Eur Food Res Technol (2002) 214:303–309
DOI 10.1007/s00217-001-0471-0

ORIGINAL PAPER

Iñigo Arozarena · Belén Ayestarán
María Jesús Cantalejo · Montserrat Navarro
Marta Vera · Irene Abril · Ana Casp

Anthocyanin composition of Tempranillo, Garnacha and Cabernet Sauvignon grapes from high- and low-quality vineyards over two years

Received: 11 June 2001 / Published online: 20 February 2002

Abstract The anthocyanin composition of Tempranillo, Garnacha and Cabernet Sauvignon grapes over two vintages was studied. Samples were obtained during berry ripening from two vineyards that differed by producing fruit of high and low quality. The proportions of the individual compounds remained practically constant within a single vineyard along the sampling period, the changes in the anthocyanin composition being principally quantitative. Different multivariate statistical methods showed that the anthocyanin profile was primarily determined by variety. This genetic dependence was particularly verified when the sums of the non-acylated glucosides, the acetates and the *p*-coumaryl derivatives, were analysed. The mean relative content corresponding to these three anthocyanin fractions was always the same within each variety, independently either of the vineyard or the vintage year considered. Relating to the individual compounds, several permanent qualitative differences between vineyards in each variety were also found, although the difference in the total anthocyanin concentration was always much higher.

Keywords Anthocyanin composition · Grapes · Tempranillo · Garnacha · Cabernet Sauvignon · Ripening

I. Arozarena (✉) · M.J. Cantalejo · M. Navarro · M. Vera · I. Abril
A. Casp
Tecnología de Alimentos,
Escuela Técnica Superior de Ingenieros Agrónomos,
Universidad Pública de Navarra, Campus Arrosadía s/n,
31006 Pamplona, Spain
e-mail: inigo.arozarena@unavarra.es
Tel.: +34-48-169849, Fax: +34-48-169893

B. Ayestarán
Area de Tecnología de Alimentos,
Dpto. de Agricultura y Alimentación, Universidad de la Rioja,
Complejo Científico Tecnológico, Madre de Dios 51,
26006 Logroño, Spain

Introduction

The color of red grapes is due to the anthocyanins. The composition of anthocyanins is primarily determined by genetic factors. Thus, a first distinction could be made between *Vitis vinifera* and others species of *Vitis* (i.e., muscadine grapes) according to the presence or lack of anthocyanidin diglucosides. In *Vitis vinifera* grapes there are only monoglucosides of five anthocyanidins: delphinidin, cyanidin, petunidin, peonidin and malvidin [1]. Except for the case of the Pinot Noir variety, which only contents unacylated anthocyanins [2, 3], these monoglucosides appear partially acylated with several acids. Two types of derived anthocyanins are found, that of acetates, and that formed by the cinnamoyl derivatives: the *p*-coumarates and, secondarily, the caffeoates. The distribution of these different compounds in grapes and the resulting wines mainly depends on variety. Likewise, anthocyanins have been used with success in many chemotaxonomical studies [4, 5, 6, 7, 8, 9]. Two clones of the same variety may present differences in the anthocyanin composition. The seasonal conditions also influence the distribution of anthocyanins in grapes [10, 11]. Several authors pointed out that the proportions of anthocyanins in the grape skin vary along with the berry ripening [12, 13, 14, 15, 16] whereas others stated that they remain practically invariable [17, 18, 19]. In any case, the accumulation pattern of different anthocyanins depends mainly on variety [11]. With regard to the soils, it has been reported that changes in their physical [14] and chemical characteristics [17] could have some influence on the anthocyanin composition of berries. Nevertheless, almost all references coincide in the fact that the non-genetic factors such as climate, soil conditions or viticultural practices have a greater effect on the concentration of anthocyanins rather than on their relative distribution.

In the present paper this anthocyanin distribution was studied over two consecutive ripening periods in Tempranillo, Garnacha and Cabernet Sauvignon grapes from

vineyards that produce fruits of different quality. The objectives of the study were to know the evolution of anthocyanins during the berry ripening, to use the anthocyanin profile of grapes to characterise them according to variety, and, if possible, according to the vineyard of origin.

Materials and methods

Vineyards

Grapes of each variety were taken from two different vineyards, one was well-known for producing high-quality fruit, while the other one was well-known for the opposite. All the six vineyards were from Navarra, in North Spain. The Garnacha high-quality grapes (GaHQ) were from Cintruénigo, while the Garnacha low-quality vineyard (GaLQ) was located in Marcilla. Both places show similar climatological conditions during the year, with very hot summers and an important hydric stress in winter. Tempranillo (TeHQ, TeLQ) and Cabernet Sauvignon (CbHQ, CbLQ) vineyards were from Arínzano, a more northerly and cooler zone than the places mentioned above.

HPLC equipment

Analysis of anthocyanins in grape skin extracts was performed in a Waters 2690 Separation Module equipped with a Waters 996 Photodiode Array Detector. Anthocyanins were separated in a Nova-Pak C_{18} (150 mm×2 mm i.d., 4 µm particle size) column. The Millenium software was used for chromatography and data management.

Reagents and commercial standards

Acetonitrile gradient HPLC grade was obtained from Merck (Darmstadt, Germany), Formic acid 98% was from J.T. Baker (Deventer, Holland), Tartaric acid was purchased from Panreac (Barcelona, Spain), and ethanol absolute gradient HPLC grade was from Scharbau (Barcelona, Spain). The anthocyanin compound malvidin-3-*O*-β-glucoside was obtained as a commercial standard from Polyphenols Laboratories (Sandnes, Norway).

Grape sampling

The study was performed over two years, 1999 and 2000. Sampling was limited to a 1 Ha area representative of each vineyard. The same 288 vines were sampled in each vineyard for both years. The sampling protocol used was the following: 3 individual berries were picked at each vine, from the top, the bottom and the middle part of one cluster randomly selected. Then, once all the vines had been sampled, a total of 864 berries was collected. This process was repeated two more times. Therefore, on each day of sampling three bags of 864 berries were obtained from each cultivar. Sampling started around 20 days after the onset of *véraison* and finished 10 days after harvest. Each vineyard was sampled on 5 to 9 dates, depending on the evolution of berry ripening.

Preparation of grape skin extracts for analysis of anthocyanins

One hundred berries were randomly selected from each group of 864 and weighed. Skins were manually separated from the pulp, weighed and introduced in a 300-mL flask with 100 mL of a "synthetic wine" [0.5% (w:v) tartaric acid in a 12:88 (v:v) ethanol:water solution, pH adjusted to 3.2]. After having removed the air with N_2 and sealed the flask, the mixture was kept stirring at 20 °C for 24 hours. This process was carried out two more times, replacing the liquid fraction by another 100 mL of synthetic wine. After a total maceration time of 72 h the three liquid fractions were brought together and the volume was adjusted to have a 300 mL extract. Finally, the three extracts obtained for each vineyard and date were put together in a whole 900 mL grape skin extract which was immediately stored under N_2 at –18 °C until analysis.

Chromatographic analysis

20 µL skin extract were injected directly into the HPLC after being concentrated in a rotavapor at 30 °C and filtered through a Millex-HV 0.45 µm filter (Millipore). A flow rate of 0.20 mL/min at ambient temperature was used. Solvent A was 5% (v:v) formic acid in water, and solvent B was acetonitrile. Proportions of solvent B varied as follows: 0–20 min, 90% to 20%; 20–28 min, 20%; and 28–35 min, 20% to 24.2%. Elution was monitored between 250 and 550 nm. The identification of anthocyanins was made on the basis of the elution order and the spectral properties of different compounds, comparing them with those found in the literature [1, 7, 20, 21, 22]. Anthocyanins were quantified at 525 nm as malvidin-3-glucoside 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.

Statistical analyses

The statistical methods employed, i.e., two-way analysis of variance, principal component and stepwise discriminant analyses were carried out with the Statgraphics Plus software for Windows 5.0. version (Manugistics, Inc.; Rockville, USA).

Results and discussion

Fifteen anthocyanins were identified: the 3-monoglucosides of delphinidin (Dpg), cyanidin (Cyg), petunidin (Ptg), peonidin (Png) and malvidin (Mvg); the acetyl derivatives of these monoglucosides (Dpac, Cyac, Ptac, Pnac and Mvac, respectively), and the following cinnamic acid esters: the cyanidin-3-monoglucoside-*p*-coumarate (Cypc), the malvidin-3-monoglucoside-caffeoate (Mvcf), and the petunidin, peonidin and malvidin 3-monoglucoside-*p*-coumarates (Ptpc, Pnpc and Mvpc, respectively). The delphinidin-3-monoglucoside-*p*-coumarate eluted at the same time as the acetate of malvidin-3-glucoside. Then, the former could not be analysed.

The whole 15 anthocyanin compounds previously mentioned were quantified in all Tempranillo samples, but some of them were not detected in several samples of the two other varieties. This was the case of the acetyl derivatives of delphinidin and cyanidin glucosides in all Garnacha samples, and the petunidin 3-aceytlglucoside in those from the 2000 vintage year. On the other hand, the *p*-coumaryl derivatives of the cyanidin and petunidin glucosides were only found in a very small proportion in some of the 2000 Cabernet Sauvignon samples.

Therefore, the different statistical methods presented in this paper were performed using data from the ten compounds detected in the whole 81 samples; that is, the five non-acylated glucosides and the five acyl derivatives of peonidin and malvidin glucosides. Moreover,

Table 1 Distribution (%) of the three anthocyanin fractions in grapes from each vineyard at the beginning of the sampling period and at harvest

		High-quality vineyard				Low-quality vineyard			
		1999		2000		1999		2000	
Variety	Fraction	*b*	*h*	*b*	*h*	*b*	*h*	*b*	*h*
	Sum_g	93.8	94.4	93.3	91.6	93.8	93.1	94.3	91.8
Tempranillo	Sum_ac	4.0	3.0	3.9	4.0	3.9	4.0	4.0	4.1
	Sum_cn	2.2	2.6	2.8	3.8	2.3	2.9	1.7	4.1
	Sum_g	96.6	96.0	94.2	95.1	97.1	96.0	95.2	95.2
Garnacha	Sum_ac	2.4	2.3	2.5	2.0	1.9	2.1	2.3	2.7
	Sum_cn	1.0	1.7	3.3	2.9	1.0	1.9	2.5	2.1
	Sum_g	67.7	69.3	67.7	70.9	68.7	71.3	70.5	73.9
Cabernet Sauv	Sum_ac	32.2	29.8	30.5	28.7	30.9	29.5	27.3	25.6
	Sum_cn	0.1	0.9	1.8	0.4	0.4	0.2	2.2	0.5

Sum_g=sum of non-acylated glucosides; *Sum_ac*=sum of acetyl derivatives; *Sum_cn*=sum of cinnamoyl derivatives; *b*=samples at the beginning of the sampling period; *h*=samples at harvest.

three additional variables were included: the sum of the non-acylated glucosides (Sum_g), the sum of acetates (Sum_ac) and the sum of cinnamoates (Sum_cn).

Evolution of the anthocyanin composition of grapes during ripening

In Table 1, the global relative content in non-acylated glucosides, acetates and cinnamoates of grapes from the six vineyards is shown at two different dates each year, one corresponding to the first sample obtained during ripening, and the other one to the harvest day. The variations observed along ripening in the three anthocyanin fractions were very small. Moreover, they were different depending on variety, vineyard and vintage year. A slight increase in the percentage of non-acylated glucosides was observed in 1999 for the Tempranillo high-quality grapes, while in 2000 this percentage decreased. Just the opposite was checked for the Garnacha high-quality grapes. The contribution of cinnamoates seemed to increase during ripening of Tempranillo berries, but this did not always happen in the case of the Garnacha and Cabernet Sauvignon varieties. On the other hand, the sum of acetyl derivatives seemed to be the anthocyanin fraction with the most constant proportion during ripening, particularly in the grapes of Garnacha and Tempranillo. In the case of the Cabernet Sauvignon variety, the proportion in acetates slightly declined from the first sample to the harvest, while the unacylated fraction always increased. This was the only regular tendency confirmed.

When the individual compounds were considered, no permanent tendency was observed (data not shown). The variations in the relative content of the several components during ripening were smaller even than those observed for the three global anthocyanin fractions. Therefore, these results did not confirm the anthocyanin biosynthetic pathway described by Roggero et al. [12]. They suggest that the cyanindin-3-glucoside is the initial anthocyanin synthesised. It is the direct precursor of peonidin and delphinidin. The latter is converted into petunidin, which finally may conduce to malvidin. Several authors have confirmed this biosynthetic pathway [13, 14, 15, 16, 23, 24] showing the evolution of the anthocyanin composition of berries during ripening. In general, they observed an intense accumulation of those compounds formed at the end of the anthocyanin synthesis process, peonidin and, primarily, malvidin. The amounts of the remained components, particularly that of cyanidin, increased more slowly, with the result that their contribution to the total anthocyanin content progressively declined along ripening.

We did not observe this evolution, probably due to the fact that we started sampling when the synthesis of anthocyanins in the berries was already in an advanced phase. This synthesis starts during *véraison*, becoming pronounced after it [25]. In this work, the first sampling of each vineyard was taken around 20 days after *véraison*, when berry juices reached 16–19°Brix, and their anthocyanin content was high. From this time to harvest, the accumulation pattern of all the anthocyanins was practically the same, and the subsequent variations in the composition of berries were quantitative instead of qualitative, in agreement with other findings previously reported [17, 18, 19].

Once it was observed that the berries did not suffer any significant change in their anthocyanin composition during ripening, raw data were statistically analysed in order to describe the differences between the varieties, the vineyards and the vintage years.

Anthocyanin characterisation of varieties

Firstly, a two-way analysis of variance was carried out according to the vineyard and vintage year factors. Mean values for each of the six vineyards and two years studied, together with their respective confidence intervals,

Table 2 Two-way analysis of variance (vineyard, vintage year). LSD-means (±95% confidence intervals) for vineyards[1] and vintage years

n[2]	TeHQ		TeLQ		GaHQ		GaLQ		CbHQ		CbLQ		1999		2000	
	13		14		13		11		16		14		37		44	
Dpg	8.4	±0.5^{a}	5.8	±0.5^{b}	2.6	±0.6^{cd}	2.3	±0.6^{cd}	2.0	±0.6^{c}	3.4	±0.5^{d}	4.2	±0.3	4.1	±0.3
Cyg	3.3	±0.3^{a}	2.2	±0.3^{b}	1.5	±0.3^{c}	0.8	±0.3^{d}	0.5	±0.3^{d}	1.4	±0.3^{c}	1.8	±0.2	1.3	±0.2
Ptg	10.1	±0.4^{a}	8.1	±0.3^{b}	4.4	±0.4^{c}	4.5	±0.4^{c}	3.1	±0.3^{d}	4.2	±0.3^{c}	5.8	±0.2	5.6	±0.2
Png	10.0	±0.4^{a}	8.9	±0.4^{b}	14.7	±0.4^{c}	10.0	±0.4^{a}	5.8	±0.4^{d}	8.7	±0.4^{b}	11.3	±0.2	8.1	±0.2
Mvg	61.4	±1.4^{a}	67.7	±1.4^{b}	72.5	±1.5^{c}	77.5	±1.6^{d}	57.5	±1.3^{e}	53.8	±1.4^{f}	63.2	±0.9	66.9	±0.8
Pnac	0.3	±0.1^{a}	0.3	±0.1^{a}	0.3	±0.1^{a}	0.2	±0.1^{a}	2.0	±0.1^{b}	2.6	±0.1^{c}	1.1	±0.1	0.8	±0.1
Mvac	2.7	±0.6^{ab}	3.3	±0.5^{b}	1.9	±0.6^{a}	2.0	±0.6^{a}	26.7	±0.5^{c}	22.4	±0.6^{d}	9.8	±0.3	9.8	±0.3
Mvcf	1.1	±0.1^{a}	1.1	±0.1^{a}	0.7	±0.1^{b}	0.8	±0.1^{ab}	0.3	±0.1^{c}	0.3	±0.1^{c}	0.7	±0.8	0.8	±0.1
Pnpc	0.2	±0.1^{a}	0.2	±0.1^{a}	0.2	±0.1^{a}	0.2	±0.1^{ab}	0.1	±0.1^{b}	0.1	±0.1^{ab}	0.1	±0.1	0.2	±0.1
Mvpc	1.4	±0.3^{a}	1.6	±0.3^{a}	1.1	±0.3^{ac}	1.4	±0.4^{a}	0.4	±0.3^{b}	0.5	±0.3^{bc}	0.8	±0.2	1.4	±0.2
Sum_g	93.2	±0.7^{a}	92.7	±0.7^{a}	95.7	±0.7^{b}	95.3	±0.8^{b}	68.8	±0.6^{c}	71.5	±0.7^{d}	86.3	±0.4	86.1	±0.4
Sum_ac	3.7	±04^{a}	4.1	±0.4^{a}	2.2	±0.4^{b}	2.2	±0.5^{b}	30.3	±0.4^{c}	27.5	±0.4^{d}	11.9	±0.3	11.4	±0.2
Sum_cn	3.1	±0.5^{a}	3.2	±0.5^{a}	2.2	±0.5^{b}	2.5	±0.5^{ab}	0.8	±0.4^{c}	1.0	±0.5^{c}	1.8	±0.3	2.5	±0.3

[1] Two vineyard means followed by the same letter indicates that they are not significantly different at 95% confidence level
[2] Number of samples.
Dpg=delphinidin-3-glucoside; *Cyg*=cyanidin-3-glucoside; *Ptg*=petunidin-3-glucoside; *Png*=peonidin-3-glucoside; *Mvg*=malvidin-3-glucoside; *Pnac*=peonidin-3-acetylglucoside; *Mvac*=malvidin-3-acetylglucoside; *Mvcf*=mavidin-3-caffeoylglucoside; *Pncp*=peonidin-3-*p*-coumarylglucoside; *Mvcp*=malvidin-3-*p*-coumarylglucoside.
Sum_g=sum of non-acylated glucosides; *Sum_ac*:sum of acetyl derivatives; *Sum_cn*:sum of cinnamoyl derivatives.
Te=Tempranillo; *Ga*=Garnacha; *Cb*=Cabernet Sauvignon.
HQ=high-quality vineyard; *LQ*=low-quality vineyard.

Table 3 Two-way analysis of variance (vineyard, vintage year). *F* ratios and *p*values for each of both factors and their interaction[1]

	Vineyard		Vintage year		Interaction	
	F ratio	*p* value	*F* ratio	*p* value	*F* ratio	*p* value
	(5.69)		(1.69)		(5.69)	
Dpg	83.7	0.0000				
Cyg	55.5	0.0000	21.4	0.0000	5.6	0.0002
Ptg	248.9	0.0000				
Png	218.4	0.0000	359.4	0.0000	29.4	0.0000
Mvg	156.4	0.0000	39.5	0.0000	10.3	0.0000
Pnac	953.3	0.0000	166.8	0.0000	62.3	0.0000
Mvac	1857.7	0.0000				
Mvcf	28.7	0.0000				
Pnpc	6.8	0.0000				
Mvpc	9.8	0.0000	17.3	0.0001		
Sum_g	1335.6	0.0000				
Sum_ac	4242.4	0.0000				
Sum_cn	19.9	0.0000	13.3	0.0005		

[1] Only significant F ratios/p values at 95% confidence level are presented.
Dpg=delphinidin-3-glucoside; *Cyg*=cyanidin-3-glucoside; *Ptg*=petunidin-3-glucoside; *Png*=peonidin-3-glucoside; *Mvg*=malvidin-3-glucoside; *Pnac*=peonidin-3-acetylglucoside; *Mvac*=malvidin-3-acetylglucoside; *Mvcf=mavidin*-3-caffeoylglucoside; *Pncp*=peonidin-3-*p*-coumarylglucoside; *Mvcp*=malvidin-3-*p*-coumarylglucoside.
Sum_g=sum of non-acylated glucosides; *Sum_ac*=sum of acetyl derivatives; *Sum_cn*=sum of cinnamoyl derivatives.

are presented in Table 2, while Table 3 shows the F ratios and p values obtained for each of the two factors and their interactions.

Significant differences, with p<0.0001, were observed between vineyards for all the variables. The largest F ratios corresponded to four of them: Sum_ac, Sum_g, and the malvidin and peonidin acetylglucosides; followed by the non-acylated monoglucosides of petunidin, peonidin, malvidin, delphinidin and cyanidin. Finally, the cinnamic acid esters, principally the *p*-coumaryl derivatives of malvidin and peonidin glucosides, presented the lowest F ratios.

On the other hand, significant qualitative differences between vintages were detected only for six variables. According to F ratios the variations from year to year particularly affected to the peonidin-3-glucoside and its acetyl derivative. In general, the proportion of these compounds was larger in 1999 than in 2000. The same

was observed for the cyanidin-3-glucoside, while the opposite occurred for the malvidin-3-glucoside, its *p*-coumaryl derivative and the sum of cinnamoates. Nevertheless, these differences between vintages were not verified in all the vineyards, as was shown by the significant interactions between vineyard and vintage year listed in Table 3. Again, the largest F ratios corresponded to the peonidin derivatives.

From the study in detail of the means in Table 2, we may conclude that the differences observed between vineyards in their anthocyanin qualitative composition must be explained fundamentally in terms of the varietal variability. Thus, Cabernet Sauvignon grapes from both the high- and the low-quality vineyards showed a very much larger relative content in acetyl derivatives, principally that of malvidin-3-glucoside, than grapes from Tempranillo and Garnacha. In turn, the mean proportion in the monoglucosides of delphinidin, cyanidin and petunidin was significantly higher in Tempranillo than in the other varieties, while Garnacha presented the greatest content in malvidin and peonidin unacylated glucosides.

These results were confirmed when a principal component analysis over the whole data set was performed. From thirteen principal components obtained, the first three with eigenvalues >1, were selected, accounting for almost 90% of the total variance (Fig. 1). Fig. 2 shows the distribution of 81 grape samples along the first two principal components (77.58% variance), and also the component weights of 13 original variables. Both principal components could be called “varietal components”, because samples were clearly grouped according to variety. The original variables with a more important weight in component 1 were the acetyl derivatives of malvidin and peonidin, the sum of acetates, the sum of non-acylated glucosides, the sum of cinnamoyl derivatives, and the malvidin-3-glucoside-caffeoate. The first three ones presented negative weights and were strongly related to the Cabernet Sauvignon variety, whose samples were located on the negative part of the first axis. Tempranillo and Garnacha samples were placed just on the opposite side. The separation of both varieties was finally achieved with the help of principal component 2. The anthocyanin compounds mainly involved in it were the non-acylated glucosides of malvidin, delphinidin, cyanidin and petunidin. The former one presented a positive weight, while the further three variables showed negative ones. Garnacha samples were located in the positive part of the PC2 axis, principally due to their high relative content in malvidin and peonidin glucosides and total non-acylated glucosides. Delphinidin, cyanidin and petunidin determined the location of Tempranillo samples in the opposite side along this axis. This variety was also closely related to the glucosides acylated with cinnamic acid. These anthocyanin profiles were consistent with the results previously obtained in grapes and wines of the same varieties from Spain [6, 9, 10, 20, 26] and from other countries [5, 22].

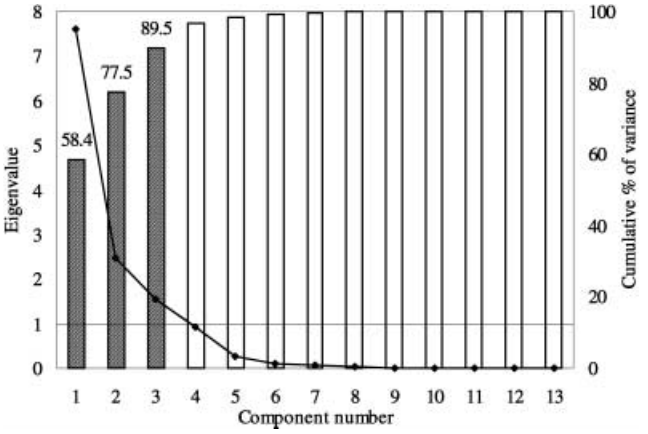

Fig. 1 Principal components scree plot

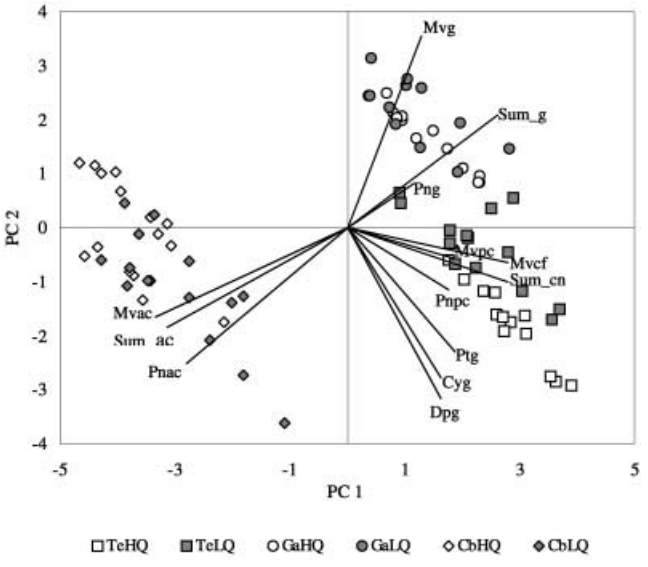

Fig. 2 Principal component analysis. Distribution of samples along PC1 and PC2 and component weights of original variables. *Dpg*=delphinidin-3-glucoside, *Cyg* cianidin-3-glucoside, *Ptg* petunidin-3-glucoside, *Png*=peonidin-3-glucoside, *Mvg*=malvidin-3-glucoside, *Pnac*=peonidin-3-acetylglucoside, *Mvac*=malvidin-3-acetylglucoside, *Mvcf*=mavidin-3-caffeoylglucoside, *Pncp*=peonidin-3-*p*-coumarylglucoside, *Mvcp*=malvidin-3-*p*-coumarylglucoside, *Sum_g*=sum of non-acylated glucosides, *Sum_ac*=sum of acetyl derivatives, *Sum_cn*=sum of cinnamoyl derivatives. *Te*=Tempranillo, *Ga*=Garnacha, *Cb*=Cabernet Sauvignon, *HQ*=high-quality vineyard, *LQ*=low-quality vineyard

Anthocyanin differences between vineyards

Some differences were found between high- and low-quality vineyards within each variety (Table 2). This was verified for the five unacylated monoglucosides, except for that of delphinidin in Garnacha and Cabernet Sauvignon, and that of petunidin in the former variety. In Tempranillo and Garnacha varieties the high-quality fruit had a lower relative content in malvidin-3-glucoside and a larger proportion in the rest of the non-acylated anthocyanins than in the low-quality grapes.

Table 4 Free anthocyanin content (mg/kg berries)[1] of grapes from each vineyard at harvest

	High-quality vineyard		Low-quality vineyard	
	1999	2000	1999	2000
Tempranillo	595.7	658.5	450.2	387.0
Garnacha	279.1	473.6	931.8	819.5
Cabernet Sauvignon	1198.4	1084.1	750.6	748.7

[1] Quantified as malvidin-3-glucoside.

Table 5 Stepwise discriminant analysis according to vineyard

Step	Selected variable	F-to-enter	% of samples correctly classified							Standardized coefficients	
			TeHQ (13)	TeLQ (14)	GaHQ (13)	GaLQ (11)	CbHQ (16)	CbLQ (14)	Total (81)	DF1	DF2
1	Sum_ac	2730.4	61.5	92.9	46.2	63.6	81.3	78.6	71.7	–5.52	–1.15
2	Ptg	112.9	100.0	85.7	61.5	36.4	87.5	78.6	76.5	–1.52	2.22
3	Pnac	33.3	100.0	85.7	69.2	63.6	93.8	100.0	86.4	3.35	0.63
4	Png	14.5	100.0	85.7	100.0	54.5	93.8	100.0	90.1	0.23	–1.39
5	Mvac	12.9	100.0	85.7	100.0	54.5	100.0	100.0	91.4	3.80	1.51
6	Dpg	8.7	100.0	92.9	100.0	90.9	100.0	100.0	97.5	1.99	–1.68
7	Cyg	11.4	100.0	100.0	100.0	100.0	100.0	100.0	100.0	–0.61	1.51
% of variance										95.5	4.2
p-value										0.0000	0.0000

Sum_ac=sum of acetyl derivatives.
Ptg=petunidin-3-glucoside; *Pnac*=peonidin-3-acetylglucoside; *Png*=peonidin-3-glucoside; *Mvac*=malvidin-3-acetylglucoside; *Dpg*=delphinidin-3-glucoside; *Cyg*=cyanidin-3-glucoside.
Te=Tempranillo; *Ga*=Garnacha; *Cb*=Cabernet Sauvignon.
HQ=high-quality vineyard; *LQ*=low-quality vineyard

The opposite was observed for the third variety. The Cabernet Sauvignon high-quality vineyard produced grapes with a greater proportion in the malvidin derivatives, including the unacylated glucoside and the acetate, than those from the low-quality vineyard.

Nevertheless, the differences in anthocyanins between vineyards were lower in terms of composition than in terms of concentration [14, 17, 19], as can be observed in Table 4. This table shows the total content in free anthocyanins of grapes from each vineyard at harvest day. The Tempranillo and Cabernet Sauvignon high-quality grapes had a larger anthocyanin content than the corresponding low-quality ones, while the opposite was observed for Garnacha. In the case of this variety, grapes from the low-quality vineyard were very much richer in anthocyanins than those from the high-quality vineyard, particularly in 1999.

Regarding again to the anthocyanin composition of grapes, the qualitative differences between vineyards within each variety tended to disappear when the further three variables listed in Table 2 (respectively, sum of non-acylated glucosides, sum of acetates and sum of cinnamoates) were analysed. This was particularly true for Tempranillo and Garnacha varieties which significantly differed one from the other in the mean total relative content of the unacylated anthocyanins and their acetyl derivatives, but no differences were found between the high- and low-quality vineyards within each of the two varieties. A higher or lower proportion of malvidin 3-glucoside seems to have been compensated by, respectively, a lower and higher percentage of the other four anthocyanin glucosides, with the result that the sum of non-acylated glucosides was vineyard-independent and remained practically constant in each variety.

In a lower extent, this compensation or equilibrium between malvidin compounds and the rest of the anthocyanins was observed in the case of Cabernet Sauvignon as well, not only within the group of unacylated glucosides, but also within that of acetyl derivatives.

Therefore, it seems that the influence of genetic factors in the anthocyanin qualitative composition of grapes became particularly evident when the variables corresponding to the sums of unacylated glucosides, acetates and cinnamoyl derivatives were considered. Thus, Garnacha grapes presented almost only non-acylated glucosides (more than 95%) with a very low proportion of acetates, while Cabernet Sauvignon was characterised by a very large content in acetyl derivatives (30% and 27%, in the high- and low-quality grapes, respectively) and a slight presence of cinnamoates (1% in both vineyards). Tempranillo showed an intermediate anthocyanin profile, although quite close to that of Garnacha. Tempranillo grapes had the largest relative content in cinnamoyl derivatives (around 3%) and an appreciable proportion of acetates (4%).

Discrimination of vineyards

Finally, the results of a stepwise discriminant analysis are presented (Table 5 and Fig. 3). This analysis was performed over the whole data set and with the vineyard as discriminant factor. Thus, five discriminant functions were obtained after seven steps. Table 5 indicates the

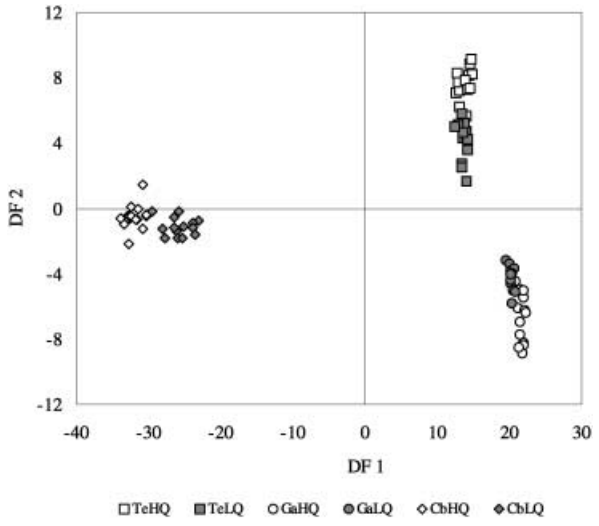

Fig. 3 Discriminant analysis. Distribution of samples along DF1 and DF2. *Te*=Tempranillo, *Ga*=Garnacha, *Cb*=Cabernet Sauvignon, *HQ*=high-quality vineyard, *LQ*=low-quality vineyard

variable that entered at each step into the discriminant model, together with its F-to-enter value, and the percentage of samples correctly classified in each vineyard. Moreover, it can be observed the standardized coefficients of the seven variables in the first two discriminant functions, which were significant with $p<0.0001$ and respectively accounted the 95.5% and 4.2% of the total variability. The model was defined by the acetyl derivatives and by the first four non-acylated glucosides. The sum of acetates was selected at first step. Considering only this variable, a 71.6% correct classification was achieved. It must be noted that the remaining 28.4% was classified as an incorrect vineyard, but not as an incorrect variety. This fact was confirmed during all the stepwise selection processes until all the samples became part of the correct vineyard. That proved again the pronounced genetic character of the anthocyanin composition of grapes.

To conclude, the distribution of 81 cases along the first two discriminant functions is shown in Figure 3. This figure may summarise all the results discussed above. Three strongly separated groups were observed, corresponding to the three varieties studied. Their location in the graph confirmed the differences and similarities that existed between them. Thus, a very large distance existed between the Cabernet Sauvignon group and those of Tempranillo and Garnacha, more closely located along the first discriminant function. The distinction between the samples from the high-quality vineyard and those corresponding to the low-quality one was possible within each variety. Therefore, some permanent qualitative differences existed between vineyards in the anthocyanin composition of grapes, although they were small in comparison with the quantitative differences. Likewise, the collection of data from, at least, another vintage year should be recommended to confirm these results.

Aknowledgements This work has been supported by Bodegas Julián Chivite Ltd. in Cintruénigo, Navarra (Spain).

References

1. Hebrero E, García-Rodríguez C, Santos-Buelga C, Rivas-Gonzalo JC (1989) Am J Enol Vitic 40:283–291
2. Mazza G (1995) Crit Rev Food Sci Nutr 35:341–371
3. Gao L, Girard B, Mazza G, Reynolds AG (1997) J Agric Food Chem 45:2003–2008
4. Roggero JP, Larice JL, Rocheville-Divorne C, Archier P, Coen S (1988) Rev Fran Oenol 112:41–48
5. Etiévant P, Schlich P, Bertrand A, Symons P, Bouvier JC (1988) J Sci Food Agric 42:39–54
6. González-San José ML, Santa-María G, Díez C (1990) J Food Compos Anal 3:54–66
7. Castia T, Franco MA, Mattivi F, Muggiolu G, Sferlazzo G, Versini G (1992) Sci Aliments 12:239–255
8. Calò A, Tomasi D, Cravero MC, Stefano R Di (1994) Riv Vitic Enol 47:13–25
9. Arozarena I, Casp A, Marín R, Navarro M (2000) Z Lebensm Unters Forsch 212:105–112
10. Cacho J, Fernández P, Ferreira V, Castells JE (1992) Am J Enol Vitic 43:244–248
11. Mazza G, Fukumoto L, Delaquis P, Girard B, Ewert B (1999) J Agric Food Chem 47:4009–4017
12. Roggero JP, Coen S, Ragonnet B (1986) Am J Enol Vitic 37:77–83
13. González-San José ML, Barrón LJR, Díez C (1990) J Sci Food Agric 51:337–343
14. Tomasi D, Calò A, Biscaro S, Vettorello G, Panero L, Stefano, R Di (1999) Bull OIV 72:321–337
15. Lanaridis P, Bena-Tzourou I (1997) J Int Sci Vigne Vin 31:205–212
16. Esteban MA, Villanueva MJ, Lissarrague JR (2001) J Sci Food Agric 81:409–420
17. Yokotsuka K, Akitoshi N, Nakazawa K, Sato M (1999) Am J Enol Vitic 50:1–12
18. Asselin C, Ballester FF (1992) Prog Agric Vitic 109:415–420
19. Brossaud F, Cheynier V, Asselin C, Moutounet M (1999) Am J Enol Vitic 50:277–284
20. Hebrero E, Santos-Buelga C, Rivas-Gonzalo JC (1988) Am J Enol Vitic 39:227–233
21. Dallas C, Laureano O (1994) Vitis 33:41–47
22. Hmamouchi M, Es-Safi N, Pellecuer J, Essassi EM (1995) Bull OIV 68:905–919
23. Jordao AM, Ricardo da Silva JM, Laureano O (1998) Vitis 37:93–94
24. Fernández-López JA, Hidalgo V, Almela L, López-Roca JM (1992) J Sci Food Agric 58:153–155
25. Jackson RS (2000) Wine Science. Principles, practice, perception. 2nd edn. Academic Press, San Diego
26. Mayen M, Mérida J, Medina M (1994) Am J Enol Vitic 45:161–166