AROMATIC DIVERSITY OF *VITIS VINIFERA* cv ALBARIÑO QUANTIFICATION OF AROMATIC POTENTIAL

DIVERSITÉ AROMATIQUE DE *VITIS VINIFERA* cv ALBARIÑO QUANTIFICATION DU POTENTIEL AROMATIQUE

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Abstract: Albariño is the most important cultivar of *Vitis vinifera* in Galicia (Spain) and northern Portugal. Musts from different geographic areas from Galicia have been analysed, in order to obtain their characterization by the contribution index. Monoterpenes were analyzed by gas chromatographic/mass spectrometry. O Rosal should be the most aromatic potential must since it contained significantly higher contribution index values of volatile compounds. The bound forms of the compounds detected are always higher than those of the free forms, as would correspond to a quality variety. The PCA shows a good differentiation among three groups of musts according to their geographic origin. The results of contribution index shown that the fruity aroma followed by the floral had the highest contribution to the overall aroma of the musts.

Résumé : L'Albariño est le cépage de *Vitis vinifera* le plus important de la Galicia (Espagne) et du Nord du Portugal. Nous avons analysé les moûts de raisins provenant des différents zones géographiques de la Galicia, pour les caractériser par l'Indice de Contribution (CI). Les monoterpènes ont été analysés par GC/MS. Le potentiel aromatique du moût d´O Rosal était le plus élevé et les CI des composés volatils étaient significativement supérieurs. Les formes liées des composés détectés étaient dominantes par rapport aux formes libres, ce qui correspond à un cépage de qualité. L'Analyse en Composantes Principales (PCA) a permis une bonne différentiation des trois groupes de moût selon l'origine géographique. Les résultats de CI ont montré que l'arôme fruité d'abord, puis l'arôme floral avaient la plus grande contribution dans l'arôme des moûts.

Keywords: Albariño must, Contribution index, free compounds, bound compounds.

Mots clés : moût d´Albariño, Indice de Contribution, composés libres, composés liées

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J. Int. Sci. Vigne Vin, 2006, **40**, n°4, 197-202

INTRODUCTION

Aroma is one of the main contributing to the quality of musts and wines. The must aroma depends on many factors, environmental, management practices and grape varieties. Aroma and flavour constituents of different grapes have been extensively studied in the last few years. The aromatic components of certain grape varieties are present in the grape berry both in free form and bound to sugars in the form glycosides (CORDONIER and BAYONOVE, 1974; WILLIAMS *et al.*, 1982). Compounds such as terpenols, terpene diols, 2-phenylethanol, benzyl alcohol and C13-norisoprenoids have been to be aglycons of such glycosides (WINTERHALTER and SKOUROUMOUNIS, 1997). The importance of each compound on the must aroma depends on the correlation between chemical composition and perception thresholds, because most of the volatile compounds were present at concentrations near or below their individual sensory thresholds (FALQUÉ *et al.*, 2001).

Terpenes, because of their high concentrations and low aroma thresholds, are the components responsible for the characteristic aroma of a must (CARBALLEIRA *et al.*, 2001). They are mainly derived from the grape, synthesized during maturation, and qualitatively and quantitatively influenced by the cultivar, soil, climate and viticultural practices (BELANCIC *et al.*, 1997; BERTRAND and ANOCIBAR-BELOQUI, 1996). The contribution of each compound to the entire aroma can be estimated by its contribution index (C.I.). Compounds with C.I. > 1 are considered to have active contribution (VERSINI, 1994). The C.I. does not take in account the depressive or synergic odour effects resulting from the interactions of different molecules present in musts, but it can serve as a first approximation to the aromatic potential contribution of each compound to the global aroma (FERREIRA *et al.*, 2002)

Albariño is the typical white *Vitis vinifera* variety from Galicia (northwest Spain) and northern Portugal, characterized by fruity and floral odours (CARBALLEIRA *et al.*, 2001; DIEGUEZ *et al.*, 2003). This variety, grape and wine, has been object of several studies (FERNANDEZ *et al.*, 1999; CARBALLEIRA *et al.*, 2001; VILANOVA and MASNEUF, 2005; VILANOVA and VILARIÑO, 2006).

The objective of this study was to determine the aromatic profile, real and potential, of musts of *Vitis vinifera* cv Albariño from the most important geographic areas in Galicia, where is grown this variety: O Condado do Tea, O Rosal y O Val do Salnés.

MATERIALS AND METHODS

I - MUST SAMPLES

The *Vitis vinifera* cv Albariño must were collected from different geographic areas from Galicia, O Condado do Tea and O Rosal y O Val do Salnés. The musts were transported to the laboratory refrigerated at 8 ºC and were supplemented with 50 mg/L SO_2.

II - DETERMINATION OF AROMATIC COMPOUNDS

To determine the identities and amounts of aromatic compounds present, the extracts were analysed by gas chromatography. All gas chromatographic analysis were performed with a Hewlett-Packard 5890 Serie II chromatograph equipped with flame ionisation detection (FID). Compounds were separated on a 50 m x 0.25 mm internal diameter fused-silica capillary column coated with a 0.20 µm film of Chrompack CP-Wax57CB.

III - FREE AND BOUND VARIETAL COMPOUNDS

Free and bound volatile compounds were fractionated by selective retention on SepPak Vac C-18, according to the procedure described by Di STEFANO (1991), with some modifications (CORTÉS, 1997). After the cartridge was activated with 5 mL of methanol and 10 mL of distilled water, the sample (100 mL of centrifuged wine, diluted with distilled water (1:1) and 1 mL of 3-octanol, 10 mg/L, like internal standard) was passed through C-18 phase. Then the cartridge was washed with 25 mL of distilled water and finally the free fraction was eluted with pentane-dichloromethane (2:1, 10 mL). The elute was dried over anhydrous sodium sulphate and concentrated to 0.5 mL, by evaporation with a stream of nitrogen, before GC analysis. The bound fraction was eluted with methanol (10 mL) and concentrated to dryness in vacuum before dissolution in citrate-phosphate buffer (pH 5.0, 5 mL). AR-2000 (Gist Brocades, France) was added and the mixture was incubated at 40 ºC for 18 h to accomplish enzymatic hydrolysis. Glycosides hydrolysed were extracted like free form fraction, and the extract was concentrated, by evaporation with a stream of nitrogen, before GC analysis. Conditions used for chromatographic analysis were: injector temperature (250 ºC), temperature program (60 ºC for 5 min, increased at 3 ºC min^{-1} to 220 ºC, 15 min isothermal), detector temperature (260 ºC), injection type (Splitless, 30s) and injection size (1 µL). Carrier gas helium at 1.20 mL/min; make-up gas: nitrogen 15 mL/min. The detector gas flow rates were: hydrogen, 40 mL/min; air, 400 mL/min.

IV - 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 250 ºC). 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.

V - DATA PROCESSING

The compounds considered as aroma contributing substances are primarily those present in concentrations higher than their odour threshold (ROCHA *et al.* 2004). To evaluate the contribution of a chemical to the aroma of a must, a contribution index (C.I.) was calculated according to Versini *et al.* (1994):

C.I. = Concentration of compound/Contribution limit. When C.I. > 1, the compound should be considered.

Each free compound was assigned to one or several aroma series, according to the principal odour descriptors found in the literature (ETIEVANT, 1991; FERREIRA *et al.*, 2001; PEINADO *et al.*, 2004).

VI - STATISTICAL ANALYSES

Differences among the Albariño musts with respect to the variables were assessed by one-way analysis of variance (ANOVA). Principal component analysis (PCA) was performed using to find the possible differentiation between musts. These analyses were accomplished using the Enterprise Guide 3 System Software (SAS Institute, Cary, NC, USA).

RESULTS AND DISCUSSION

Free and bound terpenes were determined in Albariño musts from three different geographic areas for Galicia: O Condado do Tea, O Rosal y O Val do Salnés. From the aromatic point of view, terpenols and norisoprenoids are very important aroma compounds in must. These compounds are synthesized during berry maturation and their concentration in grapes depends on various factors such as the cultivar, the region or the climatic conditions. The aromatic compounds determined in this study were α-rpineol, linalool, nerol, 2-phenylethanol, α-ionone, limonene and citronellol.

One way of quantification of the odour activity of a compound is to determine its contribution index (C.I.). Such a value is calculated by dividing the concentration of the compound in the must into its contribution limit (20 % perception threshold) (VERSINI *et al.*, 1994). This C.I. number permits the valuation of the degree of participation of each compound in the final aroma. Aromatic compounds such as linalool and terpineol described as floral; nerol as rose-like; 2-phenylethanol is described as rose-like, sweet and perfume-like; α-ionone as violet; linalool as fruity and rose-like; limonene and citronellol as fruity and citric (MEILGARD, 1975; LOPEZ *et al.*, 1999; BAYONOVE *et al.*, 2000). Contribution index average data (mean±SD) from each Albariño must are summarized in tables I and II. The contribution of Nerol, 2-phenylethanol and a-terpineol, to the aroma of Albariño musts cannot be considered important because their C.I. were lower than 1.

Table I - Contribution limit (C.L.), aromatic serie and Contributión Index (C.I.) of free compounds of Albariño musts from different areas from Galicia.
Limite de Contribution (CL), série aromatique et Indice de Contribution (IC) des composés libres des moûts d´Albariño dans les différentes zones viticoles de la Galicia.

Free			I.C. Albariño musts			
Compound	C.L. (μg/L)	Serie	Condado do Tea	O Rosal	Val do Salnés	Sig
Limonene	42	1,3	0.70±0.00	1.00±0.01	0.51±0.00	***
Linalool	10	2,4	0.66±0.04	0.94±0.01	0.35±0.10	***
Citronellol	3,6	2,3	0.49±0.03	0.62±0.10	0.51±0.11	ns
Nerol	80	2,3,4	0.53±0.01	0.59±0.00	0.63±0.00	***
α-ionone	0,52	2	11.49±0.84	16.68±0.41	13.26±0.88	***
2-phenylethanol	1500	2,4	0.11±0.00	0.12±0.00	0.12±0.00	ns

Aromatic series: 1. fruity; 2. floral; 3. citric; 4. rose (López *et al.*, 1999; Etievant, 1991; Bayonove *et al.*, 2000; Ferreira *et al.*, 2001; Peinado *et al.*, 2004)

Table II - Contribution limit (C.L.), aromatic serie and Contribution Index (C.I.) of bound compounds of Albariño musts from different areas from Galicia.

Limite de Contribution (CL), série aromatique et Indice de Contribution (IC) des composés liés des moûts d'Albariño dans les différentes zones viticoles de la Galicia.

Bound			I.C. Albariño musts			
Compound	C.L. (μg/L)	Serie	Condado do Tea	O Rosal	Val do Salnés	Sig
Limonene	42	1,3	1.10±0.02	1.28±0.00	0.29±0.06	**
Linalol	10	2,4	4.52±0.00	5.19±0.02	3.79±0.44	**
Citronelol	3,6	2,3	1.60±0.06	1.74±0.07	2.23±0.15	***
Nerol	80	2,3,4	0.14±0.00	0.18±0.00	0.16±0.00	*
α-ionone	0,52	2	29.79±0.74	50.58±0.51	29.98±3.69	***
2-feniletanol	1500	2,4	0.17±0.00	0.21±0.00	0.11±0.00	***
α–terpineol	80	2	0.01±0.00	0.03±0.00	0.00±0.00	***

Aromatic series: 1. fruity; 2. floral; 3. citric; 4. rose (*López et al.*, 1999; Etievant, 1991; Bayonove *et al*., 2000; Ferreira *et al*., 2001; Peinado *et al.*, 2004)

Table I shows the contribution index of free compounds. The major positive C.I. for Albariño must is due to α-ionone (11.49 units in Condado do Tea, 13.26 units in Val do Salnés and 16.68 units in O Rosal). Limonene has only contributed in Albariño must from O Rosal. Fruity and floral can be assumed to be with the strongest odour impact to the aroma of Albariño wines. VILANOVA and VILARIÑO (2005) found similar results in young white wines elaborated with Albariño grapes from different origin from Galicia when were analysed by sensory analysis. In aromatic varieties, the concentrations of free volatile compounds are usually above their perception thresholds. In others varieties, the release of the bound forms by chemical or enzymatic hydrolysis (MARAIS and VAN WYCK, 1986; WILLIAMS *et al.*, 1982; GUNATA *et al.*, 1990; RAPP and MANDERY, 1986) can notably increase the aroma of its wines. In order to compare the potential aromatic of different musts, contribution index of bound compounds was calculated. As can be observed in table II, the aromatic profile for each Albariño must was different. The C.I. of α-ionone, citronellol and linalool in Albariño musts from different areas was higher than 1. Limonene contributes positively to Albariño aroma in O Condado de Tea and O Rosal. High amount of monoterpenes in Albariño wines was found by FALQUÉ *et al.* (2001), which could explain the floral aroma descriptors. DIEGUEZ *et al.* (2003) studied the composition of the Albariño grape at the harvest date and they observed floral character in Albariño variety. The figure 1 shows the potential aromatic of Albariño must from different geographic areas from Galicia taking into account the C.I. values of free and bound compounds. α-ionone was the highest compound in the three areas. O Rosal should be the most aromatic must since it contained significantly higher C.I. values of volatile compounds. It can be seen that the C.I. of the bound forms of the compounds detected is always higher than those of the free forms, as would correspond to a quality variety (DIEGUEZ *et al.*, 2003)

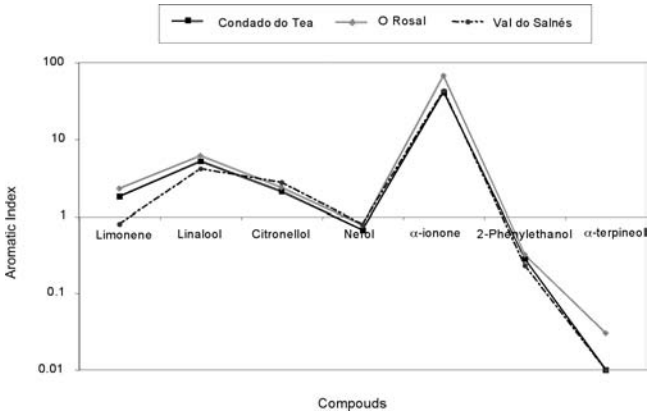

Figure 1 - C.I. values of free and bound compounds of Albariño musts from different geographic areas.

CI des composés libres et liés dans les moûts d´Albariño des diffférentes zones géographiques.

ANOVA showed significant differences to exist in the mean contribution index of the aromatic compounds in each must (tables I and II). Only free citronellol and free 2-phenylethanol do not show significant differences. The high number of significant differences for the majority of the aromatic compounds studied reflects the influence of « terroir ».

Principal component analysis (PCA) was used to identify the aromatic index of the volatile compounds that discriminated best between the musts of the three different areas (figures 2a and 2b). The first two principal compo-

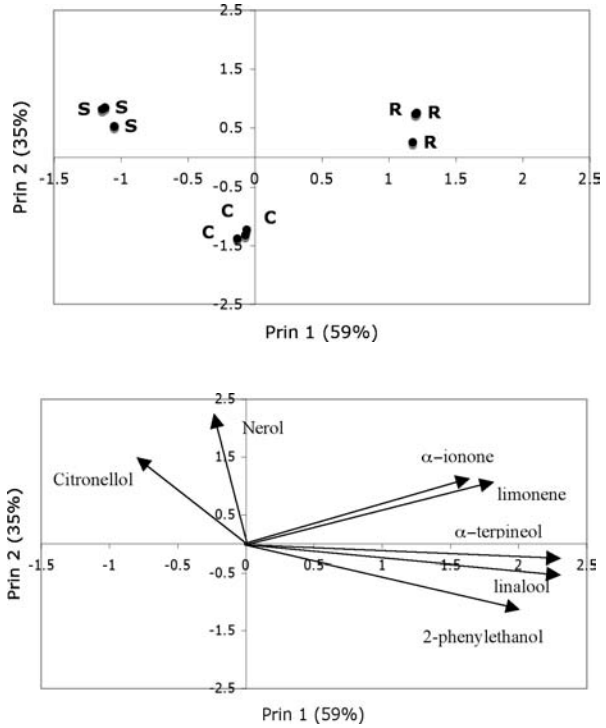

Figure 2 - Principal components analysis of Contribution Index values of Albariño musts from different geographic areas.

Analyse en Composantes Principales (PCA) des Indices de Contribution des moûts d´Albariño dans différentes zones géographiques.

nents accounted for 93.6 % of the total variance (58.8 and 34.9 % respectively). The variables that contribute most to the first principal component (Prin1) were limonene, linalool, 2-phenylethanol and α-terpineol. The second principal component (Prin2) was characterized by citronellol and nerol. According to figure 2a, it can be stated that Albariño musts from different geographic can be distinguished according to their contribution index. If we compare the variables in the same plane (figure 2b), it is clear that α-ionone, limonene and linalool are the parameters that make the highest contribution to the Albariño must from O Rosal, while Albariño musts from Val do Salnés is highly influenced by itronellol.

CONCLUSION

Some aromatic compounds were quantified in Albariño musts from different areas from Galicia and their contribution indexes (C.I.) were calculated based on published data. The C.I. of the bound forms of the compounds detected is always higher than those of the free forms, except for the nerol. Only C.I. of α-ionone, citronellol and linalool in Albariño musts from different areas was higher than 1, taking into account free and bound forms. Limonene contributes positively to Albariño aroma in O Condado de Tea and O Rosal. The PCA shows a good differentiation among three groups of musts according to their geographic origin. In our study, the results of C.I. shown that the fruity aroma followed by the floral had the highest contribution to the overall aroma of the musts.

Acknowledgements : The authors would like to thank to the Diputación Provincial de Pontevedra and CSIC (I3P Program-Fondo Social Europeo).

REFERENCES

BAYONOVE C.L., BAUMES R., CROUZET J. et GÜTAMA Z., 2000. Aromas. *En: Fundamentos científicos y tecnológicos*. Flanzy, C. Ed Mundi-Prensa, Madrid, 137-176.

BELANCIC A., AGOSIN E., IBACACHE A., BORDEU E., BAUMES R., RAZUNGLES A. et BAYONOVE, C., 1997. Influence of sun exposure on the aromatic composition of Chilean Muscat grape cultivars Moscatel de Alejandría and Moscatel Rosada. *Am. J. Enol. Vitic*., **48**, 181-186.

BERTRAND A. et ANOCIBAR-BELOQUI A., 1996. Essais d´utilisation d'enzymes pectolitiques à activés complémentaires de type glucosidase dans les vins, Influence sur l'arôme. *Rev. Fr. Œnol.*, **157**, 19-683.

CARBALLEIRA L., CORTÉS S., GIL M.L. et FERNÁNDEZ E., 2001. Determination of aromatic compounds, during ripening, in two white grape varietes, by SPE-GC *Chromatographia*, **53** (Suppl.): S350-S355.

CORDONIER R.E. et BAYONOVE C.L., 1974. Mise en évidence dans la baie de raisin var. Muscat d'Alexandrie de monoterpènes liés révélables par une ou plusieurs enzymes du fruit. *C.R. Acad. Sci.* Paris 278:3387-3390.

DIEGUEZ S.C., LOIS L.C., GÓMEZ E.F., et DE LA PEÑA M.L.G., 2003. Aromatic composition of the *Vitis vinifera* grape Albariño. LWT-*Food Sci. Technol*., **36**, 585-590.

ÉTIEVANT P.X., 1991. Wine. In: Maarase H. (Ed.), *Volatile compounds in food and beverages* (pp 483-546). Zeist, The Netherlands: TNO-CIVO Food Analysis Institute.

FALQUÉ E., FERNÁNDEZ F. et DUBOURDIEU D., 2001. Differentiation of white wines by their aromatic index. *Talanta*, **54**, 271-281

FERNÁNDEZ E., CORTÉS S.M., CASTRO M., GIL M. Y GIL M.L., 1999. Distribution of free and glycosidically bound mono-terpenes and norisoprenoides in the ski and pulp of Albariño grapes during 1998 maturation. In: Aline Lonvaud Funel (Ed.), *Oenologie 99. 6º Symposium International d'Oenologie*. Bordeaux (pp. 161-164). Paris, Tec&Doc.

FERREIRA V., ORTÍN N., ESCUDERO A., LÓPEZ R. et CACHO, J., 2002. Chemical characterization of the aroma of Grenache rosé wines: Aroma extract dilution analysis, quantitative determination, and sensory reconstitution studies. *J. Agric. Food Chem*. **50**, 4048-4054.

FERREIRA V., AZNAR M., LÓPEZ R. et CACHO, J., 2001. Quantitative gas chromatography-olfactometry carried out at different dilutions of an extract. Key differences in the

odor profiles of four high quality Spanish aged red wines. *J. Agric. Food Chem*., **49**, 4818-4824.

GUNATA Y.Z., BITTEUR S., BAUMES R., SAPIS J.C. et BAYONOVE C., 1990. Activités glycosidases en vinification. Perspectives d´exploitation des précurseurs d´arôme du raisin, de nature glycosidique. *Rev. fr. Œnol.*, **122**, 37-41.

LÓPEZ R., FERREIRA V. et CACHO, J.F., 1999. Quantitative determination of the odorants of young red wines from different grape varieties. An assessment of their sensory role, Oenologie 99. 6º Symposium International d'oenologie. Ed TEC&DOC, París, 148-151.

MARAIS J.S. and VAN WYCK C.J. 1986. Effect of grape maturity and juice treatments on terpene concentrations and wine quality of *V. vinifera* L. cv. Weisser Riesling and Bukettraube. *S. Afr. J. Enol. Vitic*., **7**, 26-43.

MEILGARD M.C., 1975. In: F. Drawert, Geruch-und Geschmackstoffe,H. Carl (Eds.), *Aroma Volatiles in Beer: Purification, Flavour, Threshold and Interaction*, Nürnberg, 1975, 211-254.

O.I.V., 1990. *Recueil des méthodes internationales d'analyse des vins et des moûts*. Office International de la Vigne et du Vin, Paris.

PEINADO R.A., MORENO J., BUENO J.E., MORENO J.A. et MAURICIO J.C. 2004. Comparative study of aromatic compounds in two young white wines subjected to pre-fermentative cryomaceration. *Food Chem.*, **84**, 585-590.

RAPP A. et MANDERY H., 1986. Wine aroma. *Experientia*, **42**, 873-884.

ROCHA SM., RODRIGUES F., COUTINHO P., DELGADILLO I. et COIMBRA MA., 2004. Volatile composition of Baga red wine. Assessment of th identification of the World-be impact odorants. *Anal. Chim. Acta*, **513**, 257-262.

VERSINI G., ORRIOLS I. et DALLA SERRA A., 1994. Aroma components of Galician Albariño, Loureira and Godello wines. *Vitis*, **33**, 165-170.

VILANOVA M. and MASNEUF-POMARÈDE I., 2005. Characterization of yeast strains from Rías Baixas (NW Sapin) and their contribution to the fermentation of Albariño wine. *Annals Microbiol*., **55**, 23-26.

VILANOVA M. et VILARIÑO F., 2006. Influence of geographic origin on aromatic descriptors of Spanish Albariño wine. *Flav. Frag. J*., **21**, 373-378.

WILLIAMS P.J., STRAUSS C.R., WILSON B.J., 1982, Use of C18 reversed phase liquid chromatography for the isolation of monoterpene glycosides and norisoprenoid precursors from grape juice and wine. *J. Chromatography*, **235**, 471-480.

WINTERHALTER P. et SKOUROUMOUNIS G.K., 1997. Glycoconjugated aroma compounds: Occurrence, role and biotechnological transformation. *In Biotechnology of aroma compounds*. R.G. Berger (Ed.), pp. 73-105. Springer-Verlag, Berlin.

Manuscrit reçu le 18 avril 2006 ; accepté pour publication, après modifications le 19 octobre 2006

