Evolution of Five Anthocyanidin-3-Glucosides in the Skin of the Tempranillo, Moristel, and Garnacha Grape Varieties and Influence of Climatological Variables

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Anthocyanins in skins of Tempranillo [Valdepeñas (California)], Moristel, and Garnacha (Grenache) skin grape varieties were separated and quantified by HPLC in two different vintages (1987 and 1988). The relationship between this evolution and the climatology data for the sampled vineyards was studied. The changes in the anthocyanin content were greater between the same varieties in different years than the different varieties in the same year. Nevertheless, differences were found between varieties. The delphinidin- and petunidin-3-glucosides were found in much higher relative content in Tempranillo grapes than in Garnacha and Moristel. Garnacha presents a higher relative content in cyanidin- and peonidin-3-glucosides, while Moristel presents higher malvidin-3-glucoside relative content.

KEY WORDS: anthocyanidin-3-glucosides, climate, cyanidin, delphinidin, malvidin, peonidin, petunidin

The color of the grape is a sensory quality which changes during ripening. Anthocyanins are red pigments which are responsible for the grape's color. They appear mainly in the skin during the ripening and increase until maturity. The nature of anthocyanins has been studied in detail, and delphinidin, cyanidin, petunidin, peonidin, and malvidin have been found in the form of 3-glucosides and, to a lesser extent, as esters of acetic, coumaric, and caffeic acids. The diglucosides are found only in non-vinifera grapes (1).

The anthocyanin content of mature grapes is very variable, and depends on the variety and on climatic conditions. Singleton and Esau (1) estimate that the average content of anthocyanins in the black grapes used for winemaking is 800 mg/kg. However, Flanzy *et al.* (2) found an average of 2.295 mg/kg. Growth conditions can alter the anthocyanin content, and some natural factors, such as the amount of potassium which is assimilable from the soil, can have a considerable influence on the anthocyanin content of grapes (3).

HPLC gives fast simultaneous separation of the different anthocyanins with no need for prior isolation. We have used this technique to systematically monitor the evolution of anthocyanins in the skins of the Tempranillo, Moristel, and Garnacha varieties of grapes during ripening.

Materials and Methods

The Vines

Geographic location: The Tempranillo [Valdepeñas (California)] and Moristel varieties studied were from the Somontano district, in the province of Huesca, which lies between latitudes 41°15' and 42°25' North and longitudes 2°45' and 4°25' East (Madrid meridian). The Garnacha (Grenache) variety was from Cervera de la Cañada (Calatayud), in the province of Zaragoza, which lies between latitudes 40°50' and 42°45' North and longitudes 1°30' and 4°05' East (Madrid meridian). Both regions have *denomination d'origine.*

Soil characteristics. ***Somontano district:*** The soil on which the Tempranillo and Moristel vines are grown is classed as brown chalky soil on marls, sandstone and unconsolidated pebble-bearing levels. The pebbles are not strongly cemented or bonded, but are simply in contact and stacked, making for greater permeability and root penetration.

Calatayud district: The vineyard soil description is: xeronrendzines on marl, sandstone, and conglomerates with areas of brown chalky soil. The destruction of part of the conglomerate makes the soil somewhat stony, both on the surface and in profile. Furthermore, the conglomerate's greater resistance to disintegration and weathering means that erosion has exposed the conglomerate on the surface, on which a high proportion of lithosols is found.

Sampling: The study was performed over two years, 1987 and 1988. Samples were taken from 500 vines from the same vineyards for seven successive dates about seven to 10 days apart, from the beginning of the ripening until the harvest. At least 2000 grapes from 500 vines were picked up in each sampling. From each vine, four clusters were collected in plastic bags. Once all the samples had been collected, they were placed in a freezer at -28°C. The frozen samples were then homogenized and stored in the freezer again until analysis.

Preparation of the extract: Pigment extracts were prepared by maceration of washed skin from 50 freshly-thawed berries with methanol + 1% HCl (4). Nine extractions were carried out with 10 mL solvent at

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room temperature under inert gas. Each maceration lasted one hour. Finally, the nine extracts were combined for a final volume of 100 mL.

For each sample of grapes, three extracts were prepared in order to obtain analytical results from the average of three determinations.

HPLC analysis: A Kontron chromatograph was used, fitted with Kontron 420 pumps, Kontron 491 mixer, Kontron 461 auto-injector, Kontron 430 UV-Vis detector. The data were treated with the program Kontron Data System 450. A Nucleosil C-120 C-18 5u (25 × 40) column was used.

The chromatographic conditions were as follows: elutant A: H_2O/formic acid (5%); elutant B: CH_3CN; flow rate: 1 mL/min; gradient - from 10% to 15% for elutant B for the first 10 minutes, from 15% to 19% of elutant B for 7 minutes, and from 19% to 40% for 17 minutes. Compounds were detected by measuring absorbance at 520 nm. Injection volume was 50 µL.

Methods

To calibrate the chromatographic response and express the results in terms of concentration, malvidin-3,5-diglucoside chloride was used. A range of solutions of malvidin-3,5-diglucoside chloride were prepared and chromatographed in the same conditions as the extracts. The results obtained were as follows:

Concentration (mg/L)	Peak area
1200	1400
720	847
360	400
240	266
120	134

Fitting these data by least squares gives a line which passes through the origin, whose equation is:

$$\text{Concentration (mg/L)} = 0.859 \bullet \text{Area}$$

with a correlation coefficient of r = 0.9998. To calculate the concentration of each anthocyanin, molecular weights must be taken into account. This means multiplying the value obtained by the molecular weight of the anthocyanin and dividing by the molecular weight of malvidin-3,5-diglucoside chloride (MW = 691.0). In this way, the concentration of each anthocyanin is given by:

$$\text{Concentration (mg/L)} = \frac{0.859 \bullet \text{Area} \bullet \text{MW}}{691}$$

Tables 1 and 2 show the concentration of each anthocyanidin expressed in milligrams per kilo of grapes.

Determination of the total anthocyanin content: To determine the total anthocyanin content, the method developed by Ribéreau-Gayon and Stonestreet for analyzing wine (5) was used. The extracts are analyzed by the same procedure as for wine, but first it is necessary to dilute the polyphenols extract so as not to go outside the linear range (the unbleached tube absorbance must not exceed 0.5 AU). Normally, extracts of mature black grapes need to be diluted four-fold, and extracts of semi-ripe grapes two-fold.

Results and Discussion

Evolution of the weight of 100 berries: Tables 1 and 2 show that the weights of the Tempranillo and Moristel grapes varied widely between one year and the next. The year 1988 gave the highest weight, due undoubtedly to rain during ripening. The Garnacha variety showed very little difference between the mature grapes of the two years, although the evolution was different. The second year gave a lower weight due to over-maturing.

Total anthocyanin content: The evolution of anthocyanins during the maturing of these grapes followed the pattern reported in the literature: it increased from the onset of ripening, reached a peak at maturity, and fell slightly during over-maturing. Large differences in the anthocyanin content were observed between the two years within each variety, 1988 being the richer year. In a particular year, the three varieties had similar anthocyanin contents at maturity.

Anthocyanidin-3-monoglucosides: The order of elution of the anthocyanins in reverse phase is related to their polarity; the more polar compounds are eluted first (5 6,7,8). The polarity of anthocyanins is directly related to the degree of hydroxylation and methylation of the benzene ring. The hydroxyl groups increase the polarity. As a result, the first anthocyanin eluted is delphinidin, followed by cyanidin, petunidin, peonidin, and malvidin (5,7,9). The retention times are as follows:

Delphinidin-3-monoglucoside	8-9 min
Cyanidin-3-monoglucoside	10-11 min
Petunidin-3-monoglucoside	12-13 min
Peonidin-3-monoglucoside	14-15 min
Malvidin-3-monoglucoside	16-17 min

Evolution of the delphinidin-3-monoglucoside content: The evolution of the delphinidin-3-monoglucoside content of the three varieties studied, are the same.

The delphinidin-3-monoglucoside contents were higher in 1988 than 1987, as was the case with total anthocyanins. The Tempranillo and Garnacha varieties had a similar anthocyanidin content, which was higher than that of the Moristel variety (Tables 1 and 2). The Tempranillo variety presents the highest relative content of this anthocyanin (Table 3).

Evolution of the cyanidin-3-monoglucoside content: The evolution of the cyanidin-3-monoglucoside content of the three varieties studied are the same.

The cyanidin-3-monoglucoside evolution differs from that of total anthocyanins. In the Tempranillo and Moristel varieties, the content of this anthocyanidin increased slightly from ripening to maturity, while remaining at a very low level. In the Garnacha variety, in 1987 the level increased from the ripening , peaked and then fell. In 1988, it continued rising until maturity, giving almost four times the 1987 level. The concentration was considerably lower than that of the other

Table 1. Analytical data for 1987 for the varieties Tempranillo, Moristel, and Garnacha.

Variety	Date	°Brix	Weight 100 berries (g)	Total anthocyanins (mg/kg)	Anthocyanidin-3-glucosides (mg/kg) Dp-3-gluc	Cy-3-gluc	Pt-3-gluc	Pn-3-gluc	Mv-3-gluc
Tempranillo									
(1987)	8/10	16.3	122	547	23.9	7.9	18.9	5.7	48.6
	8/17	19.2	121	842	33.7	5.4	29.7	5.9	86.6
	8/27	18.4	125	1033	37.5	4.6	35.2	6.1	114.0
	8/31	18.7	126	1239	52.2	7.5	47.5	7.4	143.0
	9/8	19.5	127	1674	77.8	7.5	62.3	9.7	182.0
	9/14	20.2	139	1362	82.6	10.5	70.3	12.1	206.0
Moristel									
(1987)	8/10	9.0	96	186	5.6	2.2	5.7	6.2	19.6
	8/17	13.5	107	506	13.5	2.7	16.6	8.4	74.4
	8/31	15.0	142	988	24.2	4.7	29.8	12.6	154.0
	9/8	21.4	146	1330	28.4	4.3	34.8	8.9	180.0
	9/14	21.5	143	1326	38.6	7.1	41.3	12.8	180.0
Garnacha									
(1987)	8/21	12.5	129	267	5.0	2.7	6.7	10.9	39.6
	8/28	20.2	149	507	15.8	8.7	15.8	31.4	67.8
	9/7	22.6	176	980	46.7	17.9	42.7	47.4	142.0
	9/11	25.2	168	1117	51.4	15.9	44.4	46.4	161.0
	9/18	25.2	165	1264					
	9/25	23.0	152	1172	50.8	13.4	43.0	48.0	172.0

Table 2. Analytical data for 1988 for the varietiesTempranillo, Moristel, and Garnacha.

Variety	Date	°Brix	Weight 100 berries (g)	Total anthocyanins (mg/kg)	Anthocyanidin-3-glucosides (mg/kg) Dp-3-gluc	Cy-3-gluc	Pt-3-gluc	Pn-3-gluc	Mv-3-gluc
Tempranillo									
(1988)	8/8	13.5	163	480	27.5	9.5	20.1	9.8	38.2
	8/16	17.0	172	972	50.9	12.1	40.2	13.8	101.0
	8/22	19.0	201	1230	61.2	11.3	55.6	14.6	148.0
	8/30	20.0	195	1711	76.9	16.2	74.0	19.2	208.0
	9/6	21.5	179	2037	87.9	16.5	87.1	22.5	229.0
	9/13	25.0	203	1969	92.4	17.5	76.6	21.8	239.0
Moristel									
(1988)	8/22	12.0	150	294	9.0	3.7	10.7	8.1	49.0
	8/30	15.0	179	777	27.2	8.8	30.5	21.3	120.0
	9/16	16.5	201	1268	41.7	10.0	47.2	25.6	194.0
	9/13	19.5	202	1517	50.5	13.5	56.5	27.5	247.0
	9/20	20.0	201	1615	45.8	12.1	56.8	33.7	265.0
Garnacha									
(1988)	9/2	17.5	130	472	16.5	11.1	15.0	47.5	58.8
	9/9	23.0	136	1029	38.8	21.2	33.6	81.9	151.0
	9/16	24.5	135	1428	53.2	25.0	48.2	108.0	221.0
	9/23	26.0	149	1852	80.7	33.0	72.3	122.0	293.0
	9/30	25.5	152	1887	83.2	35.1	74.5	130.0	305.0
	10/10	28.5	142	2306	92.6	42.9	98.8	156.0	355.0

Table 3. Relative content of the anthocyanidin-3-glucosides in three varieties studied during 1987 and 1988.

Variety	Anthocyanidin3-glucoside (%)				
	Dp-3-gluc	Cy-3-gluc	Pt-3-gluc	Pn-3-gluc	Mv-3-gluc
Tempranillo (1987)	21.6	2.7	18.4	3.2	54.0
Tempranillo (1988)	20.6	3.9	17.1	4.9	53.4
Moristel (1987)	13.8	2.5	14.7	4.5	64.3
Moristel (1988)	10.9	4.2	13.6	8.0	62.3
Garnacha (1987)	15.5	4.1	13.1	14.7	52.6
Garnacha (1988)	14.0	6.6	15.3	8.7	55.0

Table 4. The climatic data for the Somontano and Calatayud .

Vine location	Date	Precipitation (mm)	T. max.	T. min.	T. av.
Somontano	April, 1987	25	13.1	4.2	8.6
Somontano	May, 1987	61	12.8	5.2	9.0
Somontano	June, 1987	4	17.6	7.4	12.5
Somontano	July, 1987	16	28.0	15.3	21.6
Somontano	August, 1987	5	30.6	16.3	23.4
Somontano	Sept., 1987	32	25.4	13.4	19.4
Somontano	Oct., 1987	89	17.0	8.8	19.9
Somontano	April, 1988	178	12.1	1.9	7.0
Somontano	May, 1988	52	14.6	6.3	10.4
Somontano	June, 1988	56	19.6	10.7	15.2
Somontano	July, 1988	5	19.8	7.5	13.6
Somontano	August, 1988	4	30.0	14.3	22.1
Somontano	Sept., 1988	0	23.8	8.5	16.1
Somontano	Oct., 1988	73	20.4	5.4	12.9
Calatayud	April, 1987	22	19.1	6.7	12.9
Calatayud	May, 1987	17	19.7	7.2	13,4
Calatayud	June, 1987	29	26.0	12.7	19.7
Calatayud	July, 1987	50	27.5	15.8	21.6
Calatayud	August, 1987	0	31.7	16.2	23.9
Calatayud	Sept., 1987	12	28.8	14.2	21.5
Calatayud	Oct., 1987	52	18.6	9.0	13.8
Calatayud	April, 1988	122	15.3	6.4	10.8
Calatayud	May, 1988	106	19.1	9.6	14.6
Calatayud	June, 1988	159	21.4	11.7	16.6
Calatayud	July, 1988	33	27.8	14.3	21.1
Calatayud	August, 1988	0	27.9	14.4	22.2
Calatayud	Sept., 1988	4	26.5	10.7	18.6
Calatayud	Oct., 1988	51	20.7	7.8	14.2

anthocyanins (Tables 1 and 2).

Evolution of the petunidin-3-monoglucoside content: The evolution of the petunidin-3-monoglucoside content of the three varieties studied are the same.

Comparing the two years, 1988 had higher concentrations than 1987. In the case of Garnacha, the 1988 figure was double that of the previous year. Of the three varieties, Moristel had the lowest content of this anthocyanidin; Garnacha and Tempranillo could be said to have similar petunidin-3-monoglucoside contents (Table 1 and 2). Nevertheless, the Tempranillo variety presents the higher relative content (Table 3).

Evolution of the peonidin-3-monoglucoside content: The peonidin-3-monoglucoside content increased from the ripening to maturity in all three varieties, although the final values were lower in Tempranillo and in Moristel than in Garnacha (Tables 1 and 2). There was a considerable difference between the two years, up to three-fold. The highest values in the three varieties were found in 1988.

Evolution of the malvidin-3-monoglucoside content: In this case too, the evolution during maturation were very similar to those of total anthocyanins. Malvidin-3-monoglucoside is the anthocyanidin which is present in the highest quantities in all three varieties. Of the three, Garnacha has the highest amount, followed by Tempranillo and Moristel, both with similar values (Tables 1 and 2). The Moristel variety presents the higher relative content of this anthocyanin than the other varieties (Table 3).

As with total anthocyanins, the contents were higher in 1988 than in 1987. In the case of Garnacha, the 1988 content was double that of 1987.

Climatology: The climatic data for the Somontano district, collected by the local meteorological station, were provided by the Instituto Nacional de Meteorología. Table 4 shows precipitations and the maximum, minimum, and average temperatures.

The was more precipitation in the months of April, May, and June in 1988. During maturation, precipitation was light in both years.

Temperatures were similar in both years, and the only noteworthy feature is the abnormal temperature figure for July 1988. The grapes mature in the hottest month: August.

The climatic data for the Calatayud district, collected by the Estación Meteorológica de Calatayud,

were provided by the Instituto Nacional de Meteorología.

Table 4 shows that there was a considerable difference in precipitation between the two years. In 1988, there were abundant rains in April, May, and June, whereas it hardly rained during the maturation period. In 1987, there was very little rain all year.

Temperatures during the maturation period were slightly higher in 1987. This, combined with the low rainfall, indicates that 1987 was much drier than 1988.

Conclusions

The evolution of the anthocyanin content of grapes is heavily influenced by the year's climatic conditions, and the differences between the years in a single variety are greater than between different varieties in the same year. The five anthocyanidins studied evolve in a similar way to the total anthocyanin content.

Delphinidin-3-glucoside is most abundant in the Tempranillo variety, whereas Moristel has the lowest content of this anthocyanidin.

The cyanidin-3-glucoside content varies very little from ripening to maturity. The highest concentration is found in the Garnacha variety. The amounts found in the three varieties are very low.

The Moristel variety has the lowest petunidin-3-glucoside of the three, while Tempranillo variety has the highest relative content of this anthocyanin.

Peonidin-3-glucoside is found in very low concentrations in the Tempranillo and Moristel varieties, whereas the Garnacha variety has a much higher concentration. Its concentration in skin varies greatly from year to year.

The Moristel variety has the higher relative content of malvidin-3-glucoside.

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