
<td>1002</td>
<td>M, N, O</td>
<td>489 ± 5.56</td>
<td>460 ± 5.50</td>
<td>349 ± 6.55</td>
<td>263 ± 3.60</td>
</tr>
<tr>
<td>Hexyl acetate</td>
<td>Fruit, herb</td>
<td>1011</td>
<td>1013</td>
<td>M, N, O</td>
<td>121 ± 2.51</td>
<td>110 ± 2.00</td>
<td>64 ± 1.73</td>
<td>30 ± 0.51</td>
</tr>
<tr>
<td>Methyl octanoate</td>
<td>Orange</td>
<td>1138</td>
<td>1140</td>
<td>N, O</td>
<td>29.3 ± 1.73</td>
<td>31.0 ± 0.57</td>
<td>32 ± 1.15</td>
<td>27.9 ± 2.00</td>
</tr>
<tr>
<td>Diethyl succinate</td>
<td>Wine, fruit</td>
<td>1179</td>
<td>1176</td>
<td>M, N, O</td>
<td>115 ± 5.65</td>
<td>147 ± 8.18</td>
<td>337 ± 9.16</td>
<td>534 ± 7.93</td>
</tr>
<tr>
<td>Ethyl octanoate</td>
<td>Fruit, fat</td>
<td>1196</td>
<td>1195</td>
<td>N, O</td>
<td>294 ± 7.57</td>
<td>269 ± 2.51</td>
<td>259 ± 3.60</td>
<td>243 ± 0.57</td>
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<tr>
<td>2-Phenylacrylic acid</td>
<td>Rose</td>
<td>1249</td>
<td>1247</td>
<td>N, O</td>
<td>18 ± 1.15</td>
<td>179 ± 1.52</td>
<td>128 ± 0.57</td>
<td>98 ± 0.51</td>
</tr>
<tr>
<td>2-Phenylacrylic acid</td>
<td>Rose</td>
<td>1265</td>
<td>1259</td>
<td>M, N, O</td>
<td>92.4 ± 3.21</td>
<td>88.3 ± 0.57</td>
<td>78.1 ± 1.15</td>
<td>39.9 ± 0.36</td>
</tr>
<tr>
<td>2,6-Dimethyl-1,3-dioxan-4,6-diol</td>
<td>Bergamot</td>
<td>1270</td>
<td>1265</td>
<td>N, O</td>
<td>15.1 ± 1.73</td>
<td>18.1 ± 3.21</td>
<td>34 ± 2.08</td>
<td>42.1 ± 0.57</td>
</tr>
<tr>
<td>Geranyl ethyl ether</td>
<td>Green</td>
<td>1297</td>
<td>1288</td>
<td>N, O</td>
<td>14.5 ± 0.17</td>
<td>14.5 ± 0.98</td>
<td>23 ± 0.58</td>
<td>27 ± 1.52</td>
</tr>
<tr>
<td>2-Phenylethyl propanoate</td>
<td>Floral</td>
<td>1349</td>
<td>1349</td>
<td>N, O</td>
<td>91.2 ± 1.02</td>
<td>93.2 ± 0.50</td>
<td>75 ± 1.10</td>
<td>58.6 ± 2.02</td>
</tr>
<tr>
<td>Ethyl dec-9-enolate</td>
<td>Fruity</td>
<td>1389</td>
<td>1373</td>
<td>N, O</td>
<td>23.0 ± 0.76</td>
<td>24.2 ± 0.46</td>
<td>21 ± 0.50</td>
<td>18.2 ± 0.43</td>
</tr>
<tr>
<td>Ethyl decanoate</td>
<td>Grape</td>
<td>1380</td>
<td>1381</td>
<td>N, M, O</td>
<td>52.4 ± 0.52</td>
<td>45.4 ± 1.20</td>
<td>39 ± 0.68</td>
<td>26.3 ± 0.32</td>
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<tr>
<td>2-Phenylethyl butanoate</td>
<td>Musty</td>
<td>1440</td>
<td>1438</td>
<td>N, O</td>
<td>18.3 ± 0.26</td>
<td>15.5 ± 0.40</td>
<td>11 ± 1.01</td>
<td>8.81 ± 0.51</td>
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<td>Ethyl laurate</td>
<td>Leaf</td>
<td>1509</td>
<td>1593</td>
<td>N, O</td>
<td>36.8 ± 0.40</td>
<td>38.1 ± 1.02</td>
<td>34 ± 0.50</td>
<td>30.3 ± 2.09</td>
</tr>
</tbody>
</table>

Table 1: Mean quantitative value (µg/L) and standard deviation (SD) of volatile compounds both in ASTI (6.5 % v/v) and MOSCATO (6.5 % v/v) wines after storage at different temperatures.

(continued on next page)
### Table 1 (continued)

<table>
<thead>
<tr>
<th>Compound</th>
<th>Odour</th>
<th>RI (DB-5)</th>
<th>Identifi(^a)</th>
<th>(^\text{ASTI (6.5% v/v) (n = 12)})</th>
<th>(^\text{MOSCATO (6.5% v/v) (n = 12)})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>RI Lit(^a)</td>
<td>RI Calc</td>
<td>Control</td>
<td>5 °C</td>
</tr>
<tr>
<td>3-Methyl-1-butanol</td>
<td>Whiskey</td>
<td>727 726</td>
<td>M, N, O</td>
<td>50887(^a) ± 15</td>
<td>50225(^a) ± 14</td>
</tr>
<tr>
<td>2-Methyl-1-butanol</td>
<td>Malt</td>
<td>739 740</td>
<td>M, N, O</td>
<td>19628(^a) ± 18</td>
<td>19055(^a) ± 13</td>
</tr>
<tr>
<td>Benzyl alcohol</td>
<td>Floral</td>
<td>1032 1029</td>
<td>M, N, O</td>
<td>17.3(^a) ± 0.43</td>
<td>18.2(^a) ± 0.55</td>
</tr>
<tr>
<td>2-Phenylethyl alcohol</td>
<td>Honey</td>
<td>1110 1116</td>
<td>M, N, O</td>
<td>12019(^a) ± 13</td>
<td>11812(^a) ± 17</td>
</tr>
<tr>
<td><strong>C6 Alcohols</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(E)-3-Hexenol</td>
<td>Moss</td>
<td>853 854</td>
<td>M, N, O</td>
<td>24.1(^c) ± 0.60</td>
<td>27.5(^a) ± 1.97</td>
</tr>
<tr>
<td>(Z)-3-Hexenol</td>
<td>Grass</td>
<td>857 858</td>
<td>M, N, O</td>
<td>40.3(^a) ± 0.90</td>
<td>42.4(^c) ± 0.80</td>
</tr>
<tr>
<td>Hexanol</td>
<td>Resin</td>
<td>864 860</td>
<td>M, N, O</td>
<td>423(^c) ± 7.39</td>
<td>441(^c) ± 3.15</td>
</tr>
<tr>
<td><strong>Others</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetaldehyde</td>
<td>Pungent</td>
<td>&lt;500 &lt;500</td>
<td>M, N, O</td>
<td>9185(^a) ± 10</td>
<td>9369(^a) ± 8</td>
</tr>
<tr>
<td>Benzoaldehyde</td>
<td>Almond</td>
<td>962 964</td>
<td>M, N, O</td>
<td>8.21(^c) ± 0.51</td>
<td>10.5(^c) ± 0.28</td>
</tr>
<tr>
<td>Vitispirane</td>
<td>Floral</td>
<td>1272 1291</td>
<td>M, N, O</td>
<td>6.35(^a) ± 0.20</td>
<td>8.2(^a) ± 0.64</td>
</tr>
</tbody>
</table>

\(^a\) RI lit: retention index (van den Dool & Kratz, 1963) reported in the literature DB-5 ((5%-phenyl)-methylpolysiloxane) GC column or equivalents (Gauvin, Lecomte, & Smadja, 2004; Hognadottir & Rouseff, 2003; Ozel, Gogus, & Lewis, 2006).

\(^b\) The reliability of the identification or structural proposal is indicated by the following: (M) mass spectrum and retention time consistent with those of an authentic standard; (N) structural proposals given on the basis of mass spectral data (NIST98); (O) mass spectrum consistent with spectra found in literature.

\(^c\) Values with different letters in single sampling date group (capital and lower case for Asti and Moscato, respectively) are significantly different (estimated by analysis of variance (ANOVA) followed by Tukey’s “Honest Significant Difference” test, \(p < 0.05\)).
all the acids identified significantly correlated with the first dimension (PC1, 85.5% of the total variance), thus discriminating samples stored at different temperatures. Also the “temperature”, considered as a supplementary quantitative variable, positively correlated with PC1; in fact, it was observed that samples at 25°C were placed at the highest PC1 values, while “control” and “5 °C” wines were grouped at the lowest values (Fig. 2).

On the contrary, a negative trend was highlighted for some alcohols levels, particularly 3-methyl-1-butanol, 2-methyl-1-butanol and 2-phenylethyl alcohol (the first two molecules are

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**Fig. 2.** Graphical representation of PCA performed on aroma compounds grouped in different chemical subclasses: esters, terpenes, acids, alcohols, others, and a selection of the most characteristic compounds, based on the odour activity values (OAVs). Asti and Moscato are indicated as AC, A5, A15, A25, and MC, M5, M15, M25, for “control” wines and samples stored at 5, 15 and 25 °C, respectively.
negatively associated to PC1, the last one to PC2), depending on the temperatures considered. These molecules give to wine aroma the characteristic whiskey, malt, and honey notes; their partial or complete loss is conventionally responsible of a deterioration of the wine plain aroma. Also isobutanol, characterised by a typical solvent scent, is negatively correlated to PC2, so decreasing at the highest temperatures.

The increase of the storage temperature was well described by both PC1 and PC2, showing significant correlation coefficients (0.649 and 0.738, respectively) (Fig. 2). Butanol, propanol, benzyl and other linear C6 alcohols were positively associated to the temperature increase.

In general, Asti wines contained higher amounts of 3-methyl-1-butanol and 2-methyl-1-butanol than Moscato samples, while Moscato was richer in 2-phenylethyl alcohol, isobutanol, hexanol, propanol and butanol.

3.1.4. Others

Other key odorants for the overall evaluation of the aroma on muscat wines, such as vitispirane, acetaldehyde and benzaldehyde, were identified and quantified. Results obtained from PCA showed that all these compounds significantly contributed to differentiate samples stored in different conditions, especially vitispirane and acetaldehyde, showing a positive correlation with PC1 and the increase of the temperature. The benzaldehyde content was positively correlated with PC2 and permitted to separate Asti from Moscato wines (the highest values were observed for these last ones) (Fig. 2).

Furthermore, two “off-flavors” responsible of common wine imperfections described as “cork tones”, i.e., 2,4,6-trichloroanisole (TCA) and 2,4,6-tribromoanisole (TBA), were identified in one Asti sample stored at 25 °C. This aspect could be particularly important regarding the identification of these compounds, belonging to the haloanisoles series, generally present at trace levels, but characterised by a highly odoriferous impact (2,4,6-trichloroanisole has a very low threshold concentration of 4–5 ng/L) (Alzaga, Ortiz, Sachez-Baeza, Marco, & Bayone, 2003).

Finally, in order to assess the influence of the specific compounds identified on the overall wine aroma, the odour activity values (OAVs) were determined; OAVs were calculated as ratio of the concentration of each compound with its perception threshold (data not shown) (Sánchez Palomo, Díaz-Maroto, González Viñas, Soriano-Pérez, & Pérez-Coello, 2007).

Linalool, β-damascenone, ethyl hexanoate, ethyl butanoate, isoamyl acetate, 2-phenyl ethyl acetate, geraniol, and 2-phenyl ethanol were the most characteristic aroma compounds of muscat-based wine. The PCA performed on this restricted dataset highlighted that all these compounds are negatively associated to the increase of the storage temperature. The first principal component (PC1: 68.8% of the total variance) was positively correlated with the vectors associated to these aromatic compounds, while was inversely correlated to the supplementary quantitative variable “temperature”. Excepting for geraniol and β-damascenone, slightly more concentrated in Asti wines, all these compounds were prevalent in the Moscato samples.

3.2. Development of the post-analysis processing of data

Despite the hyphenated chromatographic method applied in this study is extremely powerful and sensitive, the interpretation of the so obtained spectra is a time-consuming step because of the complex molecular fingerprint of wine aroma. In the second part of this study we developed a mathematical approach aimed to simplify the comparison/interpretation among different chromatograms, highlighting dissimilarities in analogue wine samples analysed during storage.

Different strategies were recently developed in order to simplify (i) the recognition of peaks on 2D chromatograms; (ii) to wrap different 2D fingerprints or (iii) to automatically obtain an interpretation of the complex profile (Bandow et al., 2008; Mondello et al., 2008). An interesting approach performed exploiting 2D commercial software, specifically developed for proteomic analysis, was recently applied on comprehensive GC×GC analysis of fruit volatile compounds by Schmarr and Bernhardt (2010).

Briefly, in our study the mathematical process was organised in several steps that permitted us to directly compare different complex chromatograms, earlier graphically reconstructed starting from raw data recorded by the GC instrument. The first step was importing the 251 × 673 matrix of raw data directly from the GC software (Chrom-Card; version 2.3) and successively uploading it to Mathematica® software.

The second step was focused on alignment verification of the chromatograms, since this was a fundamental requirement for a corrected comparison process reducing variability and normalising the complex profiles obtained. For this reason we decided to use the peak of BHT (internal standard) as reference pixel, in order to determine possible “dimensional shifts” in the GC chromatograms and, in case of a shift, to align them. Actually, in our analysis no shift occurred, so there was no need of such an alignment; the GC method has proved to be extremely repeatable in this sense as the internal standard were found located in the same position (pixels) for each injection. Once it has been guaranteed the effective alignment of chromatograms, it was necessary to optimise the quality of the 2D contour plots processing data in logarithmic scale to better appreciate the different pixels contrasts of the graphical representation (Fig. 3A).

As previously described, the principal purpose of this post-analysis mathematical data-processing was to compare each other
two chromatograms in order to (automatically) appreciate the difference of the evolution of volatile aroma between two wine samples. In order to highlight the different intensity patterns concerning the comparison between two chromatograms, the next necessary step is the normalisation of two corresponding matrices (re-scaling). This last step was due to the fact that each chromatographic analysis was generally subjected to a certain degree of variability that did not make comparable two 2D contour plots without re-scaling. For this reason a chromatogram was chosen as a “reference” and the second one, designated as “test”, was scaled (with a pixel by pixel correction intensity dependent) in order to create comparable images (Fig. 3B). Therefore, it was possible to appreciate variations in aroma composition comparing “reference” and “test” chromatograms.

First, the wine samples were subjected to a panel for the sensorial analysis (data not shown), then we selected among Asti wines one sample defined “pleasant, fruity and fresh” (reported as “perfect” wine, corresponding to “control”, and used as “reference” for the chromatograms comparison), one sample considered “pleasant, but not perfect” (reported as “imperfect” wine, corresponding to “15°C 6 months storage”) and one sample considered “unpleasant” (reported as “bad”, corresponding to “25°C 6 months storage”).

Even if we finally reported data only related to Asti samples it must be highlighted that applying this approach also Moscato samples gave comparable results.

Figs. 4 and 5 (and relative tables) report the comparison between the “reference” (perfect wine) and the “test” chromatograms (imperfect and bad wines, respectively).

We compared the samples, automatically appreciating both the decreases and increases in intensity of the different peaks previously identified. In this way, since the spots present in the chromatograms correspond to different molecules, it was possible to automatically monitor changes in aromatic composition of wine samples characterised with different sensorial profiles. The green spots represent pixels that reduced their intensity whether the “reference” scaled matrix was compared with the “test” one. On the contrary red spots represent pixels that enhance their intensity between the two scaled matrices. Therefore, transferring this trend from an analytical point of view, the green area correspond to molecules that, in the test sample, decreased their content respect the reference, and red ones increased their levels. In order to consider the olfactory characteristic of different compounds, we considered strategic to select the percentage of “cut-off” relative to the positive or negative change in pixel intensity related to chromatograms comparison. After fixing a certain value of cut-off, it is possible to highlight the decrease/increase (measured as pixel intensity) opting for a certain percentage value of difference between the chromatograms (threshold value). Once the threshold value is selected, it is possible to highlight with different colours the pixels on the basis of their decrease/increase of intensity (e.g., green negative trend and red positive one). The final result is represented pointing...
out in the reference chromatogram the pixels, which decrease or increase their intensity, in green or red colour, respectively. In this way, starting with high value of percentage and thereafter descending to the lower values, the trend of the molecules that change more in the sample can be monitored.

Fig. 4 reports the comparison between the bi-dimensional fingerprints of “perfect” Asti sample and the “imperfect” one; differences were observed starting from a 30% threshold value. In this case, the tasting panel described the “perfect wine” as having an intense, floral, typically “Muscat” aroma, and a moderate fresh and fruity odour; on the contrary the “imperfect wine” scored lower fresh and fruity notes and some oxidised nuances of Muscat aroma.

It can be underlined that some representative molecules, characterised by fruity or floral odour, like linalool, isoamyl and hexyl acetates, and 2-phenethyl alcohol (and some of its esters) decreased in the “imperfect” wine. On the other hand, some molecules with waxy or rancid odour increased their content. Fig. 5 reports an analogue comparison between the same perfect Asti sample and the bad one (described by the tasting panel as having an intense, floral, typically “Muscat” aroma, and a moderate fresh and fruity odour; on the contrary the “imperfect wine” scored lower fresh and fruity notes and some oxidised nuances of Muscat aroma).

With respect to the compounds reported in Table 1, the use of this approach allowed to identify additional molecules (characterised by significant changes related to the storage conditions) that appear to be responsible for the aroma of the Muscat-based wines. More generally, this approach could be considered useful to compare wines with different flavors, as well as to refer them to one specific and well characterised “reference standard”, leading to a powerful tool for the quality control of wines.

4. Conclusions

The results of this work allowed us to conclude that the powerful combination of HS-SPME technique with GC×GC/TOF-MS system is useful for the analysis of volatiles compounds in wines. A wide range of both polar and non-polar organic compounds from wine aroma were easily extracted and identified, overcoming problems of samples complexity and co-elution from the presence of a diverse range of chemical classes in untreated real wine samples. This method could be fundamental for the wine aroma analysis, permitting the comparison of different wines, also obtained from different grape varieties as well as from different technologies.

Regarding the evolution of the aroma of muscat-based wines during 6 months storage, samples maintained at 5 °C retained almost intact their aroma; terpeneic compounds most abundant and significant are linalool, geraniol, and β-damascenone to 5 °C, while at 25 °C prevail α-terpineol and furan linalool oxides. The most high storage temperatures promote hydrolysis and then the loss
of fruity aroma. This fact allowed us to confirm a significant effect of temperature on the quality of the fruity white wines during ageing.

Furthermore, the application of an innovative mathematical approach to study bidimensional fingerprints of wine aroma permitted both to standardize the 2D patterns, comparing GC profiles, and to identify significant differences among samples.

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References


Turkish Journal of Chemistry, 30, 71–76.