ANTHOCYANIN CHARACTERIZATION OF YOUNG RED WINES FROM APPELLATION OF ORIGIN NAVARRA (SPAIN)

María José NORIEGA* and Ana CASP

Tecnología de Alimentos, Escuela Técnica Superior de Ingenieros Agrónomos, Universidad Pública de Navarra, Campus de Arrosadia, 31006 Pamplona (Spain)

Abstract

Aims: The aim of this paper is to study the anthocyanin profile of the monovarietal young red wines from the Appellation of Origin Navarra. Differentiation of wines according to age and grape variety (Tempranillo, Grenache and Cabernet-Sauvignon) was procured using the multivariate statistical methods.

Methods and results: The anthocyanin composition of wines was analysed by HPLC's technology. Then, the data were analysed statistically. In this way, principal component analysis and stepwise discriminant analysis classified correctly samples belonging to the same category. The anthocyanin 3-monoglucosides appeared as key factor in the differentiation of wines according to age. Largest relative concentrations in these anthocyanins were found in the youngest wines. In addition, a separation of wines according to variety is obtained in terms of their relative contents on the anthocyanin acetate derivatives.

Conclusion: A minimum number of anthocyanin compounds made classification of wines according to both age and grape variety possible in this work. Cabernet-Sauvignon is the variety that appeared to differ most from the rest, because it is the one that presents the major levels of the MvAc anthocyanin. The malvidin 3-monoglucoside was variable the highest discriminant power according to age. Using this variable exclusively, 80 % of correct classification was achieved.

Significance and impact of study:The importance of the anthocyanin compounds on the global quality of the young wine (1 or 2 years-old), has driven us to realize this work. It is important to know the evolution that these compounds suffer throughout the years in order to apply new technologies as for example the microoxygenation.

Key words: young red wines, origin Navarra, wines of the market, anthocyanic profile, high-performance liquid chromatography (HPLC), multivariate statistical analysis

Résumé

Objectif : L'objectif de ce travail est d'étudier le profil anthocyanique des vins rouges jeunes monocépages de l'Appellation d'Origine Navarre. La différenciation des vins selon l'âge et le cépage (Tempranillo, Grenache et Cabernet-Sauvignon) a été menée en utilisant les analyses statistiques multivariées.

Méthodes and résultats : La composition anthocyanique des vins a été analysée par la technologie du CLPH. Les données ont été traitées statistiquement. L'analyse en composantes principales et l'analyse discriminante ont classé correctement les vins de la même catégorie. Les anthocyanines 3-monoglucosides apparaîssent comme un facteur clef dans la différenciation des vins en fonction de l'âge. Les plus jeunes vins présentent les plus fortes concentrations relatives en anthocyanines. De plus, une séparation des vins selon le cépage est obtenue sur le plan des teneurs relatives des dérivés acétates des anthocyanines.

Conclusion : Un nombre minimum de composés anthocyaniques permet la classification des vins en fonction de l'âge et du cépage. Le Cabernet-Sauvignon est le cépage qui montre le plus de différences, car il a les niveaux les plus élevés en anthocyanine MvAc. La malvidine 3-monoglucoside présente le plus grand pouvoir discriminant en fonction de l'âge. En utilisant exclusivement cette variable, on classe correctement 80 % des vins.

Signification et impact de l'étude : L'importance des composés anthocyaniques sur la qualité globale du vin jeune (1 ou 2 ans) nous a amené à réaliser ce travail. Connaître l'évolution de ces composés au cours des années permet l'application de nouvelles technologies, comme par exemple la microoxygénation.

Mots clés : vins rouges jeunes, origine Navarre, vins de marché, profil anthocyanique, chromatographie liquide haute pression (CLPH), analyse statistique multivariée

manuscript received : 18 May 2005 - revised manuscript received: 8 January 2007

*Corresponding author: nodo@unavarra.es

J. Int. Sci. Vigne Vin, 2007, **41**, n°2, 111-119

©Vigne et Vin Publications Internationales (Bordeaux, France)

INTRODUCTION

Recently, most wineries of the world tend to produce high quality young wines, as well as to produce the traditional aged wines, thus expanding their range of products to satisfy the demand of the market due to the changes on the consumers' habits. In this sense, one is in the habit of the young wine destining for daily consumption, whereas aged for the big occasions.

In relation to young wines, they are distinguished to be full of floral and fruity aromas, which make the youth of the wines to be desired attributes. Therefore, a young wine should not be consumed after two or three years of the vintage date, which shows up on their label, because the quality of fruity and freshness characters, as soon as the colour, are the first to deteriorate. Among young red wine constituents, the anthocyanin phenolic compounds (belonging to the flavonoids family) play an important role. Anthocyanins are substances that take part in several physico-chemical properties, but also in some sensory characters. In this way, they are the principal components responsible for colour differences, but also they contribute to the gustative sensation of wines (Jackson *et al.*, 1978; Bertolucci *et al.*, 1989; Glories, 1999).

We can find several studies about anthocyanin composition of grape and wines. Most of these studies are focused on the characterisation of grape varieties and wines (Wulf and Nagel, 1978; Roggero *et al.*, 1986; Hebrero *et al.*, 1988; Santos-Buelga *et al.*, 1991; Ortega *et al.*, 1992; Castia *et al.*, 1992; Núñez *et al.*, 2001; Burns *et al.*, 2002). On the other hand, statistical multivariate methods of analysis are crucial tools for the interpretation and comparison of physico-chemical data of wines. Thus, the use of these techniques has developed appreciably in the last years solving the problem of wine origin identification (García-Jares *et al.*, 1993; Mazza *et al.*, 1999; Sivertsen *et al.*, 1999 ; Arozarena *et al.*, 2000).

The importance of the anthocyanin compounds on the global quality of the young wine has driven us to realize this work. The aim is to determine the anthocyanic composition of red young wines of Navarre. In this way, multivariate statistical techniques, as principal component analysis and stepwise discriminant analysis, were applied to study the clustering of young wines, according to age and variety depending on their anthocyanin profile.

MATERIALS AND METHODS

1. Materials

The samples used in this work correspond to monovarietal young red wines from the 1999 and 2000 vintages and have the « Appellation of Origin Navarra ». They were produced from two characteristic grape varieties of Spain (Tempranillo and Grenache) and the last one come from France origin: Cabernet-sauvignon. As all samples were commercial wines (i.e. bought in the market), the winemaking process for each wine is unknown. We analysed a number of 25 wines, all at the same time in year 2000. Each wine bottle sample was replicated twice and all chemical determinations were made in triplicate.

2. HPLC equipment

The high-pressure liquid chromatography (HPLC) apparatus used to analyse the anthocyanin composition, consisted of a Waters 2690 Module of Separation connected to a Waters 996 diode array detector (DAD). Anthocyanins were separated on a Nova-Pack C18 (15 cm x 0,20 cm i.d., 4 µm particle size) column. Data treatment was carried out using the Millipore 2010 Millenium software.

3. Chromatographic analysis

Anthocyanins were analysed using an established method by Hebrero *et al.* (1988). First, 30 mL of wine was dried to dryness in a rotavapor at 30 ºC. The residue was redissolved to 5 mL with a « synthetic wine », which consisted of a solution of 0,5 % tartaric acid (w/v) with 10 % ethanol, adjusted to pH 3,2. After filtration of a few millilitres of solution through a Millex-HV Millipore 0,45 µm filter. Sample volumes of 20 µL of the filtrate was used for HPLC analysis. A flow rate of 0,20 mL/min at ambient temperature was used. Solvent A was 5 % (v:v) formic acid in water (Formic acid 98 %, from J.T. Baker, Holland), and solvent B was acetonitrile (HPLC grade, from Merck, Germany). The gradient of solvent B varied as follows: 10 % to 20 %, 0-20 min; 20 % , 20-28 min; and 20 % to 24,2 %, 28-35 min. Elution was performed simultaneously at the spectrum range 250 nm-550 nm. The identification of anthocyanins was made on the basis of their elution order and spectral properties of different compounds (Wulf *et al.*, 1978; Hebrero *et al.*, 1988; Arozarena *et al.*, 2000). The quantification of each identified anthocyanin was carried out at 525 nm and expressed in grams per litre of malvidin 3-*O*-β-glucopiranoside (which was used as the standard: Refer. 1601-1. HPLC-purity, from Polyphenols Laboratories, Norway) through the calibration curves obtained within a concentration range between 1 and 900 mg/L, with linear correlation coefficients greater than 0,999. Both, standards and samples were determined in triplicate.

4. Statistical analysis

The statistical methods employed belong to statistical multivariate analysis. Analysis of variance of anthocyanin data, according to vintage and variety, was first employed as a preliminary estimation of the discriminating power

©Vigne et Vin Publications Internationales (Bordeaux, France)

Table 1 - Relative amounts of the individual anthocyanins in the analysed wines from each variety and vintage

Wine	Variety	Vintage	DfG	PtG	PnG	MvG	DfAc	PtAc	PnAc	MvAc	PtCu	PnCu	MvCu	MvCf
1	Tempranillo	1999	5,45	7,44	4,85	55,14	1,73	1,24	2,52	3,70	0,97	1,37	12,48	2,10
2	Tempranillo	1999	9,13	9,22	4,83	46,47	6,49	2,09	0,00	6,36	1,59	1,84	6,33	2,21
3	Tempranillo	1999	9,42	9,35	4,46	53,05	3,25	1,13	3,47	3,01	0,90	1,23	7,15	1,72
4	Tempranillo	1999	7,36	9,46	5,37	48,34	5,52	1,44	0,95	6,50	1,18	1,60	7,75	2,07
5	Tempranillo	1999	7,88	8,53	3,68	56,66	4,81	1,23	0,00	7,11	0,81	1,04	4,95	1,38
6	Tempranillo	1999	5,78	9,84	10,00	43,14	5,90	1,98	1,45	5,61	1,44	2,17	6,30	1,93
7	Tempranillo	1999	3,71	6,63	6,99	39,07	9,67	3,07	3,08	6,36	2,66	2,74	7,49	3,12
8	Tempranillo	1999	9,17	10,66	4,62	50,44	3,13	1,42	1,06	8,04	0,77	1,16	6,21	1,45
9	Grenache	1999	4,50	4,68	4,95	40,13	16,13	4,11	0,00	7,49	3,32	0,00	8,41	3,18
10	Grenache	1999	7,42	5,66	4,64	48,97	12,55	3,20	0,00	5,08	2,32	2,01	5,90	2,24
11	Cabernet-sauvignon	1999	6,58	6,46	6,32	43,84	5,40	3,25	2,53	10,61	1,81	1,87	5,44	1,96
12	Cabernet-sauvignon	1999	5,37	4,88	3,36	48,56	6,34	1,89	1,48	15,87	0,97	1,20	4,95	1,16
13	Tempranillo	2000	7,49	7,72	3,66	53,55	2,32	1,27	1,13	11,29	0,53	1,00	7,41	1,34
14	Tempranillo	2000	6,98	8,54	4,34	57,88	1,87	1,19	0,90	7,08	0,62	1,11	6,76	1,16
15	Tempranillo	2000	6,46	8,30	3,18	61,20	1,34	0,99	2,16	3,20	0,91	1,15	8,54	1,66
16	Tempranillo	2000	6,37	7,76	7,69	56,79	2,02	1,18	2,96	3,53	0,74	1,40	7,26	1,50
17	Tempranillo	2000	6,90	8,48	3,09	57,52	1,95	1,13	0,00	11,68	0,53	0,71	5,88	1,05
18	Tempranillo	2000	7,26	10,15	3,44	62,44	1,83	1,05	1,63	3,38	0,60	0,84	5,60	1,13
19	Tempranillo	2000	3,52	5,71	7,34	65,28	2,15	0,80	1,62	2,71	0,94	1,41	6,78	0,98
20	Tempranillo	2000	6,13	8,78	4,26	62,51	1,53	0,82	2,05	3,67	0,60	0,88	6,71	1,27
21	Grenache	2000	5,46	7,46	4,46	67,55	1,16	0,69	1,28	3,44	0,61	0,85	5,61	0,89
22	Grenache	2000	3,18	5,93	4,02	70,20	2,12	1,48	1,36	2,30	1,06	1,09	5,40	1,02
23	Grenache	2000	4,96	7,31	5,68	63,36	1,83	0,77	1,75	4,00	0,84	1,13	6,59	0,99
24	Cabernet-sauvignon	2000	6,99	7,78	2,94	51,48	2,71	1,79	1,39	14,88	0,56	0,90	6,10	0,97
25	Cabernet-sauvignon	2000	6,44	6,72	7,65	53,68	3,29	1,59	1,30	10,44	0,71	1,35	4,80	0,76

J. Int. Sci. Vigne Vin, 2007, **41**, n°2, 111-119

©Vigne et Vin Publications Internationales (Bordeaux, France)

of each variable. In addition to the application of variance analysis, a principal component analysis (PCA) carried out on the covariance matrix, was used to reduce the number of dimensions of the system in order to clarify its interpretation and to observe the possible relationships between different groups of wines (age, varieties) and specified variables. Finally, a stepwise discriminant analysis (SDA) was employed to select those compounds with the greater capacity to separate and classify wines into different categories. Application of these multivariate techniques were computed using Statgraphics Plus statistical analysis software for Windows, version 5.1 (Statistical Graphics Corporation, USA).

RESULTS AND DISCUSSION

Although 15 anthocyanins were identified, many were present in only trace amounts; therefore, for quantitative studies the levels of only 12 anthocyanins were determined: the 3-monoglucosides of delphinidin (DfG), petunidin (PtG), peonidin (PnG) and malvidin (MvG); the acetyl derivatives of 3-monoglucosides of delphinidin (DfAc), petunidin (PtAc), peonidin (PnAc) and malvidin (MvAc); the malvidin-3-monoglucoside-caffeoate (MvCf) and the petunidin, peonidin and malvidin-3-monoglucoside-*p*-coumarates (PtCu, PnCu, MvCu respectively). The delphinidin-3-monoglucoside-*p*-coumarate eluted at the same time as the acetate of malvidin-3-monoglucoside. Then, the former could not be analysed. Figure 1 shows the structure of each anthocyanin.

The relative amounts of individual anthocyanins were expressed as a percentage of the combined levels of the twelve anthocyanins identified (table 1). The 3-monoglucoside of malvidin was the major component in all of the wines. Thus had confirmed by several authors (Roggero *et al.*, 1986; Santos *et al.*, 1991; Arozarena *et al.*, 2000). Its contribution to the total anthocyanin content ranged from 39,07 % (wine 7) to nearly 70 % (wine 22). The Cabernet-Sauvignon was the variety that showed the lowest concentration of this anthocyanin, while Grenache was characterised by their high content.

Raw data were statistically analysed. Firstly, the variance analyse applied to anthocyanin data (table 2) indicated a variability between vintages. In this sense, the most important compound was MvG, with the highest *F* value. However, MvCf and DfAc also contribute to wine differentiation. With respect to relative anthocyanin composition of varieties analysed, MvAc (with a very high *F* value) and PtG were the most discriminating variables.

By application of PCA to the raw data, the results obtained showed that from twelve principal components obtained, the first four with eigenvalues greater than unity were selected, accounting for almost 83,4 % of variance (We find the variance on the new components that were successively extracted. These values are expressed as a percent of the total variance and the sum of the eigenvalues is equal to the number of original variables. And so, the variances extracted by the components are called the eigenvalues.).

R_1	R2	R3	Compound
OH	OH	Glucose	3-Glucoside of Delphinidin (DfG)
OCH_3	OH	Glucose	3-Glucoside of Petunidin (PtG)
OCH_3	H	Glucose	3-Glucoside of Peonidin (PnG)
OCH_3	OCH_3	Glucose	3-Glucoside of Malvidin (MvG)
OH	OH	Acetyl-Glucose	Acetyl 3-Glucoside of Delphinidin (DfAc)
OCH_3	OH	Acetyl-Glucose	Acetyl 3-Glucoside of Petunidin (PtAc)
OCH_3	H	Acetyl-Glucose	Acetyl 3-Glucoside of Peonidin (PnAc)
OCH_3	OCH_3	Acetyl-Glucose	Acetyl 3-Glucoside of Malvidin (MvAc)
OCH_3	OCH_3	Caffeoate-Glucose	Caffeoate 3-Glucoside of Malvidin (MvCf)
OCH_3	OH	*p*-Coumaroyl-Glucose	Coumaroyl 3-Glucoside of Petunidin (PtCu)
OCH_3	H	*p*-Coumaroyl-Glucose	Coumaroyl 3-Glucoside of Peonidin (PnCu)
OCH_3	OCH_3	*p*-Coumaroyl-Glucose	Coumaroyl 3-Glucoside of Malvidin(MvCu)

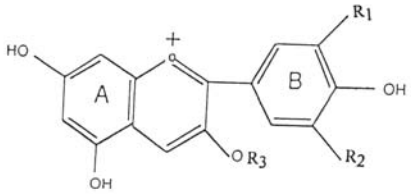


Figure 1 - Structure of anthocyanins identified

©Vigne et Vin Publications Internationales (Bordeaux, France)

Table 2 - Analyse of variance for anthocyanin measurements. Mean values (± standard errors) and F ratios for each vintage and variety

Variable	Vintage		*F* ratio	Variety[a]			*F* ratio
	1999	2000		Tempr.	Grenac.	Cabern.-s.	
DfG	6,8 ± 0,47	6,0 ± 0,45	1,5	6,8 ± 0,39	5,1 ± 0,71	6,3 ± 0,79	2,2
PtG	7,7 ± 0,48	7,7 ± 0,46	0,0	8,5 ± 0,31	6,2 ± 0,56	6,5 ± 0,63	8,9**
PnG	5,3 ± 0,51	4,7 ± 0,49	0,7	5,1 ± 0,45	4,7 ± 0,81	5,1 ± 0,91	0,1
MvG	47,8 ± 1,6	60,2 ± 1,50	30,4***	54,3 ± 2,10	58,0 ± 3,70	49,4 ± 4,20	1,2
DfAc	6,7 ± 0,83	2,0 ± 0,80	16,7***	3,47 ± 0,91	6,7 ± 1,60	4,4 ± 1,82	1,5
PtAc	2,2 ± 0,21	1,1 ± 0,20	12,6**	1,4 ± 0,21	2,1 ± 0,38	2,1 ± 0,42	2,0
PnAc	1,4 ± 0,29	1,5 ± 0,28	0,1	1,6 ± 0,25	0,8 ± 0,44	1,7 ± 0,49	1,0
MvAc	7,1 ± 1,12	6,3 ± 1,07	0,3	5,8 ± 0,67	4,4 ± 1,19	12,9 ± 1,33	13,5***
PtCu	1,6 ± 0,17	0,7 ± 0,16	13,3**	0,9± 0,17	1,6 ± 0,31	1,0 ± 0,35	1,7
PnCu	1,5 ± 0,15	1,06 ± 0,14	5,1*	1,3 ± 0,14	1,0 ± 0,25	1,3 ± 0,27	0,7
MvCu	6,9 ± 0,46	6,4 ± 0 44	0,7	7,1 ± 0,37	6,4 ± 0,67	5,3 ± 0,75	2,4
MvCf	2,0 ± 0,13	1,1 ± 0,13	23,9***	1,6 ± 0,16	1,7± 0,29	1,2 ± 0,33	0,7

aTempr.: Tempranillo; Grenac.: Grenache; Cabern.-s.: Cabernet-sauvignon.
*Significant at P value ≤ 0,05. **Significant at P value ≤ 0,01. ***Significant at P value ≤ 0,001 (LSD method).

Figure 2 shows samples projection into a subspace of maximum inertia (PC 1 and PC 4 explain the 39,5 % and 11,1 % respectively of total variance), as well as the component weights of variables. On this biplot we can visualise samples differences, positive or negative correlations between variables, and the relationships that may exist between groups of wines and anthocyanin variables. It is evident that the first eigenvector (PC 1) essentially discriminates the sampling years. This principal component was positively related to the content of anthocyanin 3-monoglucosides, especially MvG. The youngest wines (1-year-old wines) were grouped on the positive side of axis PC 1, due to high values obtained for the monoglucoside compounds (see table 2). The reverse was observed for 2-year-old wines, which have the higher levels of acylated anthocyanins.

In short, the PC 1 separating the wines by age was a function of the inversely correlated variables, MvG and the acylated derivatives PtAc and DfAc, principally, which is demonstrated when Pearson's correlations were determined (table 3).

The previous results were confirmed by the correspondent SDA, applied to all wines samples or separately to each vintage sample. These analyses allow selecting the variables with greater capacity to separate and classify wines into different categories, either age or varieties.

The results of SDA according to age, applied to all wines samples, are presented in table 4. After only two steps, a discriminant model according to age was obtained. MvG variable was selected at the first step. Using this anthocyanin variable exclusively, 80 % of correct classification was achieved and it was the variable with the highest discriminant power. At one subsequent step, the model was completed with the entry of MvAc variable. The percentage of correct classification finally obtained was 92 % for two vintages. It was observed that MvCf and DfAc which showed a substantial discriminant power from an individual point of view (table 2), were not included in this multivariate model. As it has been mentioned before, both compounds were significantly correlated between them and correlated with MvG

©Vigne et Vin Publications Internationales (Bordeaux, France)

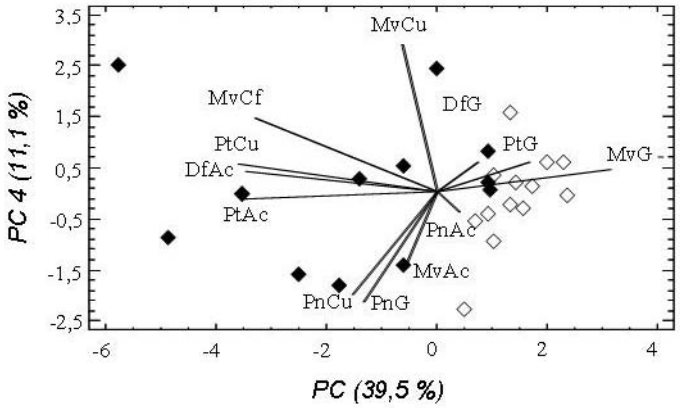


Figure 2 - Principal components biplot for PC 1 and PC 4. Vector loadings and distribution of samples according to age

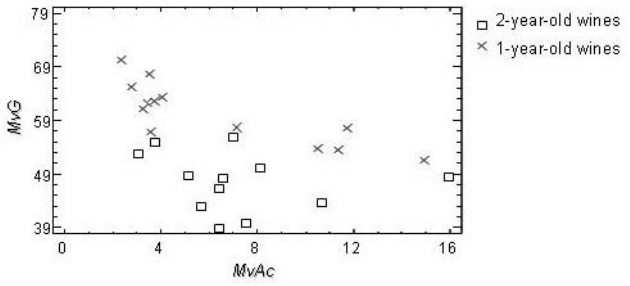


Figure 3 - Classification of wines according to age in the plane of discriminant variables

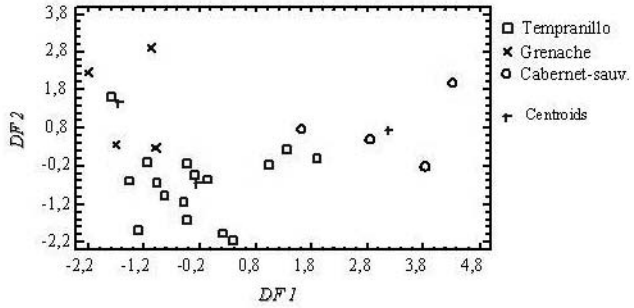


Figure 4 - Classification of wines according to variety in the plane of discriminant functions

Table 3 - Significant Pearson's correlations between anthocyanins

Pairs of variables	*r*	*P* value*
PtCu – DfAc	0,9264	0,0000
PtCu – PtAc	0,9105	0,0000
PtCu – MvCf	0,8816	0,0000
MvG – PtAc	– 0,7932	0,0000
MvCf – DfAc	0,7898	0,0000
MvCf – PtAc	0,7642	0,0000
MvG – MvCf	– 0,7561	0,0000
MvG – DfAc	– 0,7386	0,0000
MvG – PtCu	– 0,6834	0,0002
DfG – PtG	0,6704	0,0002

* P values below 0,05 indicate statistically significant non-zero correlations at the 95 % confidence level.

(table 3), therefore, it can be assumed that the presence in the model of all four variables would have been redundant.

Figure 3 shows the distribution of samples as a function of their relative MvG and MvAc contents. A clear separation of 2-years-old wines from the 1-year-old wines was observed. The first group of wines is positioned on the low part of the plot, because of their low MvG content.

On the other hand, table 5 shows the results of SDA according to variety, applied to all wines samples. By means of this analysis we attempted to differentiate the wines with regard to the variety from which they were produced. In this case, three steps were necessary to complete the discriminant model. The first variable of the model was MvAc, and although the 60 % of all wines were classified in the real category, this percentage went up to 100 % in the case of the Cabernet-Sauvignon variety. Correct classification gradually improved through the following steps, but less markedly. As seem, DF 1 was the discriminant function with the highest discriminant power according to its eigenvalues and P-value. The anthocyanin variable MvAc showed the greatest contribution to DF 1.

Figure 4 represents the distribution of wines in the plane of DF 1 and DF 2. A clear separation of Cabernet-Sauvignon wines can be seen due to relatively high MvAc content and a grouping of Tempranillo wines due to higher PtG content that the other varieties. Only, these anthocyanin compounds presented significant differences between varieties in the analysis of variance (table 2).

With the objective to separate the possible influence of vintage year and to improve the results obtained, we carried out a SDA by grape variety, for each vintage. In this way, table 6 shows the results of SDA applied to the 1999 vintage (2-year-old wines). After four steps a discriminant model includes two discriminant functions with four variables selected. These variables achieved to classify 100 % of the wines into their correct varieties.

Figure 5 display the distribution of samples along the two discriminant functions. A clear separation of the three varieties was observed. Cabernet-Sauvignon and Grenache wines were positioned on the upper part of the plot,

©Vigne et Vin Publications Internationales (Bordeaux, France)

Table 4 - Stepwise discriminant analysis according to age (1 or 2 year-old)

Selection of variables			*% of wines correctly classified*			*Standardised coefficients of DF*[a]
Step	Variable	F-to-enter	Total	2 years	1 year	DF 1
1	MvG	32,40	80	83,3	76,9	1,21
2	MvAc	4,04	92	83,3	100	0,60
				Eigenvalue of DF		1,75
				P value of DF		0,0000

[a]DF (discriminant function).

Table 5 - Stepwise discriminant analysis according to variety. Total wines

Selection of variables			*% of wines correctly classified*[a]				*Standardised coefficients of DF*[b]	
Step	Variable	F-to-enter	Total	Tempran	Grenac	CbSv	DF 1	DF 2
1	MvAc	13,50	60	43,75	80	100	1,28	-0,07
2	PtG	8,00	80	68,75	100	100	0,04	-0,98
3	PnAc	7,00	88	87,5	80,0	100	0,94	-0,39
					Eigenvalue of DF		2,50	0,90
					P value of DF		0,0000	0,0012

[a]Identification of varieties: Tempran. (Tempranillo); Grenac. (Grenache); CbSv (Cabernet-Sauvignon).
[b]DF (discriminant function).

Table 6 - Stepwise discriminant analysis according to variety in 2-year-old wines

Selection of variables			*% of wines correctly classified*[a]				*Standardised coefficients of DF*[b]	
Step	Variable	F-to-enter	Total	Tempran	Grenac	CbSv	DF 1	DF 2
1	DfAc	12,76	58,3	50	100	50	2,05	-0,33
2	MvAc	12,51	100	100	100	100	-0,93	0,96
3	MvG	4,46	100	100	100	100	1,32	1,65
4	PtAc	4,52	100	100	100	100	-0,21	2,14
					Eigenvalue of DF		11,50	6,22
					P value of DF		0,0000	0,0020

[a]Identification of varieties: Tempran (Tempranillo); Grenac (Grenache); CbSv (Cabernet-sauvignon).
[b]DF (discriminant function).

©Vigne et Vin Publications Internationales (Bordeaux, France)

Table 7 - Stepwise discriminant analysis according to variety in 1-year-old wines

Selection of variables			*% of wines correctly classified*[a]				*Standardised coefficients of DF*[b]	
Step	Variable	F-to-enter	Total	Tempran	Grenac	CbSv	DF 1	DF 2
1	MvG	10,00	84,6	75	100	100	1,02	-0,19
2	MvCf	4,59	92,3	87,5	100	100	0,48	0,93
					Eigenvalue of DF		2,31	0,83
					P value of DF		0,0018	0,0165

[a]Identification of varieties: Tempran (Tempranillo); Grenac (Grenache); CbSv (Cabernet-sauvignon).
[b]DF (discriminant function).

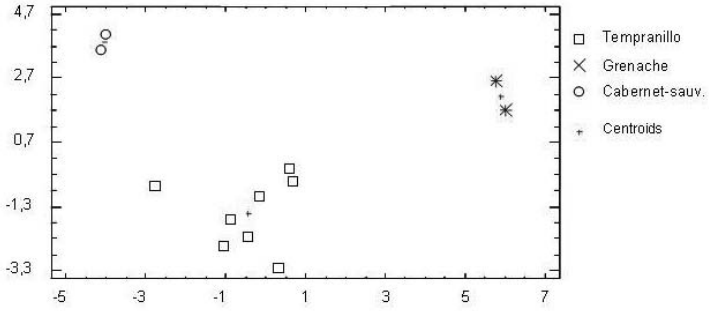


Figure 5 - Classification of 2-year-old wines according to variety in the plane of discriminant functions

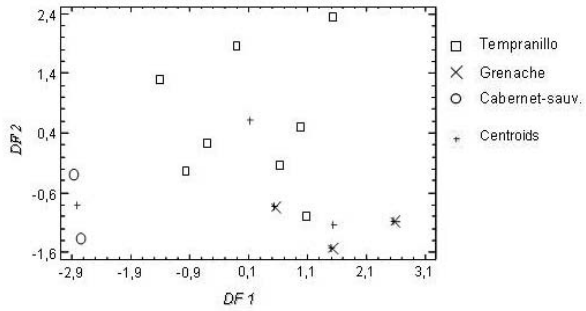


Figure 6 - Classification of 1-year-old wines according to variety in the plane of discriminant functions

whereas Tempranillo wines on the lower part. That is to say, the DF 2 allowed discriminate Tempranillo wines from the others because of their lower content of anthocyanin PtAc and their high content of MvG (table 1), since these two compounds to a great extent defined discriminant function DF 2. Moreover, DF 1 achieved a better differentiation of the three varieties. The variables DfAc (positively) and MvAc (negatively) define this discriminant function DF 1 and, therefore have he highest discriminant power. Cabernet-Sauvignon wines were positioned on the left hand side of the plot because of their high MvAc concentration. Grenache wines were positioned on the right hand side. These wines are characterised by their high DfAc content. Tempranillo wines were positioned in between.

Finally, the results of SDA according to variety, applied to the 2000 vintage (1-year-old wines) are presented in table 7. The discriminant model was completed after two steps and the variables selected were the anthocyanins MvG and MvCf. These two compounds represent a significant Pearson's correlation coefficient (-0,756). The variable MvG is the most important in this model and allowed the classification of 85 % of wines into their correct groups. The separation of varieties is showed in figure 6. DF 2 managed to differentiate Tempranillo wines from the others, because of their high MvCf content, whereas DF 1 separated Cabernet-Sauvignon samples rather well due to its low MvG content. However, no good separation of Tempranillo and Grenache wines were obtained, although Grenache wines are positioned on the right hand side of the plot because of its high MvG concentration.

CONCLUSION

The results show that a minimum number of anthocyanin compounds made classification of wines according to both age and grape variety possible in this work. Varietal differentiation of the wines was more complicated, partly due to the fact that the vintage exerted an important influence. Thus, when we individually analysed each vintage it was possible to obtain a better differentiation of wines according to variety. In this sense the Cabernet-sauvignon was the variety that showed most differences. The anthocyanins MvAc and MvG played an evident role in this differentiation, the Cabernet-sauvignon wines being characterised by their high anthocyanin acetate derivatives concentration, specifically MvAc, and their low MvG content. This is consistent with the results of other authors obtained in wines of the same varieties (Roggero *et al.*, 1986; Ortega *et al.*, 1992;

©Vigne et Vin Publications Internationales (Bordeaux, France)

Arozarena *et al.*, 2000; Núñez *et al.*, 2001; Burns *et al.*, 2002; García-Beneytez *et al.*, 2002). With regard to the age of the wine, it is tempting to speculate that the older wines are lower in the anthocyanic content because of changes in the chemical composition during aging. This fact influences, among others, in their chromatic characteristics. Therefore, the consumption of young wines could be during their first year.

In any case, the study of a greater number of samples and the possibility to apply HPLC-MS/UV analysis (Burns *et al.*, 2002) will be necessary in order to complete and to improve these results.

BIBLIOGRAPHICAL REFERENCES

AROZARENA I., CASP A., MARÍN R., and NAVARRO M., 2000. Differentiation of some wines according to variety and region based on their anthocyanin composition. *Eur. Food Res. Technol.*, **212**, 108-112.

BERTUCCIOLI S., CLEMENTI S., GIULIETTI G. and MONTEDORO G., 1989. Chemometric investigation of red wine "quality" using instrumental and sensory data. *Ital. J. Food Sci.*, **1**, 23-34.

BLOUIN J., PAPET N. and STONESTREET E., 2000. Étude de la structure polyphénolique des vins rouges par analyses physico-chimiques et sensorielles. *J. Int. Sci. Vigne Vin*, **34** (1), 3-40.

BURNS J., MULLEN W., LANDRAULT N., TEISSEDRE P. L., LEAN M. and CROZIER A., 2002. Variations in the profile and content of anthocyanins in wines made from Cabernet Sauvignon and hybrid grapes. *J. Agric. Food Chem.*, **50**, 4096-4102.

CASTIA T., FRANCO M. A., MATTIVI F., MUGGIOLU G., SFERLAZZO G. and VERSINI G., 1992. Characterization of grapes cultivated in Sardinia: chemometric methods applied to the anthocyanic fraction. *Sci. Aliments*, **12**, 239-255.

GARCÍA-BENEYTEZ E., REVILLA E. and CABELLO F., 2002. Anthocyanin pattern of several red grape cultivars and wines made from them. *Eur. Food Res. Technol.*, **215**, 32-37.

GARCÍA-JARES C., ROZES N. and MEDINA B., 1993. Caractérisation et différenciation des vins rosés d'appellation d'origine et de table, français et espagnols. *J. Int. Sci. Vigne Vin*, **27** (1), 35-51.

GLORIES Y., 1999. Substances responsables de l'astringence, de l'amertume et de la couleur des vins. *J. Int. Sci. Vigne Vin*, **33** supl. 1, 115-118.

HEBRERO E., SANTOS-BUELGA C. and RIVAS-GONZALO J.C., 1988. High Performance Liquid Chromatography-Diode Array Spectroscopy identification of anthocyanins of Vitis vinifera variety Tempranillo. *Am. J. Enol. Vitic.*, **39** (3), 227-233.

MAZZA G., FUKUMOTO L., DELAQUIS P., GIRARD B. and EWERT B., 1999. Anthocyanins, phenolics, and colour of Cabernet franc, Merlot and Pinot noir wines from British Columbia. *J. Agric. Food Chem.*, **47**, 4009-4017.

NÚÑEZ V., MONAGAS M., BARTOLOMÉ B. and GÓMEZ-CORDOVÉS C., 2001. El perfil antociánico como marcador varietal en uvas tintas de *Vitis vinifera* L. *In: VI Jornadas Científicas 2001*: Grupos de Investigación Enológica, Valencia (Spain), p. 44.

ORTEGA M. D., REYES M. J., GONZÁLEZ D. C., RIVAS J.C. and SANTOS C., 1992. Estudio de la composición antociánica de uvas tintas cultivadas en Castilla y León. *In: XIV Jornadas de Viticultura y Enología de Tierra de Barros*, Almendralejo (Spain), p. 499- 507.

ROGGERO J. C., COEN S. and LARICE J. L., 1986. Étude comparative de la composition anthocyanique des cépages. *Bulletin de Liaison-Group Polyphenols*, **13**, 380-388.

SANTOS C., MUÑOZ S. S., GUTIERREZ Y., HEBRERO E., VICENTE J. L., GALINDO P. and RIVAS J. C., 1991. Characterization of young red wines by application of HJ biplot analysis to anthocyanin profiles. *J. Agric. Food Chem.*, **39**, 1086-1090.

SIVERTSEN H., HOLEN B., NICOLAYSEN F. and RISVK E., 1999. Classification of French red wines according to their geographical origin by the use of multivariate analyses. *J. Sci. Food Agric.*, **79**, 107-115.

WULF L.W. and NAGEL C.W.,1978. High Pressure Liquid Chromatographic separation of anthocyanins of *Vitis vinifera*. *Am. J. Enol. Vitic.*, **29**, 42-49.

©Vigne et Vin Publications Internationales (Bordeaux, France)