



Analytical Methods

Markers of typical red wine varieties from the Valley of Tulum (San Juan-Argentina) based on VOCs profile and chemometrics



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ABSTRACT

We studied the VOCs profile of three red wine varieties, produced in the Valley of Tulum (San Juan-Argentina), over 4 consecutive years. Our main goal was to verify if different wine varieties could be differentiated from their VOCs profile, considering changes induced by their age, the yeast inoculated and the type of alcoholic fermentation, establishing those compounds that could be used as chemical markers of a particular variety.

Stepwise LDA of selected VOCs allowed 100% differentiation between studied wines, showing that high levels of 1-hexanol were characteristic for Malbec, while low level of ethyl caproate was characteristic for Bonarda. Using controlled fermentations, 1-hexanol, a pre-fermentative VOC, presented a similar trend in wines produced from different yeast; while other fermentative VOCs, like ethyl caproate and ethyl caprylate, presented lower levels for Bonarda but also for Syrah. To our knowledge, this is the first report on characterization of VOCs from Bonarda. Additionally, the quantitative analysis of VOCs profile, coupled to chemometrics, present a good alternative to differentiate wines from different varieties and also for studying wine fermentation.

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1. Introduction

The aroma of wine is a unique mixture of volatile compounds originating from grapes (pre-fermentative varietal aromas), secondary products formed during the wine fermentation (fermentative aromas) and ageing (post-fermentative aromas) (Swiegers, Bartowsky, Henschke, & Pretorius, 2005). At the same time, wine aroma is one of the most important factors that determine its quality and price; it is also a key attribute for consumers.

Malbec and Syrah are wine varieties recognized and appreciated all over the world for their quality, being Malbec one of the most prized wines from Argentina. Nevertheless, both varieties have been little studied (Goldner, di Leo Lira, van Baren, & Bandoni, 2011; Goldner, Zamora, di Leo Lira, Gianninoto, & Bandoni, 2009). Bonarda is a wine commonly used in combination with other varieties but rarely commercialized alone (single varietal). However,

this wine reaches good characteristics in the Province of San Juan (Argentina).

It is well known, that grapes of different varieties but growing in the same vineyard have common agricultural practices as well as similar micro-climate and meso-climate. Therefore, differences in VOCs profile of wines produced from a single vineyard should mainly reflect the grape variety used for winemaking (Falqué, Fernández, & Dubourdieu, 2002), the yeast inoculated and the type of fermentation (spontaneous or inoculated) (Domizio et al., 2007; Fleet, 2003). However, a vast number of volatile compounds, most of them volatile organic compounds (VOCs), are formed by yeast during alcoholic fermentation, impacting the flavour and the overall quality of wines (King et al., 2008). This could indicate that the analysis of VOCs profile in wines, with the help of multivariate statistics, may be a useful tool to differentiate wines from diverse varieties, establishing criteria for authentication, preventing frauds and ensuring their origin (Ferreira, Fernández, Peña, Escudero, & Cacho, 1995). To our knowledge, there is no report on the VOCs profile of Bonarda variety.

Currently, solid phase microextraction (SPME) is one of the most versatile techniques used for the analysis of VOCs in wine (Arthur, Killam, Buchholz, & Pawliszyn, 1992; Rocha, Ramalheira, Barros, Delgadillo, & Coimbra, 2001) as well as in other foods (Baroni et al., 2006). This method allows solvent-free extraction

Abbreviations: LOD, limit of detection; LOQ, limit of quantification; CV%, coefficient of variation; VOCs, volatile organic compounds; HS-SPME-GC-MS, headspace solid-phase microextraction and gas chromatography coupled to mass spectrometry.

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and pre-concentration in a single step without pre-treatment of samples, and can be conveniently used for qualitative and quantitative determination of VOCs in wine samples (Arthur et al., 1992; Câmara, Alves, & Marques, 2006b; Rocha et al., 2001).

So far, our main goal was to evaluate the combined use of VOCs profile and chemometrics to differentiate among three red wine varieties from the Province of San Juan (Argentina), contributing to both the knowledge of VOCs profile from unreported varieties (Bonarda) and the characterization of Syrah and Malbec produced in this area. Additionally, we look to verify differences produced in the same varietal elaborated using different yeast. Thus, we look for evidences on VOCs markers associated with the original grape (pre-fermentative flavours) and those linked with the fermentative process, considering changes occurring during wine ageing.

2. Materials and methods

2.1. Reagents, standards and solutions

All reagents were of analytical grade. Ultra pure water (Arium 61316-RO + Arium 611UV purification system, Sartorius, Germany) was used to prepare standard solutions, dilutions and blanks. Individual standard of each compound: ethyl caprylate, diethyl succinate, benzyl alcohol, ethyl caproate, 1-hexanol and isopentyl acetate were purchased from Sigma–Aldrich and Fluka. Standard solutions (stock solutions) containing a mixture of six standards were prepared at five different concentrations. Stock solutions were prepared by diluting commercial standards in hydroalcoholic solutions containing ethanol (12–13%) in pure water at pH 3.6, partially simulating the wine composition (Rocha et al., 2001).

2.2. Yeast strain

Four different strains of *Saccharomyces cerevisiae* were tested; two autochthonous and two commercial yeast strains (Table 1). The commercial yeast were selected in accordance with the winery's preferences and the autochthonous strains were isolated from the vineyard and selected according to their oenological properties in a previous selection study, which evaluated ethanol production, sugar consumption, glycerol production, foam and SH₂ production, killer behaviour and the organoleptic characteristics of the experimental wines obtained (data not shown).

2.3. Sampling

The sampling area (Valley of Tulum) is situated in the central-west area of Argentina, being the second wine producer of the country, with increasing exportation rate during last years. The main grape varieties growing in this area are Syrah, Malbec and Bonarda.

2.4. Commercial wine samples

A total of 54 commercial red wine samples (mono-varietal; non-blended) were provided by “Augusto Pulenta” cellar, which produces wines using grapes from their own vineyard “Doña Filomena”, having control of the provenance of grapes (Fabani, Toro, Vazquez, Díaz, & Wunderlin, 2009). Wine samples were collected after winemaking in refrigerated stainless steel tanks, followed by filtration without using bentonite or other additives, followed by bottling and stabilization at 20–22 °C during 1 year. Samples were transported to the laboratory in commercial 750 mL glass bottles and dark stored at 4–8 °C until analysis. Six wines (2006) were used for the first qualitative and semi-quantitative evaluation of VOCs profile: Syrah ($n = 2$), Malbec ($n = 2$) and Bonarda ($n = 2$). Quantitative analyses were carried out using the remainder wine set ($n = 48$), corresponding to 4 consecutive years (2006–2009): Syrah ($n = 16$; 4 – 2006, 4 – 2007, 4 – 2008, 4 – 2009), Malbec ($n = 16$; 4 – 2006, 4 – 2007, 4 – 2008, 4 – 2009) and Bonarda ($n = 16$; 4 – 2006, 4 – 2007, 4 – 2008, 4 – 2009). All analyses were performed during 2010 within 2 months.

2.5. Grape musts and scale fermentation

In order to verify differences in wines profile arising from the use of diverse yeasts (Table 1), we carried out industrial scale fermentations. So, seven different wines were produced in the same winery (Augusto Pulenta cellar), using 50,000 L stainless steel vats using Syrah, Malbec and Bonarda musts. The starters were inoculated with a volume sufficient to obtain a cellular population of 10^7 cell mL⁻¹ in the winery vat. At the end of the alcoholic fermentation, malolactic fermentation occurred spontaneously in wines. Wine samples were collected after winemaking, filtration without using bentonite or other additives, bottling and stabilization at 20–22 °C during 1 year. Twenty-one bottles (3 from each fermentation randomly selected) were transported to the laboratory and dark stored at 4–8 °C until analysis.

2.6. Analysis of VOCs in wine samples

2.6.1. HS-SPME

A manual SPME holder (Supelco, Bellafonte, PA) was used for evaluation of VOCs profiles. A 50/30 µm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) (Supelco, Bellafonte, PA) 1 cm length SPME fibre was used for fractionation of volatile and semivolatiles compounds from the headspace (HS) of properly conditioned samples. Prior to extraction, the fibre was conditioned for 1 h at 270 °C in the injection port of the GC. The absence of carry over effect was checked by extracting and injecting blanks every six chromatographic runs. All samples were analyzed in triplicate. During quantitative analyses, VOCs profile was compared with those obtained during qualitative analysis to ensure coincidence between both sets of samples; no significant differences were observed during this comparison.

Table 1
Characteristics of fermentations at industrial scale.

	Type of fermentation	Grape juice	Characteristics of yeast inoculated
Fermentation Sh1	Spontaneous	Syrah	Yeast (<i>S. cerevisiae</i> and non- <i>Saccharomyces</i>) present naturally in grapes Syrah
Fermentation Sh2	Inoculated	Syrah	Yeast isolated from grapes Syrah (<i>Yeast1</i>)
Fermentation Ma1	Spontaneous	Malbec	Yeast (<i>S. cerevisiae</i> and non- <i>Saccharomyces</i>) present naturally in grapes Malbec
Fermentation Ma2	Inoculated	Malbec	Commercial yeast (<i>Yeast2</i>)
Fermentation Ma3	Inoculated	Malbec	Yeast isolated from grapes Malbec (<i>Yeast3</i>)
Fermentation Bo1	Inoculated	Bonarda	Commercial yeast (<i>Yeast4</i>)
Fermentation Bo2	Spontaneous	Bonarda	Yeast (<i>S. cerevisiae</i> and non- <i>Saccharomyces</i>) present naturally in grapes Bonarda

2.6.2. Sample treatment

Wine bottles were let to reach room temperature, opened and analyzed in duplicate within 2 h. Wine (0.5 mL) was introduced into a 2 mL vial, adding 20 mg of NaCl and sealing with a screw cap equipped with a PTFE septum. The wine sample and headspace was pre-equilibrated for 15 min in the sealed vial and then the SPME fibre was manually inserted into the vial through the septum and exposed to the vial headspace for 15 min at room temperature (20–22 °C).

2.6.3. GC–MS

Analyses were performed on a GC (Varian Saturn 3800) The GC was equipped with a Factor Four (Varian Inc, USA, CA) capillary column VF-5; 30 m × 0.25 mm ID; DF = 0.25 µm, using an Injector 1079 with septum Varian AG3 in splitless mode. Mass detector was an ion trap (Varian 4000, USA, CA), with the filament operated at 20 mV, scanning the range m/z 40–450. The SPME fibre was desorbed at 250 °C during 5 min. The oven temperature was programmed starting at 35 °C and increased to 250 °C at a rate of 8 °C min⁻¹. The carrier gas was helium (Ultra pure grade 5.0) at a constant flow of 1 mL min⁻¹.

2.6.4. Identification and quantification of VOCs

The initial identification of VOCs (qualitative and semiquantitative) was performed during preliminary runs with young commercial wines ($n = 6$, two samples from each variety) by comparison of the GC retention times and mass spectra with both pure compound and match with spectral library (NIST MS search 2.0). The concentration of selected compounds was determined during quantitative runs (using remainder 48 wine samples from four vintages) by external calibration curves. Standard solutions were prepared to cover an analytical range close to that determined in semi-quantitative runs. All samples and standard solutions were analyzed in duplicate. The limit of detection (LOD) was taken at a signal-to-noise ratio (S/N) of 5. The limit of quantification (LOQ) was taken at a signal-to-noise ratio (S/N) of 15. The reproducibility of the result was expressed as coefficient of variation (CV%) averaged over the entire calibration curve (Table 2). The method was validated by the addition of volatile compounds to wine samples (spiked samples). For each volatile compounds the recovery percentage was determined by the ratio $(C_1 - C_0/C_2) \times 100$, where C_0 was the concentration of the compound in the wine, C_1 the concentration of the compound in spiked sample and C_2 was the concentration of the compound added to wine sample (Perestrelo, Fernandes, Albuquerque, Marques, & Câmara, 2006) (Table 2). To identify the would-be impact odorants, the aroma potential of each compound was assessed by calculating the aroma index (I) using the equation $I = c/s$ (Rocha, Rodrigues, Coutinho, Delgado, & Coimbra, 2004) where c is the concentration in the wine and s is the corresponding odour threshold (OTH) reported in the literature (Ferreira, Lopez, & Cacho, 2000; Peinado, Moreno, Bueno, Moreno, & Mauricio, 2004; Kotseridis & Baumes, 2000). Compounds that exhibit $I > 1$ contribute individually to the wine aroma and were designated as would-

Table 2

Limit of detection, quantification, coefficient of variation averaged and percentage of recovery for wine analyzed by HS-SPME-GC–MS.

Compounds	LOD (mg L ⁻¹)	LOQ (mg L ⁻¹)	CV (%)	Recovery (%)
Ethyl caproate	0.004	0.016	2.29	102 ± 7
Ethyl caprylate	0.003	0.009	3.03	104 ± 7
Diethyl succinate	0.003	0.009	0.56	102 ± 4
1-Hexanol	0.023	0.069	2.47	100 ± 6
Benzyl alcohol	0.190	0.570	8.04	109 ± 3
Isopentyl acetate	0.007	0.021	1.07	94 ± 2

be impact odorants. In addition, Rocha et al. (2004) suggested that a substance could contribute to the overall aroma, when its concentration is at least 20% of the threshold unit ($I > 0.2$).

2.7. Statistics

2.7.1. LDA

Discriminant analysis is rather exploratory in nature, while classification procedures was less exploratory in the sense that they led to well-defined rules, which can be used for assigning new objects (Johnson & Wichern, 1998). LDA in stepwise mode was performed to evaluate whether wine samples could be mathematically–statistically distinguished in according to their variety as well as to establish which parameters (VOCs) allowed distinguishing one varietal from another. Selection of the most significant variables was performed by backward stepwise analysis according to F value.

2.8. Data processing

The original data set contained six samples, two from each variety and 22 compounds on each sample. These 22 compounds were deducted from qualitative and semi-quantitative GC–MS and tentatively identified (Table 3) by comparison of their mass spectra with the NIST MS library. Stepwise LDA was applied to the remainder data set ($n = 48$ wine samples as described in Section 2.4), looking to identify those compounds enabling differentiation among wine varieties. On the basis of discriminant functions (stepwise mode), we selected seven out of 22 identified VOCs as the most representative to discriminate among different wine varieties. These seven selected VOCs were further confirmed by comparison of both retention times and mass spectra with pure commercial standards; thus, positively identified as ethyl caprylate, diethyl succinate, benzyl alcohol, ethyl caproate, 1-hexanol and isopentyl acetate. VOCs concentrations were also calculated using the remainder data set (48 commercial wine samples from four wine years), using these data for further analysis. These selected VOCs were also used to verify differences between wines from scale fermentations (21 additional samples from seven different

Table 3

VOCs tentatively identified in red wines analyzed.

Compound ^a	RT (min)
2,3-Butanediol	4.470
Ethyl butanoate	4.685
3-Furaldehyde	5.317
Ethyl 2-metil-butanoate	5.553
Ethyl 3-metil-butanoate	5.648
3-Hexen-1-ol	5.735
1-Hexanol	5.989
Isopentyl acetate	6.115
Ethanal	6.322
1-Octen-3-ol	8.681
Ethyl caproate	9.166
Hexyl acetate	9.569
3-Ethyl-4-methylpentan-1-ol	9.901
Benzyl alcohol	10.361
Phenylethyl alcohol	12.833
Diethyl succinate	14.909
Ethyl caprylate	15.437
Camphene	16.647
2-Phenylethyl acetate	17.461
Isotridecanol	19.125
1-Hexadecanol	19.399
Ethyl decanoate	21.319

^a Compounds identified by comparison with mass spectra from NIST 05 MS library.

fermentations). Besides, two-way ANOVA was used to find significant differences between levels of selected compounds evaluated from different varieties and vintages. Analysis of differences between different fermentations was performed using ANOVA test with the data set arising from scale fermentations (21 samples). The statistical package STATISTICA 7 from Statsoft (2005) was used for statistical calculations.

3. Results and discussion

3.1. Differential VOCs profile from diverse commercial red wine varieties

The average concentration of selected compounds: ethyl caproate, ethyl caprylate, diethyl succinate, benzyl alcohol, 1-hexanol

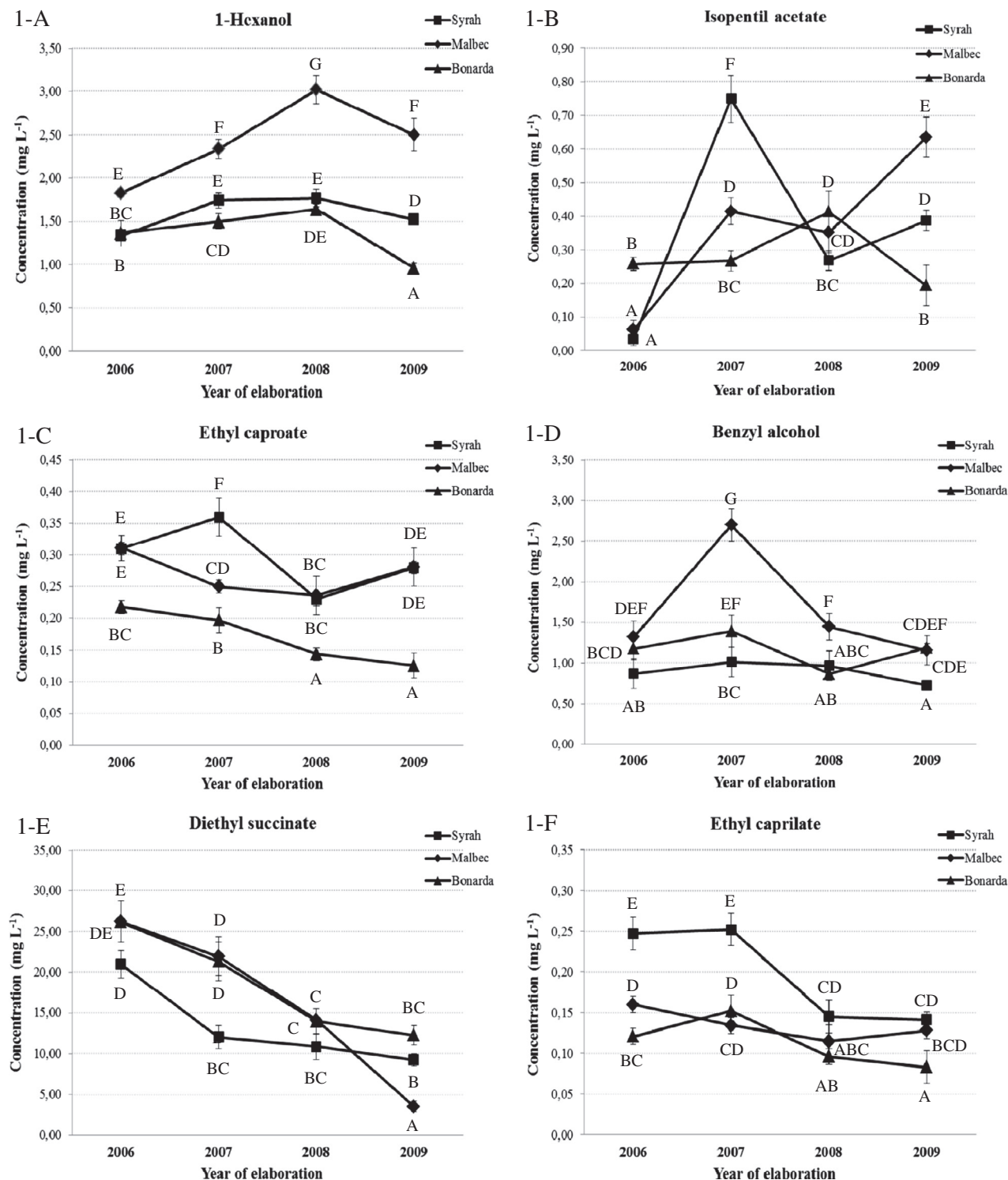


Fig. 1. Concentration of VOCs, selected by LDA, in commercial wine varieties. Values are averaged over four samples analyzed in triplicate. Different letters refer to significant difference using two-way ANOVA ($p < 0.05$).

and isopentyl acetate, analyzed in three wine varieties throughout 4 years are shown in the Fig. 1. Aroma index (*I*) for selected VOCs was calculated for each wine variety (Table 4).

Volatile esters constitute one of the most important classes of aroma compounds and are very significant in the wine aroma, associated with “fruity” and “floral” descriptors and, in the case of the red wines, playing a modulating role in their quality (Ferreira et al., 1995; Lilly, Lambrechts, & Pretorius, 2000). Ethyl esters of fatty acids are a group of volatiles compounds produced by yeast during fermentation and are formed from ethanolsis of acyl-CoA, which is an intermediate metabolite of the fatty acid metabolism. In this group of esters the ethyl group is derived from ethanol and the acid group from a medium-chain fatty acid (Pérez-Prieto, López-Roca, & Gómez-Plaza, 2003; Styger, Prior, & Bauer, 2011).

Ethyl esters analyzed during this work (ethyl caproate, ethyl caprylate and diethyl succinate) exhibited elevated aroma index (Table 4), which indicates the importance of these compounds to the whole aroma in studied wines (Gil, Cabellos, Arroyo, & Prodanov, 2006). The average content of ethyl esters found in Syrah, Malbec and Bonarda varieties from the Valley of Tulum (Fig. 1) were lower than those reported in red wines from DO “Vinos de Madrid” (Spain) (Gil et al., 2006) and Tinta Negra Mole red wines (Perestrello et al., 2006). Câmara et al. (2006b) reported that, during ageing, the concentration of ethyl esters decreased due to their evolution towards more stable compounds. In our case, the content of ethyl caproate was significantly increased along 4 years ageing in Bonarda (Fig. 1C). We did not observe a drop in the amount of ethyl caproate with ageing. Ethyl caprylate was significantly increased along 4 years ageing in Syrah (Fig. 1F), with a slight trend to augment its concentration in Malbec and Bonarda (Fig. 1F). Once more, we did not observe a declination in levels of ethyl caprylate as a consequence of ageing. Our current results agree with those reported in Chenin blanc wines (aged for 6 months) by Lilly et al. (2000), who observed that concentrations of ethyl caproate and ethyl caprylate showed an overall increase during the storage period. Furthermore, diethyl succinate presented a generalized and significant trend to increase with ageing in all wine varieties studied (Fig. 1E). It is also worthy to remark that the concentration of diethyl succinate was mostly lower in Syrah wine, however the rose of this parameter with ageing precludes its use as a chemical marker of wine variety but from ageing. The concentration of diethyl succinate observed during this work was higher than values cited by Perestrello et al. (2006) for Tinta Negra Mole red wines. Changes in the concentration of diethyl succinate during the storage have been also reported by Gonzalez-Viñas, Pérez-Coello, Salvador, Cabezudo, and Martin-Alvarez (1996) and Pérez-Coello, González-Viñas, García-Romero, Díaz-Maroto, and Cabezudo (2003). Câmara, Alves, and Marques (2006a) reported the incre-

ment of succinic acid (monoethyl and diethyl succinate) to point out wine-age. Thus, our current report confirms that diethyl succinate should not be used to evaluate differences between wine varieties but can be used as a chemical marker of wine ageing.

Acetate esters (such as isoamyl acetate, propyl acetate, hexyl acetate, phenethyl acetate), are the result of the reaction of acetyl-CoA with alcohols that are formed from the degradation of amino acids, carbohydrates and lipids (Perestrello et al., 2006; Styger et al., 2011). Isopentyl acetate (“banana” bouquet), which is produced from the amino acid leucine was present at different concentrations in all wine varieties studied (Fig. 1B). Its aroma index was greater than 1, pointing out the importance of this compound for the aroma of studied wines (Table 4). Isopentyl acetate presented variable behaviour during ageing (Fig. 1B), with a trend to decrease with ageing in both Syrah and Malbec wines (Fig. 1B), in agreement with values reported by Lilly et al. (2000) in Chenin blanc wines aged for 6 months.

Alcohols with six carbon-atoms, like 1-hexanol, are the product of the degradation of linoleic and linolenic acid esters (primarily located in the solid parts of the berry) by grape enzymes during the pre-fermentative stage (Baumes, 2000; Drawert, Heimann, Emberger, & Tressl, 1966). These alcohols contribute to “vegetal” and “herbaceous” nuances to the wine when their concentration exceed 8 mg L^{-1} (Perestrello et al., 2006). The highest concentration of 1-hexanol found during our study was about 3 mg L^{-1} (Fig. 1A), with a mean aroma index of 0.3, so not important aroma index was observed from 1-hexanol (Table 4); however, 1-hexanol was important to differentiate Malbec wines in our study (Fig. 1A). Gil et al. (2006) reported the same odour activity when analyzed red wines from DO “Vinos de Madrid” (Spain).

Benzyl alcohol is associated to “flowery” and “sweet” aromas. The highest concentration of this compound found in our case was 2.7 mg L^{-1} (Fig. 1D) in Malbec wine from vintage 2007. Other wines and vintages showed values below 1.5 mg L^{-1} but above 0.5 mg L^{-1} , which means that values observed during this work for red Argentinean wines are higher than those reported by Perestrello et al. (2006) in Tinta Negra Mole red wines. However, the aroma index for benzyl alcohol was far low ($I < 0.01$). Thus, no important contribution to wine aroma could be expected from this compound. The content of alcohols does not change significantly during wine-ageing; although the concentration of some of them could be increased because of esters hydrolysis (Câmara et al., 2006b). In our case, 1-hexanol and benzyl alcohol presented variable behaviour during ageing (Fig. 1A and D); only 1-hexanol in Malbec wines showed a trend to decrease its concentration with ageing (Fig. 1A).

Thus, considering only the odour threshold and the calculated aroma index (Table 4), ethyl caproate, ethyl caprylate, diethyl succinate and isopentyl acetate could be pointed out as chemical

Table 4
Odour descriptor, odour threshold (OTH) and aroma index (*I*) in function of red wine variety.

Compound	Odour descriptor	Odour threshold (mg L^{-1})	Aroma index (<i>I</i>)		
			Syrah	Malbec	Bonarda
Ethyl caproate	Green apple	0.014 ^A	21.43 ^c	19.29 ^{b,c}	11.43 ^a
Ethyl caprylate	Fruity, sweet, fresh	0.005 ^A	40.00 ^b	26.00 ^a	22.00 ^a
Diethyl succinate	Fruity, melon	1.20 ^B	11.09 ^a	13.72 ^a	14.43 ^a
1-Hexanol	Herbaceous, green	8.00 ^A	0.20 ^{a,b}	0.30 ^c	0.17 ^a
Benzyl alcohol	Flowery, sweet	200 ^C	<0.01 ^a	<0.01 ^a	<0.01 ^a
Isopentyl acetate	Banana	0.03 ^A	12.00 ^a	12.33 ^a	9.67 ^a

ANOVA: different letters indicates significant difference ($p \leq 0.05$) for the same compound among studied wine varieties.

^A The matrix was a 11% water/ethanol solution containing 7 g L^{-1} glycerol and 5 g L^{-1} tartaric acid, with the pH adjusted to 3.4 with 1 M NaOH (Ferreira et al., 2000).

^B Determined in 10% ($v v^{-1}$) ethanol solution adjusted to pH 3.5 with tartaric acid (Peinado et al., 2004).

^C Calculated in the Laboratory of Aroma Analysis and Enology, Department of Analytical Chemistry, University of Zaragoza, Spain. Orthonasal thresholds were calculated in a 10% water/ethanol mixture containing 5 g L^{-1} of tartaric acid at pH 3.

markers of aroma in studied wines, however their concentrations change with wine ageing, particularly with diethyl succinate (Fig. 1E), confirming that wine odour changes with ageing, but seldom appreciate through objective studies of variations in VOCs. So far, this study presents evidence on such changes.

To this point, considering the previous discussion, we observed a particular aromatic profile for each commercial red wine studied during this work. Fig. 1 shows that Syrah wines were characterized by the higher content of ethyl caprylate in comparison with both Bonarda and Malbec, even considering changes with ageing (Fig. 1F). Also benzyl alcohol (Fig. 1D) and diethyl succinate (Fig. 1E) trend to show lower concentrations in Syrah wines, though values were not always significant different with other two studied varieties. Additionally, higher values observed with ethyl caprylate in Syrah wines from the Valley of Tulum (Province of San Juan, Argentina) (Fig. 1F) could be related with the aroma of ripe fruits of wines elaborated in this region. Also from Fig. 1 we can see that 1-hexanol presented its major concentration in Malbec wines (Fig. 1A), while Bonarda was characterized by its low content of ethyl caproate (Fig. 1C). Ethyl caprylate and ethyl caproate were used by Gil et al. (2006) to differentiate red young wines from DO "Vinos de Madrid". Rocha et al. (2004) used ethyl caprylate as one of the most powerful odorant in Baga red wines. Thus, observed differences in VOCs profile could be attributed to wine variety with a light influence of ageing, with the exemption of diethyl succinate, which show significant changes with ageing. Our current results agree with Câmara et al. (2006a), who reported that years have not relevant effects on the differentiation between wines varieties.

3.2. Chemometrics of VOCs from commercial wines

The application of forward stepwise LDA on quantitative VOCs data matrix from commercial wines allows 100% right discrimination among three varieties studied, affording five descriptors: ethyl caproate (green apple), ethyl caprylate (ripe fruits-pear-sweet), 1-hexanol (herbaceous-vegetal), benzyl alcohol (flowery-sweet) and isopentyl acetate (banana). A spatial representation of studied wines in the plane defined by the first two canonical functions of SLDA is shown in Fig. 2. We observed that red wines from different varieties could be clearly differentiated, confirming our previous analysis. Commercial wines studied in this work present characteristic VOCs profile, which allowed their differentiation.

Perestrelo et al. (2006) proposed the most powerful odorants of wines on the basis of their odour description and threshold. Our present results show that some VOCs are present in wine at a level of sensorial contribution $I > 1$ (Table 4). However, other VOCs, with aroma index < 1 , were also important to fully differentiate between wine varieties. Thus, the analysis of VOCs by HS-SPME-GC-MS coupled to chemometrics is highly recommended to complement sensory analysis designed to differentiate wine varieties. So far, we conclude that the aromatic descriptors: ethyl caprylate, 1-hexanol and ethyl caproate could be used as chemical markers of variety among red wines of the studied region. Considering that three grape varieties studied grew in the same vineyard, with common characteristics of climate, viticulture and oenological factors, it was evident that the VOCs profiles of studied wines were influenced by the grape variety. So, we decided to investigate if differences in the fermentative process could affect the observed VOC pattern. Thus, we carried out scale wine-making using different methods-yeasts (Table 1).

3.3. Scale-fermentation wines. Effects of must and yeast on VOCs

The average concentrations of selected VOCs in scale-wines, fermented with different yeast, are shown in the Table 5. Ethyl esters

showed $I > 1$ (data not shown), demonstrating their importance in the aroma of wines (Gil et al., 2006; Rocha et al., 2004).

S. cerevisiae is usually the specie that produces ethyl esters. Rojas, Gil, Piñaga, and Manzanares (2003) reported that levels of ethyl esters did not decrease when using non-*Saccharomyces* in mixed cultures. The same behaviour was observed when comparing the results of spontaneous fermentation and musts inoculated with selected yeasts, which presumably dominate the fermentation. Moreover, recent studies demonstrated that differences between chemical compounds synthesized by different strains of *S. cerevisiae* are not very important and these are just simple quantitative attributes (Loscos, Hernandez-Orte, Cacho, & Ferreira, 2007; Hernández-Orte et al., 2008).

Our current results show that diethyl succinate was present at highest concentration in all scale-wines analyzed (Table 5), showing values close to those observed with young commercial wines (Fig. 1E). However, ethyl caproate and ethyl caprylate had the highest aromatic rates, contributing largely to the fermentative aroma descriptors associated with "fruit" and "flowers" in wines fermented either with different yeast or spontaneously. These last results agree with a previous report (Perestrelo et al., 2006).

Scale-wines obtained from spontaneous fermentations (Bo2 and Sh1) did not show significant differences regarding the content of ethyl caproate and ethyl caprylate (Table 5). Malbec scale-wines (Ma1, Ma2 and Ma3) exhibited the highest values of ethyl caproate and ethyl caprylate, showing significant differences with Bonarda and Syrah (Table 5). Yeast2 and Yeast3 produced wines with quantities of ethyl caproate similar to those reported by Lambrechts and Pretorius (2000).

The content of isopentyl acetate, also known as "banana" bouquet, exceeded the aromatic index (data not shown) and was the highest in Sh2, Ma1 and Ma2, and lowest in Bo2 wines (Table 5). The average content of isopentyl acetate in fermentations inoculated with *S. cerevisiae* showed significant differences depending on the yeast used, showing the same behaviour in natural fermentations (Table 5), being within the values reported by Patel and Shibamoto (2003).

Scale-Malbec had the highest concentration of 1-hexanol but with $I < 1$ (data not show). Thus, 1-hexanol does not contribute to the overall flavour of scale-wines but it is important to differentiate Malbec from Syrah and Bonarda (Table 5). This last result is coincident with that obtained with commercial wines (Fig. 1A). 1-Hexanol is a compound formed by the action of enzymes during the pre-fermentation. However, some authors reported differences in the amount of 1-hexanol for wines fermented with different yeast strains (Herraiz & Ough, 1993; Pérez-Coello, Briones Pérez, Ubeda Iranzo, & Martín Alvarez, 1999). We did not find significant differences in 1-hexanol between scale-wines elaborated with different yeast. Conversely, we observed significant differences between grape varieties (Table 5 and Fig. 1A). This last discussion reinforces the use of 1-hexanol as a good chemical marker (VOC) for differentiating between wine varieties, though its aroma index is low and could be not appreciated during sensory analysis.

The higher content of benzyl alcohol was recorded in scale-wines made from Malbec grapes, showing significant differences between the type of yeast used and the variety of grape. The aroma index was < 1 for wines analyzed, so this compound not contributes greatly to the overall flavour of wine. So far from the previous discussion, benzyl alcohol varies with both grape variety and yeast used during the fermentation; accordingly, it cannot be regarded as a good chemical marker to differentiate wine variety.

So far from the previous discussion, it seems that levels of pre-fermentative VOCs, like 1-hexanol, are not affected by the yeast used during the fermentation but mainly dependent from grape variety. On the other hand, it looks that VOCs formed during fermentation, like ethyl caprylate and ethyl caproate are affected by

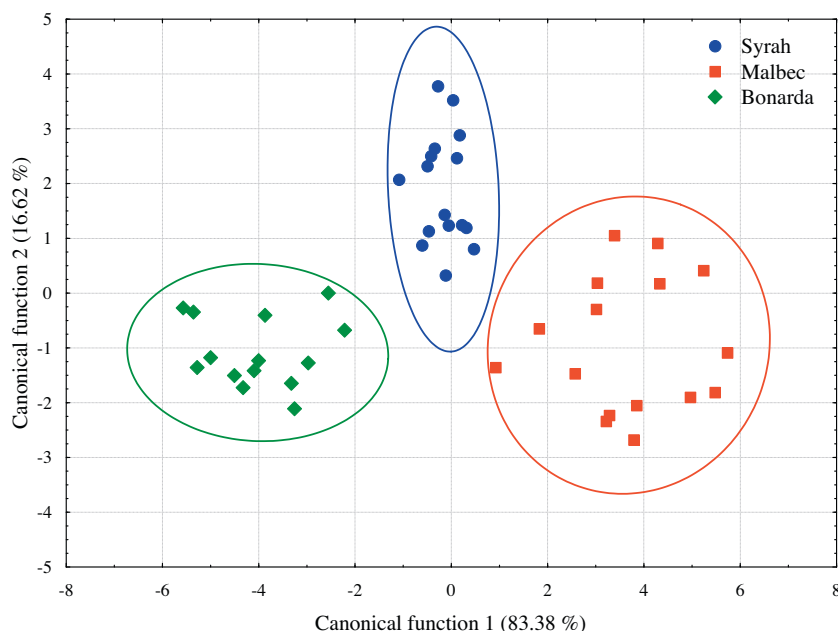


Fig. 2. Distribution of commercial wines, according to their variety, in the plane defined by first two canonical functions, calculated by LDA.

Table 5

VOCs in scale-red wines fermented using different yeasts.

Wine	Volatile organic compounds (VOCs)					
	Ethyl caproate	Ethyl caprylate	Diethyl succinate	1-Hexanol	Benzyl alcohol	Isopentyl acetate
Sh1	0.29 ± 0.02 ^b	0.13 ± 0.01 ^a	4.01 ± 0.37 ^a	1.71 ± 0.06 ^a	0.57 ± 0.06 ^{a,b}	0.41 ± 0.01 ^{b,c}
Sh2	0.26 ± 0.02 ^{a,b}	0.12 ± 0.01 ^a	4.69 ± 0.88 ^{a,b}	1.56 ± 0.13 ^a	0.65 ± 0.19 ^{a,b,c}	0.53 ± 0.02 ^d
Ma1	0.45 ± 0.06 ^{c,d}	0.19 ± 0.02 ^b	7.58 ± 0.36 ^c	2.00 ± 0.11 ^b	0.70 ± 0.03 ^{b,c}	0.54 ± 0.05 ^{d,e}
Ma2	0.47 ± 0.03 ^d	0.23 ± 0.02 ^c	7.57 ± 0.25 ^c	2.17 ± 0.19 ^b	0.76 ± 0.02 ^c	0.59 ± 0.05 ^e
Ma3	0.41 ± 0.02 ^c	0.20 ± 0.02 ^b	5.13 ± 0.24 ^b	1.95 ± 0.11 ^b	0.72 ± 0.02 ^c	0.44 ± 0.03 ^c
Bo2	0.29 ± 0.01 ^b	0.13 ± 0.01 ^a	7.41 ± 0.40 ^c	1.50 ± 0.08 ^a	0.63 ± 0.03 ^{a,b,c}	0.30 ± 0.01 ^a
Bo1	0.23 ± 0.02 ^a	0.12 ± 0.01 ^a	8.88 ± 1.09 ^d	1.50 ± 0.15 ^a	0.53 ± 0.03 ^a	0.36 ± 0.02 ^b

Wines produced at industrial scale fermentations according to Table 1. Sh1, Syrah (spontaneous fermentation); Sh2, Syrah (fermentation with yeast 1, inoculated); Ma1, Malbec (spontaneous fermentation); Ma2, Malbec (fermentation with yeast 2, inoculated); Ma3, Malbec (fermentation with yeast 3, inoculated); Bo1, Bonarda (fermentation with yeast 4, inoculated); Bo2, Bonarda (spontaneous fermentation). Values are expressed in mg L⁻¹, averaged over three samples each analyzed in triplicate. Different letters refer to significant difference using one way ANOVA ($p < 0.05$).

the yeast used. Although these last VOCs can be used to differentiate wine varieties, particularly Bonarda trends to have the lowest values of ethyl caproate, changes induced by wine ageing should be considered. The last is the case with diethyl succinate, which should be avoided as chemical marker for wine variety. It is also worthy to remark that the odour threshold of compounds could be useful for sensory analysis but it should be complemented with the use of instrumental analysis, namely HS-SPME-GC-MS to detect variations in VOCs having low aroma index, like 1-hexanol.

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