Effect of the 'terroir' on the volatiles of *Vitis vinifera* cv. Albariño

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Abstract:

The effect of the 'terroir' on volatiles of *Vitis vinifera* cv Albariño was studied. Twelve commercial Albariño wines from Galicia (Spain) were analyzed. The content of varietals and fermentative volatile compounds was determined by gas chromatography. The numerous significant differences found for most of the aromatic compounds studied show the influence of the 'terroir'. The Albariño wines from northern Galicia showed the highest total concentration of volatiles analyzed. The volatiles components showing the greatest differences in Albariño wines from different areas were terpenes and higher alcohols. Among the terpenes found, geraniol was markedly abundant in the north, while nerol and linalool were most abundant in the south. Among the alcohols, 2-phenyl ethanol and benzyl alcohol showed the highest concentrations in the south and in the north, respectively. The cluster analysis and PCA revealed two clearly defined main groups of Albariño wines from different 'terroirs'. Albariño wines from the south were more heterogenic than those of the north.

Keywords:, Albariño variety, Aroma compounds, Characterization, Terroir, Volatile composition

INTRODUCTION

Aroma is one of the most important factors determining wine character and quality. More than 800 volatiles compounds, such as alcohols, esters, organic acids, phenols, thiols, monoterpenes and norisoprenoids, have been found in wines.[1] The varietal flavor of wines is due to the present of monoterpenes, norisoprenoides and thiols. [1] Among the volatiles derived from yeast metabolism are the esters, alcohols and acetates. Ethanol and glycerol are quantitatively dominating alcohols, followed by higher alcohols and esters.[2]

Albariño, *Vitis vinifera L.*, is a grape variety used for the production of one of the highest quality white wines in Spain. This variety is authorized in three Appellations of Origin Controlled (AOC) from Galicia: Rías Baixas, Ribeiro and Ribeira Sacra. Differences in climate and soils between north and south of Galicia (distance 180 km) make the wines from the different zones individual in their own right.

The north is rocky, with alluvial topsoils, and located at sea level where two "rias" (deep river estuaries) can be found. Further south, along the east-west border with Portugal marked by along the Miño River, there is a fluvial area. The vineyards here are terraced on the sides along the river, which opens into a wide estuary. The topsoil is correspondingly alluvial and granite bedrock and slate come to the surface.

The Atlantic climate Galicia, with wet winters and sea mists, varies greatly from zone to zone. The northern region is the coolest, given its position on the coast. Although the hottest region is found in the south, occasionally temperatures reaching above 40ºC, this region harbours cold winters with frost and annual rainfall reaching up to 2000 mm.

The Albariño variety is characterized by a high intensity of floral descriptors, free monoterpenes being responsible for these floral notes. [3,4] This variety, both as a grape and as a wine, has been the focus of previous studies. [3,5,6,7,8,9] Young white wines elaborated with Albariño grapes from Galicia contain high concentrations of terpenes and have strong fruity and floral odours. [3,10]

The present study analyzes the volatile composition of Albariño variety grown in the northern and southern of Galicia, to determinate the influence of the "terroir" on wine volatiles. Multivariate techniques of data analysis were used for these comparisons.

MATERIALS AND METHODS

Wine samples

Twelve commercial Albariño wines were obtained from different producers in Northern and Southern of Galicia. All wines were produced from 100% of grapes from specified area. The wines were fermented at controlled low temperature (18ºC) in stainless steel tanks. Production techniques were uniform for all wines studied. The wine samples were collected and storage at 10ºC prior to the analysis one year after.

Sample preparation

Varietal compounds

Free and bound terpenes were fractionated by selective retention on SepPak Vac C-18 (1 g, purchased from Waters), according to the procedure described by Di Stefano, [11] with some modifications. [5] The cartridges were sequentially conditioned with methanol (5 mL) and distilled water (10 mL). A sample of 100 mL of wine diluted with 100 mL of distilled water and containing 1 mL of internal standard (3-octanol at 10 ppm in ethanol), washing the residual with 25 mL of distilled water. The free fraction was eluted with 10 mL of pentane-dichloromethane (2:1) and the solution was dried over anhydrous sodium sulphate and, prior to GC analysis, concentrated to 0.5 mL, by evaporation under nitrogen stream. The bound fraction was eluted with 10 mL of methanol and concentrated to dryness at the Rotavapor before dissolution in 5 mL of citrate-phosphate buffer (pH 5.0). 200 µL of enzyme solution with β-glycosidase activity (0.5 g of AR-2000 (Gist Brocades, France) in 5 mL of the same buffer) was added, and the mixture was incubated at 40ºC for 18 h to accomplish enzymatic hydrolysis. After the addition of the same internal standard (3-octanol), the aglycons were extracted on SepPak Vac C-18 (1 g), according to the procedure described, previously, to the free forms. Before GC analysis, the organic phase was dried with sodium sulphate and concentrated to 0.5 mL by evaporation with a stream of nitrogen.

Fermentative compounds

In the determination of methanol and higher alcohols, due to their high concentrations in wines, 1 mL of an internal standard solution (1 g of 4-methyl-2-pentanol per 1 L of ethanol) was added to 10 mL of the sample, prior to GC analysis.

Extraction of esters and acetates was carried out according to the method described by Bertrand[12]: 2 mL of 3-octanol (50 mg/L) as internal standard and 1 mL of sulphuric acid (1/3) were added to 50 mL of wine. Each sample was extracted three times with 4, 2 and 2 mL of diethyl ether-hexane (1:1, v/v).

Chromatographic analysis

The analyses were carried out using a Hewlett Packard 5890 Series II Gas-Chromatograph equipped with an HP 6890 Automatic Injector and a Flame Ionisation Detector (hydrogen, 40 mL min^{-1}; air, 400 mL min^{-1}). The compounds were separated on a CHROMPACK CP-WAX 57CB (polyethylene glycol stationary phase; 50 m × 0.25 mm id with 0.25 μm film thickness) fused-silica capillary column.

Instrumental conditions

For analysis of varietal compounds were: column temperature, 60ºC for 5 min, rising to 200ºC at 3ºC/min, then 200ºC for 25 min; injector temperature: 250ºC; detector temperature: 260ºC; make-up gas: nitrogen 25 mL min^{-1}; injection mode, Splitless (30 s); volume injected, 1.0 µL; carrier gas: helium at 1.07 mL min^{-1}.

For methanol and higher alcohols were: column temperature, 60ºC for 15 min, rising to 200ºC at 3ºC/min; injector temperature: 250 ºC; detector temperature: 260ºC; make-up gas: nitrogen 25 mL min^{-1}; injection mode, split; split ratio, 1:1; volume injected, 1.0 µL; carrier gas: helium at 1.07 mL min^{-1}.

For esters and acetates were: column temperature, 55ºC for 15 min, rising to 200ºC at 3ºC/min; injector temperature: 250 ºC; detector temperature: 260ºC; make-up gas: nitrogen 25 mL min^{-1}; injection mode, Splitless (30 s); volume injected, 1.0 µL; carrier gas: helium at 1.07 mL min^{-1}.

Identification and Quantification

Aromatic compounds were identified by comparing retention times with those of pure compounds and confirmed by GC-MS using a HP5890 Series II coupled to HP 5989 A mass spectrometer in the EI mode (ionization energy, 70 eV, source temperature 250ºC). The acquisition was made in scanning mode from m/z 10 to 1000 at 5 scan/s. Internal standards were used to quantify concentrations of individual compounds.

Statistical analyses

Significant differences among the wines from the two geographic areas were carried out by analysis of variance (ANOVA). An UPGMA cluster analysis was calculated as classificatory procedure, based on a similarity matrix constructed using Euclidean distance. Principal component analysis (PCA) was performed using to find the possible differentiation between wines. These analyses were accomplished using the Enterprise Guide 3 System Software (SAS Institute, Cary, NC, USA).

RESULTS AND DISCUSSION

The aromatic composition of the wines produced with Albariño must from North and South of Galicia (Spain) is shown in Tables 1-3. The concentrations and the deviations standard for 41 variables correspond to the average of six samples from each area analysed in duplicate.

In general the total concentration of volatiles showed that Albariño wine from south of Galicia was higher than Albariño wine from north. The wines elaborated with this variety showed different volatile profile, 42 compounds with significant differences according to the origin, twenty one compounds were significant different at 99.9% level, four compounds with significant differences at 99% level and five at 95% level.

The terpene content (Table 1) is considered to be a positive quality factor, because contribute to its varietal aroma.[13] The concentration of free terpenes was significantly different between the two areas. When we observed the significant different free monoterpenes, we founded that Albariño wines form south of Galicia showed the highest content except geraniol, α-pinene and theaspirane. Among the most relevant free monoterpenes found, geraniol was markedly the most abundant in the north; this is well above its perception threshold of 130 μg/L.[14] Nerol and linalool were the most important terpenes in the south, linalool with levels over the perception threshold, 50 μg/L.[15]

Geraniol and linalool are considered to be the most important of the monoterpenes as they are present in greater concentrations and have flavour thresholds than other major monoterpenes. [16,17]

In general, the presence of norisoprenoids is also considered to be a quality factor and typical from each variety, as they supply an agreeable scent of tea, fruits, floral, mainly rose and violet.[16,18] Also, although usually present in very low amounts (a few μg/L), as their perception threshold is very low, they play an important part in the aroma.[19] Among the free norisoprenoids that were identified (Table 1) α-ionona and β-ionona has only been identified in Albariño wine from south of Pontevedra, booth presents in amounts above their perception threshold, 2,6 μg/L and 5μg/L respectively.[20,21] Theaspirane was only present in Albariño wine from north.

A total of 12 bound compounds were identified in Albariño wines (Table 1). Significantly differences were found in all compounds except bound geraniol and eugenol. Albariño wines from south of Galicia are richer in bound aromatic compounds than in free compounds, due to the presence of β-pineno, linalool, nerol, and α-ionona.

Alcohols and esters were the largest groups made up more than 95% of the free volatiles. Albariño wines from Galicia were characterized by their higher content in some of esters responsible for the good aroma quality.[22] Higher alcohols and esters, produced during alcoholic fermentation, play an important role in the flavour of the wines, depending on the types of compounds and their concentrations. [23]

Among the alcohols (Table 2), 2-phenyl ethanol and benzyl alcohol, which are know aromatic alcohols, were found at high concentrations. The amount of 2-phenyl ethanol in Albariño wine from south of Galicia was higher than the north; the yeast from several grape precursors mainly forms this compound during the fermentation. It has a floral, rose like aroma.[24] Fernández [6] reported that 2-phenylethanol was the most important bound compound of Albariño grapes. Despite its herbaceous connotation, benzyl alcohol appears in higher concentrations in the Albariño wine from north, that seem related to the maturation index of the grape.

The Albariño wine from north and south of Galicia contained 59.17 and 58.53 mg/L of esters and acetates, respectively (Table 2). Higher levels were observed for ethyl acetate, isoamyl acetate, ethyl hexanoate, ethyl octanoate, ethyl decanoate and ethyl succinate in Albariño wines from the north. Vilanova [8] determined the role of the selected *Saccharomyces cerevisiae* strains on the volatile composition of Albariño wines and they haven't found significant differences among wines studied for ethyl and hexyl acetate. These compounds are important in young wine aroma and are synthesized during must fermentation. [1] They are among key compounds in fruity flavour of wines. [25,26]

The very high number of significant differences for the majority of the aromatic compound studied showed the influence of the ‘terroir’.

The first multivariate approach to the data was made by cluster analysis (Figure 1). Two clusters could be observed, one including all samples of north of Galicia (N1 to N6) and other including all samples of south (S1 to S6). The second cluster was more heterogeneous.

Principal component analysis (PCA) was applied to all the samples of wines studied to obtain a more simplified view of the relationship among the volatile compounds analysed. Figure 2 shows the PCA performed on the data. The first two principal components (Prin 1 and Prin 2) accounted for 78.26% of the variance (63.28% and 14.99%, respectively). Free α-pineno, free geraniol, bound α-pineno, bound limoneno, methanol, 1-propanol and ethyl octanoate were the variables that contributed mostly to the positive first axis. The negative part of this axis was mostly influenced by free α-ionona, bound β-pineno and bound nerol. The second component is mainly built up with the contribution of free β-pineno, free linalool, free nerol, free β-ionona, bound terpinen-4-ol and acetal for the positive axis and 2-phenyl ethanol for the negative axis.

A good separation of the wines from different areas was observed. The second axis was highly responsible to the separation between Albariño wines.

CONCLUSIONS

A comparative study was conducted on Albariño wine elaborated from musts from northern and southern Galicia (NW Spain). The influence of ‘terroir’ on varietal and fermentative volatile compounds was studied. Data obtained from gas chromatography showed that differences were present in wine volatiles. The Albariño wine from the north showed higher contents in total free terpenes and acetates. Total higher alcohols and ethyl esters characterized the Albariño wine from the south. 2-phenyl ethanol followed by benzyl alcohol was the most abundant compounds in Albariño wines. Clustering analysis and PCA showed a good separation of the wines from different 'terroir'. Albariño wines from the south were more heterogenic than those from the north. Differentiation of these wines was possible. This behavior could be due to the predominance of terroir' over the varietal character of the wines.

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Table 1. Concentration in free and bound terpenes and norisoprenoides (μg/L) in Albariño wines from Galicia region.

Compounds	North	South	North/South
α-pinene	95.81***	0.00^{ns}	***
β-pinene	14.28***	92.92^{ns}	***
Limonene	18.72***	18.33***	ns
Linalool	23.37***	64.2 ^{ns}	**
Terpinen-4-ol	24.25**	19.67**	ns
α-Terpineol	9.33***	33.91***	***
Citronellol	8.13***	9.51**	ns
Nerol	4.98^{ns}	151.58*	***
Geraniol	153.92***	26.47*	***
Theaspirane	14.29***	0.00^{ns}	***
α-ionone	0.00^{ns}	9.12^{ns}	***
β-ionone	0.00^{ns}	20.56^{ns}	**
Eugenol	20.07**	12.71**	ns
Total free terpenes	*1757.15*	*458.99*	
α-Pineno	179.03*	0.00^{ns}	***
β-Pineno	0.00^{ns}	170.42^{ns}	***
Limonene	22.04**	0.00^{ns}	***
Linalool	14.27***	103.40^{ns}	***
Terpinen-4-ol	6.76***	2.80^{ns}	*
α-Terpineol	15.22***	30.5^{ns}	*
Citronellol	5.79^{ns}	18.05^{ns}	*
Nerol	17.63**	364.24*	***
Geraniol	58.07^{ns}	71.23*	ns
α-Ionone	0.00^{ns}	178.60***	***
β-Ionone	0.00^{ns}	56.39*	***
Eugenol	22.29^{ns}	23.90*	ns
Total bound terpenes	*341.10*	*1019.54*	

The data are mean values of duplicates. Significance at which means differ as show by analysis of variance: *, **, *** denote significance at p<0,05, p<0,01, p<0,001 respectively; ns: not significance

Table 2. Concentration in alcohols, acetates and ethyl esters (mg/L) in Albariño wines.

Compounds	North	South	North/South.
Acetal	10.80***	3.21^{ns}	*
Metanol	67.92**	38.21^{ns}	***
1-Propanol	32.34***	15.22^{ns}	***
1-Butanol	0.00^{ns}	3.26***	ns
Benzyl alcohol	590***	45.46^{ns}	*
2-Phenyl-ethanol	8057.91***	59220.41^{ns}	***
Total alcohols	*8758.97*	*59325.77*	
Ethyl acetate	44.19***	25.35^{ns}	***
Isoamyl acetate	4.18***	0.89^{ns}	**
Hexyl acetate	0.22***	0.50	**
Ethyl lactate	4.19***	13.99**	ns
Ethyl butyrate	0.69^{ns}	15.31**	ns
Ethyl hexanoate	1.69***	1.00^{ns}	***
Ethyl octanoate	2.44^{ns}	0.56^{ns}	***
Ethyl decanoate	0.72***	0.31^{ns}	***
Diethyl succinate	0.76***	0.62^{ns}	ns
Ethyl laurate	0.09***	0.00^{ns}	***
Total esters and acetates	*59.17*	*58.53*	

The data are mean values of duplicates. Significance at which means differ as show by analysis of variance: *, **, *** denote significance at p<0,05, p<0,001 respectively; ns: not significance

Figure 1. Dendogram of clustering analysis of wine samples from Galicia (Northern: N1-N6 and Southern: S1-S6).