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RAPID COMMUNICATIONS

Resveratrol and Piceid as Varietal Markers of White Wines

Keywords: *White wines; resveratrol; piceid; characterization*

INTRODUCTION

The production of the polyphenol *trans*-resveratrol (*trans*-3,5,4′-trihydroxystilbene) is positively correlated with resistance of the grapevine to cryptogamic diseases (Langcake, 1981; Barlass *et al.*, 1987; Dercks and Creasy, 1989), and it is also considered a good marker for gray mold resistance (Jeandet *et al.*, 1992). In grapevine, this compound is synthesized in leaves (Langcake and Pryce, 1977) and in grape skins (Creasy and Coffee, 1988; Jeandet *et al.*, 1991).

Resveratrol is synthesized in response to microbial infection or stress (Langcake and Pryce, 1976; Langcake, 1981). However, it is also produced after chemical treatments, such as herbicide or fungicide application (Macheix *et al.*, 1990), following the application of carbohydrate and galacturonic acid inductors (Blaich and Bachmann, 1980), and by UV light exposure (Blaich *et al.*, 1982; Langcake and Pryce, 1976; Pool *et al.*, 1981).

The presence of resveratrol in white wines has been the object of several studies (Siemann and Creasy, 1992; Lamuela and Waterhouse, 1993; Mattivi, 1993; Roggero and Archie, 1994; Jeandet *et al.*, 1993; McMurtrey *et al.*, 1994; Pezet *et al.*, 1994; Vrhovsek *et al.*, 1995; Romero-Pérez *et al.*, 1996) for the positive physiological properties attributed to this compound. However, to our knowledge, no study has used resveratrol as a wine varietal marker. Previously, Bavaresco *et al.* (1993) and Jeandet *et al.* (1992) reported differences in the production of resveratrol in vine varieties, since the presence of this compound is an indicator of the resistance of the plant to infections.

Singleton and Trousdale (1983) show that the patterns of phenolic substances are influenced by the genetics of the grapevine and "revealed a similarity within variety and difference between varieties in Chenin blanc, French Colombard, Semillon and Thompson Seedless wines, according to the polyphenolic profile determined by HPLC".

In 1991, Somers and Pocock related that the distribution and expression of phenolic constituents can differ significantly in white grape varieties, especially the hydroxycinnamates, whereas the flavonoid concentration in wine is more affected by the winemaking practices. More recently, de la Presa Owens *et al.* (1995a,b) reported differentiation of Xarel.lo with respect to Macabeo and Parellada musts and wines due to the hydroxycinnamic esters content determined by HPLC.

Our initial purpose was to evaluate the levels of resveratrol and piceid (3-β-glucoside of resveratrol) isomers in the Spanish varietal white wines available in the market. However, we observed a different chromatographic profile characteristic for each variety. Surprisingly, wines obtained from different wineries, vintages, and appellations could be grouped within varieties by the amount of resveratrol and piceid present. In this paper, 26 white wines from 7 different varieties (Albariño, Chardonnay, Macabeo, Parellada, white Riesling, Sauvignon blanc, and Xarel.lo) were differentiated and classified by the analysis of *trans*-resveratrol, *trans*-piceid, *cis*-resveratrol, and *cis*-piceid, total phenols, absorbance at 320 nm, and the hydroxycinnamic acids *trans*-ferulic, *trans*-coutaric, and *trans*-*p*-coumaric. The analytical variables were subjected to analysis of variance (ANOVA) and principal component analysis (PCA).

MATERIALS AND METHODS

Samples. Twenty-six varietal white wines [Albariño (n = 4), Chardonnay (n = 4), Macabeo (n = 5), Xarel.lo (n = 4), Parellada (n = 4), white Riesling (n = 3), and Sauvignon blanc (n = 2) were purchased from local markets or obtained from different wineries. The wines analyzed were from different Spanish appellations and vintages.

Standards. For the identification of the peaks by HPLC, *trans*-resveratrol and *trans*-ferulic and *p*-coumaric acid standards were purchased from Sigma (St. Louis, MO). *trans*-

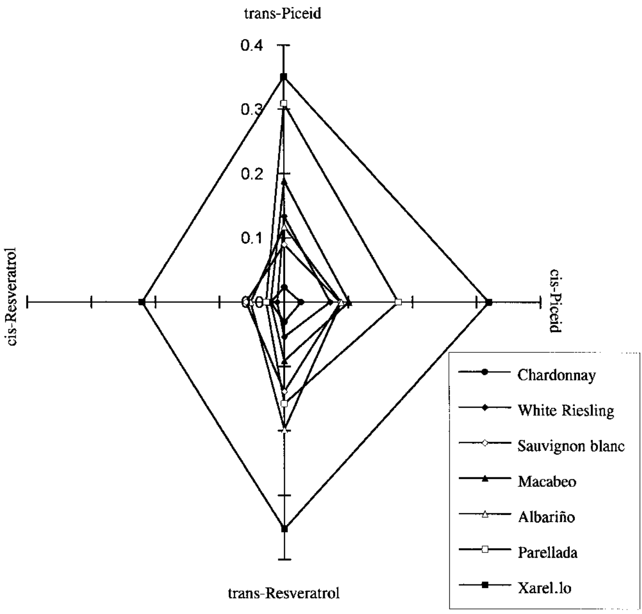

Figure 1. Comparison of descriptive profiles of different varieties of white wines.

Coutaric acid was provided by Vernon L. Singleton and Andrew L. Waterhouse (Department of Viticulture and Enology, University of California, Davis). Piceid extract was obtained from *Polygonum cuspidatum* as previously described (Waterhouse and Lamuela-Raventós, 1994). The *cis* isomers of resveratrol and piceid were obtained by exposure of *trans* forms to sunlight.

Analytical Methods. *HPLC Analysis.* Resveratrol monomers were determined according to the method described by Romero *et al.* (1996) using a Hewlett-Packard (HP) 1050 gradient liquid chromatograph equipped with an HP 1040M diode array UV−visible detector, coupled to an HP 79995A Chem Station. The sample was injected by a Rheodyne injection valve (Model 7125) with a 100 mL fixed loop. The column used for the stationary phase, at 40 °C, was a Nucleosil (Tracer) reversed phase column, C_{18} 120 (25 × 0.4 cm), 5 μm particle size with a precolumn of the same material. The HPLC conditions were as described previously (Lamuela-Raventós *et al.*, 1995). The hydroxicinnamic acids (*trans*-ferulic, *trans*-coutaric, and *p*-coumaric) were also determined according to method.

Total phenols were determined with the Folin−Ciocalteu reagent, according to the procedure described by Singleton and Rossi (1965).

Absorbance at 320 nm, for the hydroxycinnamate determination, was measured in a 1 mm cell pathway with an HP 8452A diode array spectrophotometer (Somers and Ziemelis, 1985).

All measurements were performed in duplicate.

Statistical Analysis of Data. Significant differences among wines for each of the constituents were assessed with a one-way ANOVA using STATGRAPHICS 7.0. The same program was used to perform PCA to obtain differences or groupings among wines according to variety by means of the variables analyzed. It is therefore possible to determine which variables contribute most to such differentiation.

RESULTS AND DISCUSSION

The samples analyzed were obtained from free run juice, since the levels of resveratrol in macerated white wines could be *ca.* 10 times the nonmacerated ones (Jeandet *et al.*, 1995a).

Table 1. Confidence Intervals (95%) for Means and Significance Levels of the Compounds That Contribute to Characterization

variable	Chardonnay $n = 4$	Sauvignon blanc $n = 2$	white Riesling $n = 3$	Macabeo $n = 5$	Albariño $n = 4$	Parellada $n = 4$	Xarel.lo $n = 4$	p
total resveratrol (mg/L)	0−0.225	0−0.369	0.057−0.390	0.189−0.447	0.253−0.522	0.235−0.523	0.802−1.089	<0.001
absorbance at 320 nm	2.64−4.11	1.72−3.80	5.42−7.12	3.93−5.25	4.51−5.98	3.72−5.19	3.30−4.77	<0.001
trans-piceid (mg/L)	0−0.119	0−0.176	0.033−0.235	0.111−0.268	0.031−0.206	0.222−0.397	0.263−0.438	<0.001
cis-piceid (mg/L)	0−0.044	0−0.0176	0−0.066	0.002−0.055	0.010−0.069	0.050−0.110	0.114−0.174	<0.001
trans-resveratrol (mg/L)	0−0.128	0−0.069	0−0.169	0.002−0.181	0.098−0.298	0.059−0.258	0.252−0.452	0.002
trans-coutaric acid (mg/L)	3.817−7.891	1.982−7.745	7.588−12.293	7.21−10.856	8.838−12.913	8.637−12.712	6.868−10.943	0.005
trans-ferulic acid (mg/L)	0.008−0.217	0.238−0.617	0.153−0.462	0.008−0.230	0.056−0.323	0−0.230	0−0.236	0.03
total phenols (mg of GAE/L)	143.42−214.58	104.18−204.82	175.25−257.42	173.98−237.62	229.17−300.33	159.17−230.33	152.42−223.58	0.01

In the resveratrol chromatographic analysis, we observed a considerable similarity within variety in the HPLC profile at 306 and 316 nm. The peaks responsible for this characterization were resveratrol and piceid isomers and the hydroxycinnamic acids content, as reported previously by Singleton and Trousdale (1983).

These differences in resveratrol and piceid isomers observed among varieties are shown in Figure 1. The mean content ratings for the seven varieties are plotted on a polar coordinate or "cobweb" graph. In this diagram, the center represents low content, which increases with distance from the center. The mean scores for each parameter in the axis have been connected to yield a content profile of resveratrol for each variety. According to this figure, Xarel.lo wines had the highest content of these compounds and Chardonnay had the lowest. The levels of resveratrol in white wines obtained from free run juice seem to be correlated with the resistance of the varieties used to fungal diseases, since Xarel.lo is the most resistant, followed by Parellada, while Chardonnay is one of the most susceptible. These results are in agreement with those of Jeandet *et al.* (1995b), who correlated the resistance of grapevines with grape resveratrol production.

For the analysis of the data, each parameter was examined by ANOVA to establish that the parameter varied significantly across the varieties (see Table 1). All of the compounds considered were significantly different by ANOVA, except *cis*-resveratrol and *trans*-*p*-coumaric acid. Albariño wines had higher content of *trans*-coutaric acid and total phenols. Chardonnay wines had the lowest concentration of total resveratrol, *trans*-piceid, *cis*-piceid, *trans*-resveratrol, and *trans*-ferulic acid. White Riesling and Albariño wines had higher absorbance at 320 nm. Sauvignon blanc wines were higher in *trans*-ferulic acid and lower in absorbance at 320 nm, *trans*-coutaric acid, and total phenols. Xarel.lo wines had the greatest resveratrol content (total resveratrol, *trans*-piceid, *cis*-piceid, and *trans*-resveratrol).

To obtain differences or groupings among white wines according to variety, by means of variables analyzed, PCA was performed, and the patterns were examined. In Figures 2 and 3, the 26 white wines and the 10 variables are plotted on the first two PCs. In Figure 2 it is observed that varieties were clearly separated. To our knowledge, this is the first time this analysis has been applied successfully.

Results show that total resveratrol, *trans*-resveratrol, *trans*-piceid, *cis*-resveratrol, *cis*-piceid, *tran*s-coutaric acid, and *trans*-coumaric acid were highly loaded on the first PC and the absorbance at 320 nm, total phenol content, *trans*-*p*-coumaric acid, and *trans*-*p*-coutaric acid were loaded on the second PC (Figure 3).

These compounds were also found to depend on variety when ANOVA was performed, except for *cis*-resveratrol and *trans*-*p*-coumaric acid. The first two PCs accounted for 39% and 23% of the variance, respectively, allowing the grouping of the wines by the variety.

Resveratrol and piceid isomers, like other phenols, such as hydroxycinnamic acids, have been shown to be chemotaxonomic wine markers, distinguishing varieties in white wines from different appellations, wineries, and vintages.

Although good characterization according to variety was obtained by the analysis of resveratrol and piceid isomers, a larger sample of wines should be examined to confirm our findings and to eliminate the possible biases.

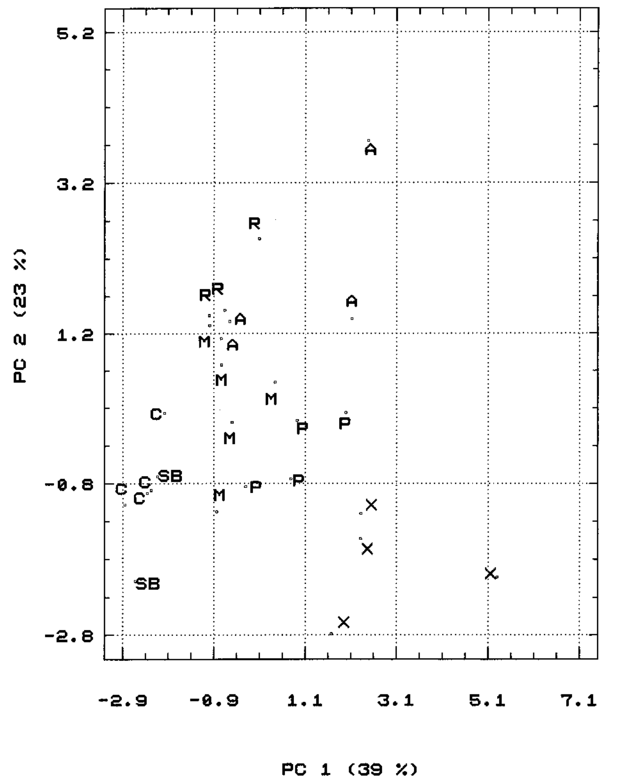

Figure 2. Bidimensional plot of the two PCs of white wines analyzed according to variety. A, Albariño; C, Chardonnay; M, Macabeo; P, Parellada; R, white Riesling; S, Sauvignon blanc; X, Xarel.lo.

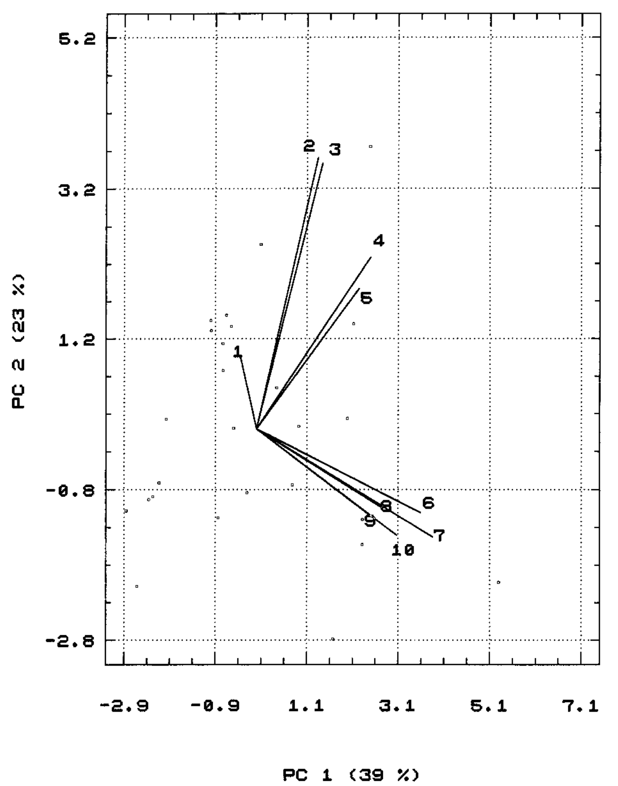

Figure 3. Parameters analyzed loadings on the first two PCs. 1, *trans*-ferulic acid, 2, absorbance at 320nm, 3, total phenols, 4, *trans*-coutaric acid, 5, *trans*-coumaric acid, 6, *trans*-resveratrol, 7, total resveratrol, 8, *trans*-piceid, 9, *cis*-resveratrol; 10, *cis*-piceid.

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