Influence of Polyphenol Levels on the Perception of Aroma in *Vitis vinifera* cv. Malbec wine

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The aim of this study was to explore the effect of two ranges of polyphenols naturally present in Malbec wine, high concentrations (4.5-7.2 g/L) and low (1.4-3.2 g/L), on the perception of aroma. Samples with a maximum ethanol level of 13.5% were taken from the fermentation tanks before the clarification and filtration process. A Quantitative Descriptive Analysis of wines was assessed by ten trained assessors, and HS-SPME-GC-MS and physicochemical analyses were performed. The intensities of fruity ($P < 0.01$), citrus ($P < 0.01$), strawberry ($P < 0.05$), cooked fruit ($P < 0.01$) and floral ($P < 0.01$) aromas decreased when the level of polyphenols increased. Neither volatile compounds nor physicochemical analyses were significant in the two groups of wines.

INTRODUCTION

Flavour results from the integration of aroma, taste and chemosensory information within the brain; it is a multisensory stimulus that, in wine, is greatly dependent on the volatile substances present both in the sample matrix and in the headspace. Polyphenols are major non-volatile components in red wine that contribute to mouth-feel properties or interact with volatile compounds in solution (Aronson & Ebeler, 2004). The polyphenol content of wine has been widely recorded as responsible for astringency and bitterness (Arnold & Noble, 1978; Gawel, 1998; Monteleone *et al.,* 2004; Vidal *et al.,* 2004; Lesschaeve & Noble, 2005; Condelli *et al.,* 2006), but its effect on aroma perception has been evaluated to a lesser extent.

Several studies that focused on the study of volatile compounds, their relation with perceived aroma and the effect of certain polyphenols have been carried out. Dufour and Bayonove (1999) investigated the influence of catechins and a wine with a highly condensed tannin fraction on the volatility of aromatic substances using a dynamic headspace technique; although the tannin fraction induced a slight decrease of benzaldehyde volatility and a salting out of limonene, it had no effect on the volatility of isoamyl acetate and ethyl hexanoate. More recently, Aronson and Ebeler (2004) investigated the action of polyphenol concentrations in the headspace and perceived aroma intensity by Headspace Solid Face Microextraction (HS-SPME) and sensory methods. They found that gallic acid significantly decreased the volatility of 2-methylpyrazine while naringin had less effect. Furthermore, when tannins were added to wines, an effect on flavour volatility was detected by

gas-chromatography (GC) analysis, but this was less evident through sensory evaluation.

Lund *et al*. (2009) found that the perception of isobutyl methoxypyrazine, 3-mercaptohexanol and ethyl decanoate was largely suppressed by the addition of polyphenols, while the perception of 3-mercaptohexanol was accentuated with a caffeic acid addition in Sauvignon Blanc wines. Notably, the mentioned studies were conducted in aqueous "model wine" solutions or in a base wine with additional wine-derived compounds. All authors have stressed the need to imitate the real context, but none of the studies were assessed with authentic wine samples, except the recent work by Sáenz-Navajas *et al.* (2010), who studied the effect of the non-volatile matrix on the aroma perception of white and red wines by combining different volatile and non-volatile extracts.

SPME has become a popular method for selectively extracting and concentrating analytes without the use of solvents (Pawliszyn, 1999); the fibres being available in different coating combinations enlarges the field of possible applications (Dietz *et al.,* 2006). This is a simple method that allows a parallel with the process of olfaction and making use of multivariate analysis tools such as Principal Component Analysis makes good differentiation and characterisation possible.

The aim of the current study was to explore the effect of two ranges of polyphenols, high (4.5-7.2 g/L) and low (1.4-3.2 g/L), that are naturally present in Malbec wine on the perception of aroma. The cut-off values for high and low polyphenol content were determined according to levels found in non-commercial and commercial wines respectively (Faitová *et al.,* 2004).

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MATERIALS AND METHODS
Wine samples
The results of our previous work have shown that the ethanol level is an important factor that could affect the detection of a wine's volatile compounds and the perception of aroma attributes when it surpasses 14.5% (Goldner *et al.,* 2009). For this reason, twenty-eight Malbec wine samples (*Vitis vinifera* cv. Malbec) were selected from a set of fifty-six with an ethanol percentage of less than 14.5%. The ethanol (AOAC, 1990) ranged from 10.5% to 13.5%. Grapes were harvested during March 2004 at 23 to 25°B, from vineyards of between 10 and 12 years maturity; fermentation times had been 7 to 10 days, maceration times 13 to 21 days and no malolactic fermentation or wood treatment had occurred (These data were supplied by each winery). Samples were obtained from fermentation tanks after maceration and before clarification and filtration, and were provided by twenty-eight different wineries from seven viticulture regions in Argentina (seven from Alto Río Mendoza; five each from Patagonia, San Juan, Valle de Uco; three from Mendoza del Este; two from Mendoza del Sur; and one from Valles Calchaquíes). The following specifications (AOAC, 1990) were applied: 20.9 to 32.3 g/L dry extract, 3.56 to 5.93 g/L tritatable acidity, 1.80 to 4.10 g/L reducing sugars and 3.28 to 4.21 pH.

The samples were classified into two groups according to their total polyphenol concentration (Folin-Ciocalteau method), taking into account information from Faitová *et al.* (2004): polyphenol levels found in commercial wines (1.4-3.2 g/L; samples No. 1 to 12) and polyphenol levels up to (4.5-7.2 g/L; samples No. 13 to 28). These, we will call "low" and "high" when referring to these two ranges respectively.

First, a sensory experiment was conducted in order to avoid esters hydrolysis or phenolic changes; then, samples were frozen at -18°C until chromatography and physicochemical determinations were completed.

Total polyphenols
Total polyphenols were determined by the Folin-Ciocalteau method (Folin-Ciocalteau reagent, Merck KgaA Darmstadt, Germany) and the results were expressed in gallic acid equivalent (GAE) g/L. Absorbance (spectrophotometer Shimadzu PharmaSpec UV-1700) of each wine (5 mL) diluted 1:10, as well as the absorbance of gallic acid standards in concentrations of 0, 0.05, 0.10, 0.15, 0.25, 0.50, 1.00, 1.50, 2.50, 5.00, 7.00 and 7.50 g/L, were determined at 760 nm against a reagent blank. Measurements were taken in duplicate and averaged. A calibration curve showing the absorbance/concentration of gallic acid was used to determine the GAE for wines.

HS-SPME isolation of volatile compounds
A Supelco fibre holder (Bellefonte, PA, USA) with a 100 μm polydimethylsiloxane (PDMS) coated fused-silica fibre, was used for adsorbing volatile compounds from the head space of properly conditioned samples. Before extraction, the fibre was conditioned for 30 min at 250ºC in the injection port of the gas chromatograph. Samples (8 mL) were placed into a 20-mL amber glass vial (Varian), saturated with sodium chloride (2.0 g) and then capped with a septum. Each wine sample was heated to 40ºC and sonicated for 30 min in an ultrasonic bath (Branson 2510) with the fibre introduced into the glass vial

headspace (Goldner *et al.,* 2009). After equilibration, the fibre was removed from the sample and the analytes were thermally desorbed in the injection port of the GC-FID-MS instrument for analysis.

GC–FID-MS analysis
Aroma compounds were analysed by means of a GC-FID-MS apparatus with a special configuration (Retta *et al.,* 2009): A Perkin Elmer Clarus 500 equipped with one injector (split/splitless) was connected using a flow splitter to two capillary columns (J&W, Scientific): a) polyethylene glycol PM *ca*. 20.000, and b) 5% phenyl-95% methyl silicone, both 60 m x 0.25 mm with 25μ of fixed phase. The polar column was connected to a FID, while the non-polar column was connected to a FID and a quadrupolar mass detector (70 eV) by a vent system (MSVent™). The whole system operated at a constant flow of 1.87 mL/min., with Helium being used as the gas carrier. The injector temperature was set at 255ºC for splitless injection. The sampling time was 3 min. The column temperature was programmed according to the following: 40ºC for 5 min, increasing by 6ºC/min to 230ºC and maintained for 13 min. Both FID temperatures were 240ºC, and the temperatures for the transference line and ionic source were set at 180ºC and 150ºC, respectively. Mass range (*m/z)* was 40-350 Da.

Identification of the compounds was taken from the retention indices (relative to C_8-C_{24} *n*-alkanes) obtained from both columns and compared with those of reference compounds, and by comparison of mass spectra using the usual libraries (Wiley/Nist, 2005; Adams, 2007). The mass spectra were obtained from reference compounds. The relative percentage contribution of the compounds was calculated from the total ion chromatograms by a computerised integration, assuming all of the response factors were 1.

Sensory analysis
Panel training
Ten paid blinded judges (four females and six males, of 21 to 55 years old) from the panel of the Staffing and Training Group (a Buenos Aires consulting company), were trained in descriptive analysis of Malbec wine. During the training period (five sessions of two hours) judges performed the following tasks: 1) aroma identification using extracts diluted in water with 2% ethanol (IRAM 20006, 1996); 2) aroma identification using standard solutions in wine; 3) use of structure scale. These panellists had prior experience in quantitative descriptive analysis of milk, mayonnaise, dairy and perfumery products.

Descriptive analysis
The Descriptive Analysis (Stone & Sidel, 1993; ASTM, 1992) was made using a 9-point intensity scale ranging from low to high. The panel leader received the scores orally (to simplify the work of blinded judges) and recorded them on a sheet of paper). Samples were tested in a conditioned room on individual tables. All samples (50 mL) were from a single bottle (750 mL) and were presented at 18 ± 2°C in transparent tulip-shaped glasses, covered with glass petri dishes and identified by random three-digit codes. A randomised incomplete block design was used to evaluate all the wines (the original set of fifty-six) and eight samples were presented for the session in the morning (2.5 h) and this was duplicated in the afternoon (2.5 h). The following

TABLE 1
ANOVA of the mixed model of sensory data.

	ANOVA *P*-values					
	Replication	**Wine**	**Assessor**	**R×W**	**R×A**	**W×A**
df	1	27	9	27	9	243
Fruity	0.521	0.122	0.001**	0.265	0.902	0.024*
Citrus	0.962	0.001**	0.169	0.113	0.307	0.006**
Strawberry	0.281	0***	0***	0.624	0.735	0.008**
Plum	0.007**	0.316	0***	0.063	0.947	0.066
Raisin	0.008**	0.026*	0.001**	0.721	0.738	0.006**
Spicy	0.129	0.006**	0.004**	0.444	0.432	0.270
Cooked fruit	0.470	0.002**	0.126	0.968	0.897	0.012*
Floral	0.847	0.014*	0.024*	0.091	0.366	0.014*
Honey	0.595	0***	0.005**	0.213	0.690	0.122
Herby	0.031*	0***	0***	0.026*	0.826	0.037**
Sweet pepper	0.295	0.256	0.058	0.295	0.204	0.150

*$p < 0.05$ **$p < 0.01$ ***$p < 0.001$
df: degrees of freedom
Error df: 243
R: replication
W: wine
A: assessor

attributes were selected for descriptive analysis: fruity, citrus, strawberry, plum, raisin, cooked fruit, floral, honey, herby, spicy and sweet pepper. (For more details of how these attributes were selected, see Goldner and Zamora, 2007.)

Data analysis
The assessors' performance was studied using an ANOVA model, in which the assessor was considered a random factor, wine and replication as fixed factors and with double replication X assessor, replication X wine and wine X assessor interactions. An analysis of variance (ANOVA) was carried out to assess attributes, volatile compounds and physicochemical characteristics that were significantly different among wines where polyphenol levels were considered a fixed factor. A Pearson correlation was calculated between sensory and GC data. The Principal Component Analysis (PCA) of both the average panel and GC data was evaluated to compare the relationship among sensory attributes and volatile compounds. A covariance matrix was used and the minimum eigenvalue was set at 1. All data were processed using Infostat version 2009p.

RESULTS AND DISCUSSION
Panel performance
The results of the ANOVA of sensory data are summarised in Table 1. Sources of variation were replication, wine, assessor and double interactions. Each wine was evaluated by ten assessors for duplication (28 wines X 10 assessors X 2 replications = 560 observations). Replication X assessor interactions were non-significant, indicating that assessors were consistent in their judgments. The judges showed good reproducibility; replication X wine interactions were non-significant except for herby aroma. Assessors displayed significant ($P < 0.05$) effect in most of the attributes; this means they evaluated samples in a different way, but their assessments were consistent between two replications. Wine X assessor interactions were significant ($P < 0.05$) for citrus, strawberry, raisin, cooked fruit, floral and herby attributes. Tang *et al*. (1999) and Zamora and Guirao

TABLE 2
Mean sensory attributes from two polyphenol ranges of 28 wines.

Attribute	Mean sensory attribute from polyphenol ranges (g/L)	
	4.5-7.2	1.4-3.2
Fruity	2.18±0.14	2.93±0.19**
Citrus	1.37±0.14	2.32±0.28**
Strawberry	2.46±0.19	3.20±0.33*
Plum	2.93±0.13	3.34±0.29
Raisin	2.22±0.17	2.25±0.34
Spicy	2.54±0.23	2.05±0.28
Cooked fruit	1.73±0.15	2.78±0.31**
Floral	1.85±0.11	2.68±0.29**
Honey	2.13±0.24	2.54±0.29
Herby	2.45±0.30	1.97±0.30
Sweet pepper	2.47±0.21	1.96±0.26

* $p < 0.05$ ** $p < 0.01$

(2002) obtained similar results in their sensory measurements and demonstrated that this could happen when samples are very similar in their sensory properties and panellists are unable to differentiate between them easily. Added to that are the individual assessor's existing differences, which is common in sensory studies (Brockhoff & Skovgaard, 1994; Naes & Langsrud, 1998; Kreutzmann *et al.,* 2007).

Effect of polyphenol concentration on aroma perception
The ANOVA of the sensory, volatile compounds and physicochemical data showed those variables that affected polyphenol levels. Differences in physicochemical data were not significant among polyphenol ranges: pH [$F_{(1, 26)} = 0.011$], dry extract [$F_{(1, 26)} = 2.768$], tritatable acidity [$F_{(1, 26)} = 1.182$], reducing sugars [$F_{(1, 26)} = 0.011$] and density [$F_{(1, 26)} = 0.011$]. The ethanol percentage was not a significant factor in the selected wines [$F_{(1, 26)} = 0.362$], thus it was ensured that the range of ethanol selected (10.5 to 13.5%) was suitable for studying the incidence of polyphenol levels in aroma perception (Escudero *et al.,* 2007; Goldner *et al.,* 2009).

The effect of the polyphenol content was classed as suppressing or accentuating, depending on whether the

intensity values increased or decreased between the ranges. The perception of red wine aroma was affected by polyphenol levels in five attributes (one at $P < 0.05$ and four at $P < 0.01$, Table 2). The aroma intensity of fruity, citrus, strawberry, cooked fruit and floral attributes all decreased when the levels of polyphenol increased; the aroma suppression observed indicated polyphenol interaction with volatile species. This was in agreement with Sáenz-Navajas *et al.* (2010) who reported an intense effect of the non-volatile matrix on the aroma perception of wine. These authors also stated that the presence of a red wine matrix with the highest polyphenol content brought about significant increases in dry fruit, vegetal, animal and undergrowth notes and significant decreases in fruitiness in yellow, citrus and exotic fruits. Our results showed a tendency to the accentuation of spicy, herby and sweet pepper aromas but this was not significant (Table 2).

Seventeen aroma compounds were identified: ethyl acetate, isobutanol, n-pentanol, 3-methyl butanol, 2-methyl butanol, toluene, furfural, ethyl isovalerate, hexanol, isoamyl acetate, ethyl hexanoate, 2-phenyl ethanol, diethyl succinate, ethyl octanoate, ethyl phenylacetate, vitispirane and ethyl decanoate. Applying an ANOVA of the GC data showed that the polyphenol content on volatile substances detected by HS-SPME was not significant, except for 2-methyl butanol, which displayed a relative minor area when the polyphenol level increased.

The polyphenols naturally present in wine affected the olfactory perception, but it was not evident by HS-SPME-GC-MS. Aronson and Ebeler (2004) found similar results under different conditions: the effect of polyphenol addition on the detection by HS-SPME was non-significant at low concentrations of flavour in model solutions.

Here it is important to take into account the type of sample. This experiment was conducted on samples with a polyphenol content that was not modified with added tannins, gallic acid or others; the wide range of polyphenol concentration was naturally present in the complex matrix of the wine and could

have been due to climate conditions, region of origin (Frankel *et al.,* 1995) or vinification technology (Villariño *et al.,* 2006). In the present study, the suppressing effect of polyphenols on fruitiness and floral aromas was found to be noteworthy.

Since the extraction efficiency of red wine aroma compounds by HS-SPME strongly depends on their polarity, their affinity to the fibre-coated phases, the temperature and time on the equilibrium (De la Calle García *et al.,* 1998; Baptista *et al.,* 2001), it is probable that, in the results reported in this paper, interactions between phenol and volatile compounds were not significant enough to be measured by SPME fibre. The PDMS fibre used in this study is considered by many authors as the most suitable for adsorbing volatile compounds from wines and other alcoholic beverages (Kafkas *et al.,* 2006). Dietz and co-workers (2006) stated that, in general, a wide range of possible solutions exist for a given analytical problem in SPME. Therefore, it is known that fibres with an additional adsorbent phase such as DVB or Carboxen are the most effective (Shirey, 1999), and it is usually necessary to combine different methods to obtain a complete extraction of volatile compounds (Mamede & Pastore, 2006). Sáenz-Navajas *et al.* (2010) confirmed that the red wine non-volatile matrix has a higher retention power, which reduces the volatility of the compounds; scores of Gas Chromatography-Olfactometry data tended to be smaller on red wines with the highest total polyphenol content. The effects resulting from the presence of matrices from red wines are more complex, slightly weaker and more matrix-dependent.

The Pearson correlation allows evaluation of the variables influenced by the polyphenol levels in a linear way; coefficients among sensory attributes are displayed in Table 3, the polyphenol concentration always being incorporated. Fruity aroma showed positive coefficients among strawberry, plum, cooked fruit, floral and honey aromas, but negative coefficients with herby aromas, whereas spicy aromas displayed a positive correlation with herby and sweet pepper attributes. Moreover, this coefficient allowed the evaluation of the variables

TABLE 3
Pearson correlations between sensory descriptors and polyphenol content of 28 selected wines.

|	Poly-phenols	Fruity	Citrus	Strawberry	Plum	Raisin	Spicy	Cooked fruit	Floral	Honey	Herby
Fruity	**-0.555****										
Citrus	**-0.378***	0.289									
Strawberry	-0.284	**0.624*****	0.283								
Plum	-0.215	**0.508****	0.149	**0.515****							
Raisin	-0.076	0.267	0.191	0.293	**0.564***						
Spicy	0.133	-0.314	**-0.616****	-0.181	-0.030	0.226					
Cooked fruit	-0.306	**0.526****	0.008	0.249	**0.611***	**0.505***	0.135				
Floral	**-0.469***	**0.403***	0.210	**0.492****	**0.485***	0.369	-0.126	**0.592***			
Honey	-0.199	**0.475***	-0.252	**0.483****	**0.440***	**0.605***	0.287	**0.563****	0.374		
Herby	0.138	**-0.500****	-0.051	-0.355	-0.206	-0.273	**0.532****	0.000	-0.206	-0.216	
Sweet pepper	0.309	-0.305	**-0.548****	**-0.402***	-0.168	0.204	**0.718****	0.198	-0.150	0.270	**0.438***

* p < 0,05 ** p < 0,01 *** p < 0,001
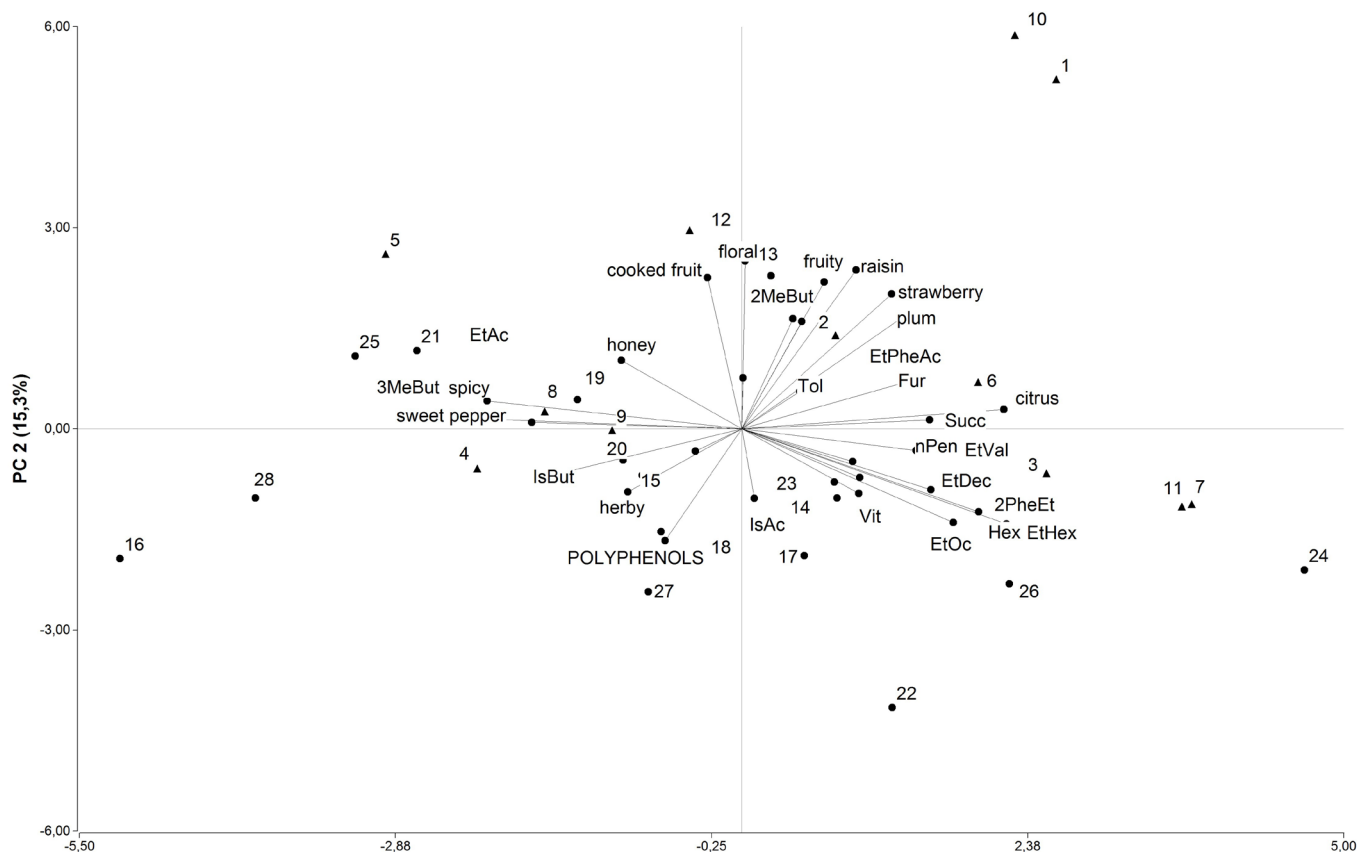
FIGURE 1
Principal component analysis of the aroma attributes and volatile compounds of 28 wines at two polyphenol levels
(Low: samples 1 to 12 and High: samples 13 to 28).

influenced by polyphenol levels: fruity, citrus, cooked fruit and floral aromas correlated in a negative way.

The Pearson coefficients among aroma and GC data showed positive correlation between fruity ($P < 0.05$), plum ($P < 0.05$), cooked fruit ($P < 0.01$) and floral ($P < 0.05$) aromas with 2-methyl butanol; citrus with vitispirano ($P < 0.05$); strawberry with ethyl succinate ($P < 0.05$) and floral and plum with ethyl phenyl acetate ($P < 0.05$) (Data not shown). This accompanies the affirmation by Goyert *et al.* (2007): olfactory receptors do not act as detectors of isolated molecular features, but more likely recognise entire molecules closely associated with perceived olfactory qualities.

Principal component analysis of sensory and GC data
This multivariate technique of data analysis has been used to explain wine differentiation and to obtain more information on the variables that mainly influence similarities and differences. Principal component analysis (PCA) of sensory and GC variables accounted for a 74.7% of the variance among samples. The results obtained for all studied samples of wines were projected onto a two-dimensional plot defined by the first two Principal Components, PC2 vs. PC1, where the polyphenol concentration was always taken into account, as is shown in Fig. 1. Citrus, strawberry, plum, fruity, floral, raisin, n-pentanol, hexanol, toluene and furfural attributes were positively correlated along PC1. In addition, most esters were always associated with the above attributes, denoting their relation with fruitiness aromas

(Escudero *et al.*, 2007), contrary to sweet pepper, spicy, herby, 3-methyl butanol, isobutanol, ethyl acetate and the polyphenol concentration. It can be seen that wines with low polyphenol levels (from No. 1 to 12) clustered with most of the volatile compounds, fruitiness aromas, floral, cooked fruit and honey attributes as opposed to the majority of wines with high polyphenol levels (from No. 13 to 28), which clustered around 3-methyl butanol, isobutanol, ethyl hexanoate, and vegetable aromas along with polyphenol content.

CONCLUSIONS
The aroma of Malbec wine was modified with increasing polyphenol levels denoting interaction effects between polyphenols naturally present and volatile substances. One consequence of this interaction was the suppressing effect on fruitiness aromas when polyphenols ranged from 4.5 to 7.2 g/L for samples with 13.5% maximum ethanol. Changes in headspace and matrix concentration could be related to relative changes in sensory intensity but the effect was not significant, neither by HS-SPME-GC-MS nor by physicochemical composition. The results suggest that winemakers could monitor polyphenol concentration and make decisions during the wine process, in order to improve the fruitiness aroma. The results are limited to two ranges of polyphenols and Malbec wine samples; they may be confirmed by further studies on other sets of samples.

ABBREVIATIONS

EtAc = ethyl acetate; EtDec = ethyl decanoate; EtHex = ethyl hexanoate; EtOc = ethyl octanoate; EtPheAc = ethyl phenylacetate; EtVal = ethyl isovalerate; Fur = furfural; Hex = hexanol; IsAc = isoamyl acetate; IsBut = isobutanol; nPen = n-pentanol; Succ = diethyl succinate; Tol = toluene; Vit = vitispirane; 2MeBut = 2-methyl butanol; 2PheEt = 2-phenyl ethanol; 3MeBut = 3-methyl butanol.

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