Vintage Discrimination of Lebanese Syrah Wine Using Headspace Solid Phase Microextraction-GC-MS and Chemometrics

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ABSTRACT

The aroma of wine determines its acceptability by consumers. Thus, it is important to know the nature and quantities of the compounds present in the wine mixtures. Analysis of aromatic compounds highly depends on the extraction methods. The extraction method adopted for this study is the Micro Extraction in Solid Phase (SPME), using a Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) fiber type. This task requires first the optimization of the SPME extraction through a central composite design. The factors considered are extraction time, extraction temperature and amount of NaCl added. After optimization, 5 vintages of wine Syrah-Adyar were analyzed using gas chromatography coupled with mass spectrometry in order to study vintage effect. Digital chromatogram simulator has been used and treated by chemometrics. Results showed that three flavors seem to differ from vintage to another: ethyl acetate, 3-methyl-1-butanol and ethyl octanoate.

Keywords: Aroma, Wine, HS-SPME, GC-MS, vintage.

Introduction

The Syrah red wine changes its aromatic style depending on the vintage, ripening and winemaking. It provides red wine with a good alcoholic degree, medium acidity, suitable for aging because of its tannic potential. These are often very high quality wines. They usually have an intense, dense and dark bluish color. They are also distinguished by their aromatic complexity and finesse of their tannins (Encyclopédie des Cépages de France, 2012).

Wine is a complex matrix that contains volatile compounds and where minor compounds play an important role in its organoleptic quality (Noguerol et al., 2009). As they are contained in very complex and variable composition of matrices, volatiles are difficult to analyze.

In fact, analysis depends critically on the technique used for the isolation of volatiles, as it affects the representativeness of the isolate (Chin et al., 2012).

Hence, the extraction is an important preliminary step in the analysis of these compounds. Several techniques are usually used; the most commonly are liquid-liquid extraction, solid-phase extraction and solid phase microextraction. The latter seems to be the most advantageous because it is: fast, simple, inexpensive, requires no solvent and sample preparation is often limited to sampling (Andujar-Ortiz et al., 2009).

The headspace method, in conjunction with GC–MS analysis, provides a high recovery, good linearity to analyze wine volatiles, over a wide range of concentrations and with a high sensitivity (Câmara et al., 2009).

In general, red wine have been extensively analyzed in terms of chemical composition, and especially by headspace solid phase microextraction for origin characterization and flavor analysis (Noguerol et al., 2009; Castro et al., 2008; Pozo-Bayón and Reineccius, 2009 and Tao and Zhang, 2010), origin comparison (Sagratini et al., 2012; Câmara et al., 2007 and Setkova et al., 2007), and off-flavor identification (Vlachos et al., 2007; Pizarro et al., 2007; Kotseridis et al., 2008 and Pizarro et al., 2008).

Due to the complexity of the matrix, there is not a single SPME fiber consistent with all the analytes present in the wine. The organic analytes of interest in the analysis of wine are polar and non-polar, and they have, therefore, affinity for all existing fiber.

However, in recent years the use of different fibers based on the combination of various adsorbents/absorbent polymers, such as divinylbenzene/Carboxen / Polydimethylsiloxane (DVB/CAR/PDMS) and Carboxen / Polydimethylsiloxane (CAR / PDMS), have become popular since they can extract a wider range of analytes (Andujar-Ortiz et al., 2009). Indeed, a DVB/CAR/PDMS showed the best results for the analysis of the aromatic fraction in wines (Castro et al., 2008).

Thus, in the study of Chin et al. (2012), fourteen additional volatiles were detected in Syrah wine with PDMS/DVB/CAR fiber, in comparison with the polyacrylate fiber.

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The SPME fiber chosen for the application of the SPME method in our work is the PDMS/CAR/DVB; since this three phase fiber is used for complex matrices, such as wine. In fact, the three phases allow the trapping of molecules by different principles: adsorption, dipole, molecular weight. It is also recommended by the supplier (Supelco, 1999) for the analysis of volatile products. Rebière et al. (2010) suggests the use of this fiber for sufficient extraction of a wide range of molecules in wine matrices (molecular weight 40-275).

To our best knowledge, there is no report describing the analysis of volatile compounds in a Lebanese Syrah wine. This work aims to discriminate different vintages of Lebanese Syrah wine based on their volatile composition, and using an optimized HS-SPME-GC-MS method. Temperature and duration of extraction for HS-SPME, as well as NaCl percentage were optimized. Compounds were tentatively identified using GC–MS and retention index matching.

Materials and Methods

Reagents and standards:

A synthetic wine solution was prepared so as the composition is as close as possible to that of real wine (Zhang et al., 2011). The synthetic wine was obtained by dissolving 6 g/L of tartaric acid in a water/ethanol mixture (12% v/v) and the following concentrations of standards chemicals: 1-propanol (57.7 mg/L), isobutanol (34.3 mg/L), 3-methyl-1-butanol (60 mg/L), Cis-3-hexanol (20.1 mg/L), 1-hexanol (15.7 mg/L), 2-phenyl-ethanol (25.5 mg/L), ethyl acetate (47 mg/L), ethyl butyrate (0.5 mg/L), isoamyl acetate (20 mg/L), ethyl hexanoate (5 mg/L), ethyl octanoate (6 mg/L), ethyl nonanoate (7 mg/L), ethyl decanoate (5 mg/L), 2-phenethyl acetate (10 mg/L), decanoic acid (1.8 mg/L), hexanoic acid (7 mg/L), linalool (0.345 mg/L), ionone (0.7 mg/L). Finally the pH was adjusted to 3.7 (pH detected for Syrah wine) with a concentrated solution of NaOH (1 molar). SPME sampling parameters were optimized for these components.

Standard volatile compounds, ethanol and tartaric acid are provided by the companies Sigma-Aldrich (USA, Germany, Netherlands, Switherland), Fulka (Netherlands, Switherland), SAFC (Germany, China), and Merck (Germany).

The DVB/CAR/PDMS fiber is recommended by the supplier to extract volatile compounds from the headspace (Supelco, 1999). The SPME fiber was conditioned at 270 °C for 1 h in the GC injector, according to the manufacturer’s recommendations. In addition, this fiber has given very satisfactory results in various studies, compared to other types of fibers (Noguerol-Pato et al., 2009 and Boutou, and Chatonnet, 2011).

Optimization of SPME Parameters:

The optimization was performed using a central composite design with 3 factors: Temperature of extraction, time of extraction, NaCl percentage (Table 1).

<table>
<thead>
<tr>
<th>Objective</th>
<th>Response surface</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variables</td>
<td>3</td>
</tr>
<tr>
<td>Experiments</td>
<td>17</td>
</tr>
<tr>
<td>Coefficients</td>
<td>10</td>
</tr>
<tr>
<td>Responses</td>
<td>1</td>
</tr>
</tbody>
</table>

Regarding the experimental domain, the operating values used are presented in Table 2.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Unit</th>
<th>Center</th>
<th>Variation step</th>
</tr>
</thead>
<tbody>
<tr>
<td>U1</td>
<td>Temperature</td>
<td>°C</td>
<td>45</td>
</tr>
<tr>
<td>U2</td>
<td>Duration</td>
<td>min</td>
<td>30</td>
</tr>
<tr>
<td>U3</td>
<td>NaCl</td>
<td>%</td>
<td>20</td>
</tr>
</tbody>
</table>

The responses measured for each compound were expressed as peak area and resolution for all chromatograms acquired in the HS-SPME optimization experiments (data not shown). Once the experiments completed and results introduced into the Nemrod W 2007 software, the search for the optimum provided us with the following data: Temperature: 40 °C, extraction time: 35 min, [NaCl]: 20%.

Sampling:

Five vintages (2006, 2007, 2008, 2009, and 2010) for a Lebanese Syrah wine were studied. Two samples were taken from each vintage and each sample was analyzed three times.
A volume of 10 ml of wine was placed into a 20 ml vial, with 2g NaCl, 5 μl of a standard solution of 2-octanol (82 mg/L in ethanol) (Canuti, et al., 2009), and a magnetic bar. Preconditioning of the sample at the extraction temperature was set for 5 minutes (Noguerol-Pato et al., 2009). After preconditioning, the SPME fiber is inserted into the headspace for 35 min. The sample is heated in a water bath at 40°C, using a temperature controller. The magnetic stirring is set at 500 rpm.

**Chromatographic conditions:**

Chromatographic analyses were performed with an Agilent 6890N gas chromatograph and an Agilent 5975N mass spectrometer. The chromatograph is equipped with a split/splitless injector. A capillary column HP-5MS (30m×0.25mm i.d., 0.25 μm film thickness) from J&W Scientific (Folsom, CA, USA) was used. Helium at a flow of 1.7ml/min was used as carrier gas. Oven temperature was programmed as follows: 40°C for 2 min, heated at 5°C/min to 150°C, heated to 250°C at 10°C/min and kept for 2 min; finally raised to 300°C at 10°C/min and held for 5 min. Injection was performed in the splitless mode. An inlet of 0.75mm i.d. was used and the injector temperature was held at 250°C. The mass spectrometry in the electron impact mode (MS/EI) at 70 eV was recorded in the range m/z 40 to 450 u.m.a.

**Chemometric analysis:**

A Principal Component Analysis (PCA) was applied to the normalized areas (used to improve the signal) of the compounds identified by SPME-GC-MS. The total number of measures was 60. Thus, the PCA allows us to study the sources of variability in this data set, and to establish a relationship between vintage samples and identified compounds. The standard normal variate (SNV) was applied in order to give the same weight to all chemical compounds.

PCA is an exploratory chemometric method that seeks orthogonal directions of maximum individuals’ dispersion in the space of original variables. Usually, these directions are the most interesting, but they are in fact rarely easy to interpret, since they almost always contain contributions of several factors (Serrano-Lourido et al., 2012).

Hence, another method was applied to improve the analysis and the discrimination between groups, the Discriminant Factorial Analysis. The purpose of this method, as in PCA, is to reduce the number of data dimensions, seeking those who separate classes best. But the DFA seeks to best separate the groups of individuals, instead of seeking the directions of greatest variability as in PCA (Bertrand and Dufour, 2006 and Lebart et al., 2000). In addition, DFA can detect some information unobservable in PCA (Gouti et al., 1998 and Devaux et al., 1988).

**Results and Discussion**

**Principal Component Analysis:**

Preliminary extraction and GC-MS analysis of Lebanese Syrah wines used in this study showed the presence of more than 60 volatile compounds in the chromatographic profile. The development of an HS-SPME–GC–MS procedure for the profile study of diverse range of volatile compounds in different vintages was the first challenge in our method development. The procedure was required to be suitably robust to offer accurate quantification over different vintages.

The ACP is calculated with four main components restoring almost all the information of the original variables. The first 2 principal components account for about 70% of the total variance and discriminate adequately between the wines according to vintage as shown in figure 1.

The PCA scatter plot PC1-PC2 (Figure 2) showed clear differentiation between vintages along the first component (PC1) axis. 2006 and 2007 vintages are located negatively on PC1, while 2009 and 2010 vintages are located in the positive part of the axis. For 2008 vintage, it was separated from the others and situated in the positive area of PC2 axis.

The complementary PCA loadings plot revealed the contribution of 2 variables for the separation by PC1, the 3-methyl-1-butanol that contributes positively on PC1 and the ethyl acetate that contributes negatively. Thus the 3-methyl-1-butanol contributes positively to the 2009 and 2010 vintages wine composition, and negatively to 2006 and 2007 vintages wine composition and vice versa for the ethyl acetate.

3-methyl-1-butanol or isoamyle alcohol is the major alcohol found in wine, produced by the yeast or following the degradation of the amino acids (Ribéreau et al., 2006 and Ivanova et al., 2013), it could also be present in the grape through the transformation of leucine by Ehrlich pass (Nechita, 2010). The amyl alcohols, C₅H₁₀OH, are the most abundant after alcoholic fermentation. There are three main isomers, 1-pentanol or amyl alcohol, 3-methyl 1-butanol or isoamyle alcohol, and 2-methyl-1-butanol. These alcohols are called
fermentation alcohols (Bakker and Clarke 2011), resulting mainly from alcoholic fermentation (Vilanova and Oliveira, 2012) and particularly that of Syrah (Zhang et al., 2011), conducted with Saccharomyces cerevisiae yeast type (Romano et al., 2003).

![Scatter Plot PC1 vs. PC2 after PCA on GC-MS data.](image1)

**Fig. 1:** Scatter Plot PC1 vs. PC2 after PCA on GC-MS data.

![Profile of PC1 loadings obtained by PCA on GC-MS data.](image2)

**Fig. 2:** Profile of PC1 loadings obtained by PCA on GC-MS data.

Therefore, the presence of the 3-methyl-1-butanol indicates changes in the fermentation procedures or in the yeasts species developed during fermentation in 2008, 2009 and 2010 vintages, or it is the slight difference in the growing temperature between June and September for the different vintages that made the difference. It is well known that temperature, especially growing season temperature, is the dominant variable prevailing grapevine phenology and it has a major influence on grape composition and development (Webb et al., 2007).

Indeed, the climates these years were not markedly different in the region, but the average temperature shows some differences as shown in table 3. This table shows the monthly average temperature (°C), between June and September (www.wunderground.com).

**Table 3:** Monthly average temperature between June and September for the 5 years studied.

<table>
<thead>
<tr>
<th></th>
<th>June</th>
<th>July</th>
<th>August</th>
<th>September</th>
</tr>
</thead>
<tbody>
<tr>
<td>2006</td>
<td>25</td>
<td>27</td>
<td>28</td>
<td>27</td>
</tr>
<tr>
<td>2007</td>
<td>25</td>
<td>27</td>
<td>28</td>
<td>27</td>
</tr>
<tr>
<td>2008</td>
<td>25</td>
<td>28</td>
<td>28</td>
<td>27</td>
</tr>
<tr>
<td>2009</td>
<td>26</td>
<td>28</td>
<td>28</td>
<td>26</td>
</tr>
<tr>
<td>2010</td>
<td>26</td>
<td>29</td>
<td>29</td>
<td>28</td>
</tr>
</tbody>
</table>
As we observed, average temperatures for 2006 and 2007 were slightly lower than those for 2008, 2009 and 2010, thus, contributing to the synthesis of the acetates precursors, while the higher temperatures benefits the synthesis of higher alcohols precursors.

The higher alcohols are believed to contribute more to the intensity of the odor of the wine than to its quality (Etiévant, 1991).

As for the ethyl acetate, it is the most common ester in wine due to its formation from ethanol, predominant in wine, and acetic acid [Sumby et al., 2010], by enzymatic esterification. Its concentration increases with aeration and acetic acid content of the medium (Antonelli et al., 1999). It gives to the wine pleasant and fruity notes at low concentrations (Ribereau-Gayon, 1978), but it is responsible for the "acetic" notes at high concentrations (Rojas et al., 2001; Plata et al., 2003 and Lambrechts and Pretorius, 2000).

It has also been reported that a high concentration of ethyl acetate generally has a suppressive effect upon the formation of other compounds responsible for a fruity aroma, even before the sensory threshold has been reached (Etiévant, 1991).

This discrimination between wines from different vintages might be the result of different chemical reactions and composition development during conservation. Indeed, we observed the importance of ethyl acetate in the composition of older wines, 2006 and 2007 vintages. This is consistent with previous findings where ethyl acetate has been found to increase with ageing (Câmara et al., 2005).

**Discriminant Factorial Analysis:**

In order to improve the discrimination between groups and to confirm the PCA observations, a discriminant analysis is performed. The discriminant Factorial Analysis (DFA) is a descriptive and explanatory method, related to PCA, which is applied to quantitative data where a typology or partition is already defined.

The DF1-DF2 scatter plot (Figure 3) shows a clear separation between the groups according to the vintage along DF1. The 2006 and 2007 vintages are located on the negative side of the DF1 axis, while those of 2008, 2009 and 2010 vintage are positively positioned along this axis.

![Fig. 3: Scatter Plot FD1 vs. FD2 after DFA in function of vintage.](image)

The loadings plot (Figure 4) shows the influence of ethyl octanoate in this discrimination between groups along DF1. Thus this compound contribute positively to the 2006 and 2007 vintage wine composition, while it is in minor content in 2008, 2009 and 2010 vintage wine.

Along DF2, the loadings plot (Figure 5) reveals the influence of a second variable which is the ethyl acetate. Thus, 2006, 2007 and 2008 vintages contain a superior amount of this compound than in 2009 and 2010 vintages. This finding confirms the results observed in ACP.

Usually, most volatiles decrease during wine storage due to oxidation or hydrolysis (Roussis et al., 2013). The most important decrease were observed for ethyl decanoate, ethyl octanoate, ethyl hexanoate, ethyl acetate and isoamyle acetate (Patrianakou, and Roussis, 2019). Thus, this discrepancy between our results and others leads us to conclude that these differences are mainly due to the vintage.
Various indicators and tests are performed, which are used to judge the value and relevance of the results obtained. Indeed, according to the classification map (Table 2), we can see that up to 100% of samples are well classified in their groups.

**Fig. 4: Profile of DF1 loadings obtained by DFA in function of vintage.**

**Table 4: Samples classification map.**

<table>
<thead>
<tr>
<th>Year</th>
<th>2006</th>
<th>2007</th>
<th>2008</th>
<th>2009</th>
<th>2010</th>
<th>%Fit</th>
</tr>
</thead>
<tbody>
<tr>
<td>2006</td>
<td>5.0000</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
<td>100.0000</td>
</tr>
<tr>
<td>2007</td>
<td>0.0000</td>
<td>6.0000</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
<td>100.0000</td>
</tr>
<tr>
<td>2008</td>
<td>0.0000</td>
<td>0.0000</td>
<td>6.0000</td>
<td>0.0000</td>
<td>0.0000</td>
<td>100.0000</td>
</tr>
<tr>
<td>2009</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
<td>6.0000</td>
<td>0.0000</td>
<td>100.0000</td>
</tr>
<tr>
<td>2010</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
<td>0.0000</td>
<td>6.0000</td>
<td>100.0000</td>
</tr>
</tbody>
</table>

**Fig. 5: Profile of DF2 loadings obtained by DFA in function of vintage.**

**Conclusion:**

This work shows the first study on volatile compounds of Lebanese Syrah wine. A total of 55 to 60 volatile compounds were identified in these wines from different vintages. An optimized HS-SPME was performed using a central composite design. The results showed a better extraction with a temperature of 40°C, extraction time of 35 min, and salt concentration of 20% of NaCl.

The results showed that primarily three flavors seem to be the basis of the difference between the vintages: ethyl acetate, 3-methyl-1-butanol, ethyl octanoate.

Further work is needed to evaluate the contribution of hydrolysis and oxidation to the decrease in aroma esters during wine storage and to put further markers to discriminate between vintages.

**References**


