beverages

MDPI

Article

Phenolic Composition and Color of Single Cultivar Young Red Wines Made with Mencia and Alicante-Bouschet Grapes in AOC Valdeorras (Galicia, NW Spain)

Eugenio Revilla [1,*], Manuel M. Losada [2] and Encina Gutiérrez [1]

[1] Departamento de Química Agrícola y Bromatología, Universidad Autónoma de Madrid, 28049 Madrid, Spain; encina.gutierrez@estudiante.uam.es

[2] Departamento de Química y Tecnología de Alimentos, Universidad Politécnica de Madrid, 28040 Madrid, Spain; manuelmaximino.losada@upm.es

* Correspondence: eugenio.revilla@uam.es; Tel.: +34-914-974-832

Academic Editor: Shao Quan Liu

Received: 12 May 2016; Accepted: 19 July 2016; Published: 25 July 2016

Abstract: Single cultivar wines made with two different red grape cultivars from AOC Valdeorras (Galicia, NW Spain), Mencia and Alicante Bouschet, were studied with the aim of determining their color and phenolic composition. Two sets of analyses were made on 30 wine samples of 2014 vintage, after malolactic fermentation took place, to evaluate several physicochemical characteristics from these wines related to color and polyphenols. Several parameters related with color and the general phenolic composition of wines (total phenols index, color intensity, hue, total anthocyans, total anthocyanins, colored anthocyanins, chemical age index, and total tannins) were determined by UV-VIS spectrophotometry. Those analyses revealed that Alicante Bouschet wines presented, in general, a higher content of polyphenols and a more intense color than Mencia wines. Using HPLC-DAD, five anthocyanin monoglucosides and nine acylated anthocyanins were identified in both types of wine; each type of wine showed a distinctive anthocyanin fingerprint, as Alicante Bouschet wines contained a higher proportion of cyanidin-derived anthocyanins. Multivariate statistic studies were performed to both datasets to explore relationships among variables and among samples. These studies revealed relationships among several variables considered, and were capable to group the samples in two different classes using principal component analysis (PCA).

Keywords: young red wines; phenolic composition; color; anthocyanins; HPLC

1. Introduction

Color, astringency, bitterness, and body of red wines, as well as their ability for aging, are related to their phenolic composition, and especially to anthocyanins, flavan-3-ols, and their derivatives [1]. In young red wines, anthocyanins extracted from grapes are the main coloring molecules [2], despite that as soon as extraction from berry skins begins, they are partially transformed into anthocyanin-derived compounds, like pyranoanthocyanins, and into polymeric pigments by reaction with flavan-3-ols [3]. It has been estimated that about 25% anthocyanins may have polymerized with flavan-3-ols by the end of alcoholic fermentation, and about 40% after one year's aging, but these figures may change depending on grape composition, winemaking practices and procedures used for aging and maturation of wines [4]. Thus, sensory characteristics of young red wines and their ability for aging related to their phenolic composition are affected by multiple factors. They include the genome expression of grapevines in the growing area (influenced by soil and weather conditions, among other natural factors), and a number of viticultural and enological technologies, which explain the diversity of wines that can be obtained from a grape cultivar in a small production area.

Several *Vitis vinifera* cultivars are used for making red wines of Apellation d'Origine Contrôlée (AOC) Valdeorras (Galicia, NW Spain) [5]. The distinctive sensory characteristics of these wines are mainly related to Mencia grapes, a well-adapted cultivar which is considered the main red grape cultivar in Valdeorras, and also in other viticultural areas of Galicia. Other grape cultivars are grown in Valdeorras area, being Alicante Bouschet the most commonly used among them. Alicante Bouschet, developed in the nineteenth century by Henri Bouschet by crossing Garnacha grapes with Petit Bouschet grapes, is one of the few grape cultivars with red-colored berry flesh, which also are known as teinturier cultivars; thus, pressing of Alicante Bouschet grapes yields a red colored must, and this character permits obtaining deep colored wines. This cultivar is known as Garnacha Tintorera in many Spanish viticultural areas, and it was introduced in NW Spain by the end of nineteenth century, after the phylloxera attack [6]. In Valdeorras, like in many other viticultural areas, both in Spain and abroad, Alicante Bouschet wines are commonly used in blending to increase the color of less colored wines made with other grape cultivars [5]. Anyway, it is possible to use this cultivar for making single variety wines.

Studies on the phenolic composition of Mencia and Alicante Bouschet grapes and wines are scarce in the current literature. Recently, the phenolic composition of Mencia grapes and wines from different areas in NW Spain has been studied [7–10], but those studies do not include grapes and wines produced in AOC Valdeorras. The phenolic composition of Alicante Bouschet grapes and wines from central Spain has been recently described [11], but data on the phenolic composition of Alicante Bouschet grapes and wines from Galicia is not available in the current literature, except a recent study on Garnacha Tintorera-based sweet wines made with raisined grapes [12,13]. Thus, there are no data available on the phenolic composition of young red wines made with Mencia and Alicante Bouschet grapes in the area of AOC Valdeorras. For this reason, it has been considered of interest to examine this question, taking into account that the weather conditions in Valdeorras (a mediterranean-continental climate, with oceanic influence) can cause abundant rainfall in September and October. These weather conditions (which may affect pH of must, estimated alcoholic degree and the extent of fungal diseases, including *Botrytis cinerea* infections) can risk the adequate accumulation of anthocyanins in grape skins and, hence, can give rise to poor-colored Mencia red wines, and blending of Mencia wines with other wines made with Alicante Bouschet grapes may be a tool of choice to obtain valuable red wines.

2. Materials and Methods

2.1. Reagents and Standards

Water was purified through a Milli-Q system (Millipore, Bedford, MA, USA). Solvents (HPLC grade) were from Merck (Darmstad, Germany), and trifluoroacetic acid, (−)-epicatechin and methylcellulose from Sigma-Aldrich (Tres Cantos, Spain). Other reagents used for general analysis of wines were purchased from Panreac (Mollet del Valles, Spain). Standards of anthocyanins were extracted from grape skins, using the procedure of Bakker and Timberlake [14], and their identity was determined by HPLC-MS, as described elsewhere [15].

2.2. Wine Samples

Thirty samples of single cultivar young red wines made in 2014 from different wineries of AOC Valdeorras were studied. Those wines, made with similar enological procedures (the most remarkable differences are related to length of maceration and cap management, variable depending on grape cultivar, quality of grapes, and winery requirements), were analyzed in April 2015, once malolactic fermentation took place. Among them, 20 wines were made with Mencia red grapes, that is the major grape cultivar grown in AOC Valdeorras, and the other ten wines, with Alicante Bouschet teinturier grapes, that are grown as a complementary cultivar in that area. Some analytical data of those wines, determined using International Organization of Vine and Wine methods [16], were as follows: alcoholic degree, 11.50–13.50; total acidity (tartaric acid equivalents), 4.0–8.0 g/L; volatile acidity (acetic acid equivalents), 0.20–0.45 g/L; total sulfur dioxide, 40–100 mg/L. Differences in the general composition of both sets of wines were irrelevant.

2.3. Spectrophotometric Analysis

Several parameters related to wine color and to the general phenolic composition of wines were determined using the procedures described by Zoecklein et al. [17], based on those proposed by Somers and Evans [18], as well as others proposed to determine color intensity (420 + 520 + 620) [19] and total anthocyans [20]. The different parameters were determined as follows:

- Total phenol index: absorbance at 280 nm after diluting 2% in water (A_{280}), using the equation:

$$\text{Total phenol index} = (50 \times A_{280}) - 4$$

- Color intensity (420 + 520): sum of absorbances at 420 an 520 nm
- Color intensity (420 + 520 + 620): sum of absorbances at 420, 520 and 520 nm
- Hue: ratio of absorbance at 420 nm to absorbance at 520 nm
- Total anthocyans (monomeric anthocyanins plus polymeric pigments derived from anthocyanins), based on the measure of absorbance at 520 nm after diluting 1% in HCl 1 M ($A_{520\text{-H}}$), using the 18.9, using the equation:

$$\text{Total anthocyans} = 18.9 \times A_{520\text{-H}}$$

- Total anthocyanins, using the equation:

$$\text{Total anthocyanins} = 18.9 \times [A_{520\text{-H}} - (5/3) \times A_{520\text{-SO2}}]$$

  where $A_{520\text{-SO2}}$ is the absorbance at 520 nm after adding 30 μL of 20% sodium disulfite to 2 mL wine.
- Colored anthocyanins (anthocyanins presented under the flavilium ion form), using the equation:

$$\text{Colored anthocyanins} = 18.9 \times (A_{520} - A_{520\text{-SO2}})$$

- Chemical age index (degree of replacement of monomeric anthocyanins by polymeric pigments): ratio of $A_{520\text{-SO2}}$ to $A_{520\text{-H}}$

The concentrations of total anthocyans, total anthocyanins, and colored anthocyanins were expressed in malvidin-3-*O*-glucoside equivalents. The content of total tannins was determined after their precipitation with methylcellulose [21], and their concentration was expressed in (−)-epicatechin equivalents. Measurements were carried out using a Boeco S-22 UV-VIS spectrophotometer (Boeckel, Hamburg, Germany). Samples were analyzed by duplicate.

2.4. HPLC-DAD Analysis of Anthocyanins

The anthocyanin profile of wines was determined by HPLC-DAD, considering the relative content of 14 anthocyanins, following a procedure described previously [15,22]. The liquid chromatograph consisted of a 600 quaternary pump, a 717 automatic injector, a TC2 controller for a column oven, a 996 photodiode array detector, and a Millennium 32 workstation (Waters, Milford, MA, USA). The separation was carried out using a Waters Nova-Pak C18 steel cartridge (3.9 mm × 250 mm) filled with 5 μm particles, and a Waters Sentry Nova-Pack C18 guard cartridge (3.9 mm × 20 mm), both thermostated at 55 °C. Two mobile phases were used: water/acetonitrile (95:5) adjusted to pH 1.3 with trifluoroaectic acid (solvent A), and water/acetonitrile (50:50) adjusted to pH 1.3 with trifluoroacetic acid (solvent B). Gradient elution was performed at a 0.8 mL/min flow rate, with the following program: linear gradient from 15% B to 35% B in 20 min, from 35% B to 50% B in 10 min, 50% B for 6 min, from 50% B to 100% B in 5 min, 100% B for 5 min, and 100% B to 15% B in 1 min. Samples (20 µL) were injected in triplicate. Spectra were recorded every second between 250 and 600 nm, with a bandwidth of 1.2 nm. Samples, standard solutions, and mobile phases were filtered before analysis through a 0.45 µm pore size membrane.

2.5. Statistical Analysis

Statistical analyses were performed using the Statgraphics Centurion XVI version 16.1.18 statistical package (Statistical Graphics Corp., Warrenton, VA, USA).

3. Results and Discussion

3.1. General Phenolic Composition and Color of Wines

Tables 1 and 2 display the results obtained in the spectrophotometric analysis of Mencia (M) and Alicante Bouschet (T) wines, and Table 3, the mean values with their standard deviation, and the range values of each parameter determined by spectrophotometry for both groups of wines. As can be noted, the grape cultivar used for making wines dramatically affects several parameters related to color and to the general phenolic composition of wines measured by spectrophotometry. Mean values for most parameters were significantly different ($p < 0.05$), taking into account the results of the LSD Fisher test, after submitting data to one-way ANOVA (Table 3).

Table 1. Total phenols, color intensity, hue, and chemical age in Mencia (M) and Alicante Bouschet (T) young red wines. Results are mean values of two replications.

Sample	Total Phenols Index	Color Intensity (420 + 520)	Color Intensity (420 + 520 + 620)	Hue	Chemical Age Index
M1	33.2	7.06	10.58	0.541	0.076
M2	27.6	6.69	10.14	0.537	0.079
M3	31.1	8.53	12.21	0.514	0.089
M4	36.7	8.84	12.66	0.566	0.067
M5	35.8	8.95	12.72	0.592	0.101
M6	39.2	11.03	15.03	0.512	0.094
M7	46.8	12.98	17.21	0.479	0.099
M8	33.3	8.00	11.46	0.429	0.059
M9	35.1	7.58	8.53	0.608	0.100
M10	38.8	8.99	10.12	0.563	0.107
M11	35.5	7.77	8.75	0.600	0.107
M12	31.7	7.32	8.24	0.592	0.113
M13	37.6	9.61	10.77	0.535	0.117
M14	27.9	5.72	6.43	0.631	0.100
M15	33.0	7.99	8.91	0.554	0.100
M16	36.7	6.76	7.68	0.685	0.054
M17	37.2	6.78	7.68	0.688	0.049
M18	39.6	7.75	8.73	0.610	0.104
M19	49.2	11.47	13.26	0.609	0.130
M20	39.0	9.51	10.80	0.551	0.111
T21	68.7	20.99	23.33	0.521	0.094
T22	52.5	12.97	14.63	0.595	0.066
T23	80.2	24.43	28.08	0.491	0.133
T24	69.6	22.61	25.44	0.490	0.119
T25	43.2	12.16	13.73	0.543	0.143
T26	54.5	16.71	18.78	0.492	0.115
T27	65.6	18.84	21.07	0.538	0.076
T28	75.1	21.18	23.72	0.521	0.078
T29	74.7	21.04	23.54	0.505	0.076
T30	71.4	19.72	22.09	0.514	0.078

Table 2. Total anthocyans, total and colored anthocyanins, and total tannins in Mencia (M) young red wines. Results, expressed in mg/L, are mean values of two replications.

Sample	Total Anthocyans	Total Anthocyanins	Colored Anthocyanins	Total Tannins
M1	357	312	59	806
M2	301	261	59	506
M3	335	285	77	719
M4	421	374	78	954
M5	346	288	71	1076
M6	433	365	97	1014
M7	459	383	120	1436
M8	391	353	83	924
M9	367	305	82	598
M10	442	363	99	606
M11	367	301	84	611
M12	346	281	79	588
M13	416	334	109	568
M14	314	261	60	453
M15	399	332	89	538
M16	849	772	30	480
M17	892	820	32	549
M18	399	329	83	864
M19	486	381	122	1622
M20	440	359	106	1159
T21	1498	1265	121	529
T22	1254	1116	71	650
T23	849	661	287	2689
T24	803	644	268	2047
T25	438	334	137	1189
T26	662	535	197	1859
T27	1415	1236	124	1722
T28	1596	1390	139	2116
T29	1641	1433	139	2070
T30	1496	1301	130	2041

Table 3. Mean values, standard deviations, and range values for analytical parameters related to general phenolic composition and color of young red Mencia and Alicante Bouschet wines. Mean values in the same row followed by a different letter are significantly different ($p < 0.05$).

Parameter	Mean Value and Standard Deviation		Range Values	
	Mencía	Alicante Bouschet	Mencia	Alicante Bouschet
Total phenol index	36.2 ± 5.3a	65.6 ± 11.7b	27.6–49.2	43.2–80.2
Color intensity (Sudraud)	8.47 ± 1.79a	19.06 ± 4.00b	5.72–12.98	12.16–24.43
Color intensity (Glories)	10.59 ± 2.68a	21.44 ± 4.55b	6.43–17.21	13.73–28.08
Hue	0.570 ± 0.063a	0.521 ± 0.032b	0.429–0.688	0.490–0.595
Chemical age index	0.093 ± 0.022a	0.098 ± 0.027a	0.049–0.130	0.066–0.143
Total anthocyans	438 ± 156a	1165 ± 436b	301–892	438–1641
Total anthocyanins	373 ± 150a	991 ± 404b	261–820	334–1433
Colored anthocyanins	81 ± 25a	161 ± 68b	30–122	71–287
Total tannins	803 ± 327a	1691 ± 690b	453–1622	529–2689

Thus, total phenols index, as well as color intensity (420 + 520), color intensity (420 + 520 + 620), total anthocyans (which include anthocyanins and polymeric red pigments), total anthocyanins (free anthocyanins) and colored anthocyanins were higher in most Alicante Bouschet wines (samples T21 to T30) than in Mencia wines (samples M1 to M20). The most remarkable exceptions were two deep-colored Mencia wines (M7 and M19), which were as rich as wine T25, made with Alicante Bouschet grapes, for the following parameters: color intensity (420 + 520), color intensity (420 + 520 + 620), total phenols index and total anthocyanins.

Moreover, two samples of Mencia wine (M16 and M17) contained high levels of total anthocyans and total anthocyanins, even higher than several Alicante Bouschet wines, but this fact was not reflected in color intensity, despite all the wines presented similar pH. This can be explained because in those Mencia wines the concentration of total tannins (which include oligomeric flavan-3-ols, as well as polymeric pigments formed by reaction of anthocyanins and flavan-3-ols) was too low (less than 600 mg/L). Thus, copigmentation of free anthocyanins with oligomeric flavan-3-ols could be negatively affected, leading to low values of color intensity, despite the relatively high content of total anthocyans and total anthocyanins [23].

The content of total tannins was higher in most Alicante Bouschet wines than in Mencia wines; the exceptions were Mencia wines M7 and M19, which were as rich as many Alicante Bouschet wines, and Alicante Bouschet wines T21 and T22, which contained very low levels of total tannins. Mencia wines M7 and M19 presented a high level of total tannins and a high color intensity, and this fact is probably due to copigmentation of free anthocyanins with oligomeric flavan-3-ols [23], despite their low level of total anthocyans and total anthocyanins. Alicante Bouschet wines T21 and T22, with a remarkable low level of total tannins, presented a high content of total anthocyanins and total anthocyanins, as reflected in color intensity. Thus, it is quite probable that maceration of skins and seeds during making of these wines was too short, as the extraction of flavan-3-ols from skins and seeds is not as quick as the extraction of anthocyanins from skins [5].

Hue and chemical age are parameters used to evaluate the extent of wine maturation [17,18]. Values of hue (between 0.429 and 0.688) are those expected for young red wines, Alicante Bouschet wines presenting more similar values (between 0.490 and 0.595) than Mencia wines. Chemical age index, which refers to the increasing dominance of wine color by oligomeric and polymeric red pigments, was quite variable, but in most cases ranged between 0.070 and 0.120, as can be expected in young red wines [18]; one-way ANOVA revealed that chemical age was statistically equal ($p < 0.05$) for both types of wines (see Table 3). The variations observed in values of hue and of chemical age index are probably due to differences in the extent of polymerization, which depends on the phenolic composition of wines and in other factors, like the extent of oxygenation of wines once the alcoholic fermentation has taken place.

Results obtained by UV-VIS spectrophotometry (nine variables) were submitted to principal components analysis (PCA), an unsupervised pattern recognition method that seeks trends and groupings without prior knowledge of the identity of the samples, that it is used to determine the variability of a dataset, and to order data by their importance. Thus, it is possible to obtain m principal components, that are lineal combinations of the m variables considered, capable of explain the total variance of the data matrix [24]. Two principal components, with eigenvalues higher than 1, explained 89.64% of total variance; each principal component was affected mainly by several variables. Thus, component 1, which explained 66.34% of variance, was affected mainly by seven variables, the exceptions were hue and chemical age index. On the other hand, component 2, which explained 23.30% of total variance, was affected mainly by five variables, and the weight of three variables (color intensity (420 + 520), color intensity (420 + 520 + 620) and total phenols index) was very small (Table 4).

The plot of principal components 1 and 2 obtained by PCA is displayed in Figure 1. As can be noted, Mencia wines are placed in the left side of the plot, and most Alicante Bouschet wines in the right side of the plot. Most Mencia wines are located forming a quite compact cluster, reflecting than those wines presented similar values for the variables considered, the exception were wines M16 and M17, located in the upper left corner of the plot. These wines contained a higher amount of total anthocyans and total anthocyanins, but a low amount of total tannins. Alicante Bouschet wines are more dispersed: wine T25 presented a phenolic composition quite similar to most Alicante Bouschet wines, and is located among them, the other nine were generally too rich in color and total tannins, and are clearly separated from Mencia wines. Additionally, linear discriminant analysis (LDA) was performed for data of general phenolic composition and color of wines. This analysis showed clearly that all wines were correctly classified in two different classes (Mencia and Alicante Bouschet);

the standardized coefficients of the discriminant function had an absolute value higher than one for all the analytical variables considered, except total tannins and hue. The differences observed for color and general phenolic composition among the Alicante Bouschet wines under study may be explained by two different, independent factors: the degree of maturation of grapes and the extent of pomace maceration during winemaking. Several authors consider that the content of tannins in whole grapes decreases during maturation [25,26]; thus, the degree of maturation of grapes used for winemaking may affect in some extent the content of tannins in wines. On the other hand, the extraction of tannins during winemaking follows different kinetics than the extraction of anthocyanins: these colored molecules are extracted in the first steps of winemaking, and a maximum is reached in the first week of pomace maceration [5], meanwhile, the extraction of tannins proceeds more slowly, and can be enhanced by several technological procedures, such as extended maceration [27]. For these reasons, and taking into account that Alicante Bouschet wines made in AOC Valdeorras are many times used for blending with Mencia wines to increase their color, it is quite probable that an appropriate management of winemaking could lead to Alicante Bouschet wines with various phenolic profiles, depending on the style of wines required by cellars.

Table 4. Weight of the nine variables determined by spectrophotometry for Mencia and Alicante Bouschet wines in the principal components 1 and 2.

Variable	Principal Component 1	Principal Component 2
Total phenols index	0.410265	0.079909
Color intensity (420 + 520)	0.415471	−0.006487
Color intensity (420 + 520 + 620)	0.410975	−0.029371
Hue	−0.238126	0.271978
Chemical age index	0.056074	−0.589626
Total anthocyans	0.322148	0.425857
Total anthocyanins	0.307412	0.457513
Colored anthocyanins	0.338592	−0.364930
Total tannins	0.346240	−0.217331

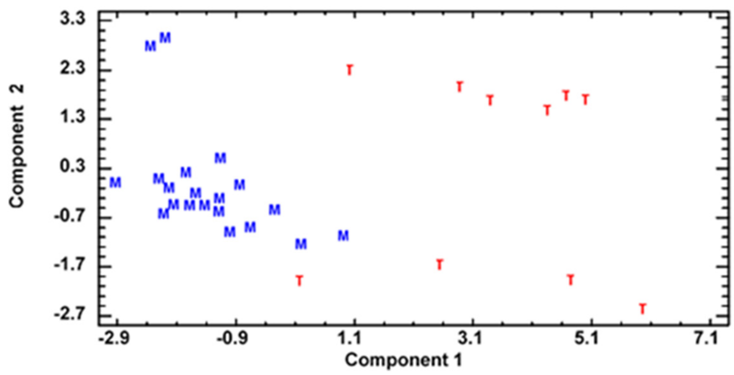

Figure 1. Dispersion plot of principal components 1 and 2 for spectrophotometric data obtained for Mencia (M) and Alicante Bouschet (T) wines.

3.2. Anthocyanin Fingerprint of Wines

The anthocyanin fingerprint or anthocyanin profile of a wine has been defined as the proportions of different anthocyanins presented in wine after HPLC analysis, and it has been proposed as a tool to assess the varietal origin of single cultivar wines [28,29]. To evaluate the anthocyanin fingerprint of wines, the relative content of 14 anthocyanins has been considered (Tables 5 and 6):

- 3-*O*-Glucosides of delphinidin (DpGl), cyanidin (CyGl), petunidin (PtGl), peonidin (PnGl) and malvidin (MvGl)

- Acetyl derivatives of DpGl, PtGl; PnGl and MvGl (DpGlAc, PtGlAc, PnGlAc and MvGlAc)
- *p*-Coumaryl derivatives of DpGl, PtGl; PnGl and MvGl (DpGlCm, PtGlCm, PnGlCm and MvGlCm)
- Caffeoyl derivative of MvGl (MvGlCf).

Table 5. Relative content (%) of anthocyanidin monoglucosides in Mencia (M) and Alicante Bouschet (T) young red wines. For key to substances, see text.

Sample	DpGl	CyGl	PtGl	PnGl	MvGl
M1	2.65	0.10	5.18	5.53	61.02
M2	3.13	0.13	5.30	4.44	62.90
M3	4.34	0.22	5.57	8.70	58.40
M4	4.65	0.24	6.22	6.03	59.46
M5	4.75	0.28	6.33	6.68	61.01
M6	8.24	0.27	8.05	7.97	56.16
M7	4.26	0.21	5.77	9.13	59.51
M8	3.99	0.16	6.63	4.89	65.75
M9	4.42	0.21	6.32	7.64	58.78
M10	6.71	0.20	8.48	8.17	56.82
M11	4.49	0.24	5.80	7.86	59.02
M12	4.13	0.07	6.20	5.15	59.80
M13	3.30	0.30	4.86	11.85	56.68
M14	3.50	0.13	5.39	5.09	61.96
M15	4.03	0.37	5.00	14.48	55.96
M16	5.52	0.51	7.18	7.21	57.84
M17	5.71	0.51	7.39	6.42	57.72
M18	3.04	0.25	4.40	13.41	56.81
M19	4.69	0.34	6.39	8.72	60.04
M20	4.53	0.44	5.66	12.62	57.73
T21	3.54	1.21	3.84	29.03	44.34
T22	4.92	1.21	4.54	24.10	48.30
T23	5.37	0.92	5.59	20.00	52.31
T24	3.91	0.94	4.32	26.60	48.94
T25	3.93	0.60	4.60	18.99	53.59
T26	4.40	0.93	4.44	24.15	49.93
T27	4.60	1.42	3.58	29.77	43.14
T28	4.67	1.55	3.76	30.48	43.18
T29	4.64	1.45	3.93	29.71	43.69
T30	4.44	1.48	3.87	29.97	43.33

Table 6. Relative content (%) of acylated anthocyanidins in Mencia (M) and Alicante Bouschet young red wines. For key to substances, see text.

Sample	DpGlAc	PtGlAc	PnGlAc	MvGlAc	DpGlCm	PtGlCm	PnGlCm	MvGlCm	MvGlCf
M1	0.72	1.01	2.76	11.16	0.67	0.21	1.75	6.75	0.49
M2	0.69	0.87	2.63	10.94	0.63	0.22	1.41	6.24	0.47
M3	0.63	0.94	2.66	9.28	0.56	0.28	2.11	5.86	0.45
M4	0.92	1.22	2.69	10.14	0.50	0.18	1.77	5.50	0.46
M5	0.81	1.03	1.89	9.75	0.35	0.10	1.41	5.15	0.48
M6	0.63	0.99	2.34	6.42	0.91	0.15	1.71	5.62	0.54
M7	0.64	1.07	2.06	7.40	0.43	0.41	2.46	6.04	0.62
M8	0.75	1.02	1.69	8.86	0.45	0.23	1.06	4.09	0.42
M9	0.87	1.02	2.57	9.62	0.45	0.14	2.01	5.50	0.45
M10	0.69	0.93	2.22	6.54	0.75	0.16	2.00	5.78	0.57
M11	0.84	1.01	2.59	9.33	0.47	0.16	2.09	5.61	0.48
M12	0.71	0.99	2.55	10.08	0.80	0.25	1.85	6.80	0.61
M13	0.37	0.71	3.31	7.22	0.68	0.20	3.49	6.56	0.48
M14	0.73	0.93	2.51	10.32	0.66	0.19	1.85	6.25	0.48

Table 6. *Cont.*

Sample	DpGlAc	PtGlAc	PnGlAc	MvGlAc	DpGlCm	PtGlCm	PnGlCm	MvGlCm	MvGlCf
M15	0.51	0.73	2.58	6.82	0.41	0.17	2.77	5.77	0.42
M16	0.97	0.96	1.62	9.99	0.50	0.15	1.79	5.24	0.53
M17	0.93	1.03	1.01	9.96	1.63	0.15	1.75	5.30	0.51
M18	0.75	0.69	2.37	6.52	0.62	0.17	3.72	6.78	0.47
M19	0.85	0.93	1.37	8.02	0.47	0.13	2.05	5.56	0.42
M20	0.76	0.82	1.38	6.48	0.47	0.13	2.99	5.55	0.44
T21	0.32	0.47	2.85	3.11	1.25	0.30	3.75	5.33	0.65
T22	0.44	0.64	1.57	4.47	1.28	0.17	3.02	4.89	0.45
T23	0.36	0.61	1.24	3.68	1.40	0.19	3.03	4.82	0.50
T24	0.38	0.46	1.02	2.57	1.45	0.09	3.70	5.27	0.33
T25	0.47	0.62	1.67	4.22	0.80	0.12	3.92	6.03	0.44
T26	0.40	0.43	1.49	3.34	0.59	0.09	3.76	5.67	0.38
T27	0.25	0.37	1.84	3.10	1.23	0.41	4.12	5.50	0.65
T28	0.26	0.36	1.83	2.99	1.25	0.31	3.69	5.11	0.57
T29	0.29	0.39	1.80	2.95	1.18	0.24	3.90	5.29	0.53
T30	0.16	0.37	2.00	3.15	1.30	0.33	3.79	5.16	0.65

Additionally, Table 7 displays the mean values with their standard deviation, and the range values of each parameter determined by HPLC for both groups of wines; mean values were significantly different ($p < 0.05$) in most cases, taking into account the results of LSD Fisher test, after submitting data to one-way ANOVA. In both types of wine, MvGl was the major anthocyanin, but its relative amount was always higher in Mencia wines (55%–66%) than in Alicante Bouschet wines (43%–54%), because Alicante Bouschet wines contained an important amount of PnGl (18%–31%), as it has been reported previously [11,30]. This fact is a consequence of the abundance of PnGl in Alicante Bouschet grapes, both in the skins and, especially, in the berry flesh, like in some other teinturier grapes [30]. Moreover, Alicante Bouschet wines contained a higher amount of CyGl than Mencia wines (0.6%–1.6% and 0.1%–0.5%, respectively), as this pigment is the biosynthetic precursor of PnGl [31]. Finally, most Mencia wines contained a higher proportion of PtGl than Alicante Bouschet grapes, because PtGl is the biosynthetic precursor of MvGl, which is formed after its *O*-methylation [31].

Table 7. Mean values, standard deviations and range values for the relative content of 14 anthocyanins in young red Mencia and Alicante Bouschet wines. Mean values in the same row followed by a different letter are significantly different ($p < 0.05$).

Anthocyanin	Mean Value and Standard Deviation		Range Values	
	Mencía	Alicante Bouschet	Mencia	Alicante Bouschet
DpGl	4.50 ± 1.30a	4.44 ± 0.53a	2.65–8.24	3.54–5.37
CyGl	0.26 ± 0.12a	1.17 ± 0.31b	0.07–0.51	0.60–1.51
PtGl	6.11 ± 1.05a	4.25 ± 0.59b	4.40–8.48	3.58–5.59
PnGl	8.10 ± 2.93a	26.28 ± 4.28b	4.44–14.48	18.99–30.48
MvGl	59.17 ± 2.48a	47.08 ± 4.04b	55.96–65.75	43.14–53.59
DpGlAc	0.74 ± 0.14a	0.33 ± 436b	0.37–0.97	0.16–0.47
PtGlAc	0.94 ± 0.13a	0.47 ± 0.11b	0.69–1.22	0.36–0.64
PnGlAc	2.24 ± 0.58a	1.17 ± 0.49b	1.01–3.31	1.02–2.85
MvGlAc	8.74 ± 1.64a	3.36 ± 0.59b	6.42–11.16	2.57–4.47
DpGlCm	0.62 ± 0.28a	1.17 ± 0.27b	0.35–2.63	0.59–1.45
PtGlCm	0.19 ± 0.07a	0.22 ± 0.11a	0.10–0.41	0.09–0.41
PnGlCm	2.10 ± 0.68a	3.67 ± 0.36b	1.06–3.72	3.02–4.12
MvGlCm	5.80 ± 0.65a	5.31 ± 0.18b	4.09–6.80	4.82–6.03
MvGlCf	0.49 ± 0.06a	0.52 ± 0.12a	0.42–0.62	0.33–0.65

Acylation of anthocyanins follows different trends in both types of wines. Certainly, the acylated derivatives of MvGl were the major acylated anthocyanins in both types of wines, but acylation was more intense in Mencia wines (18%–24%) than in Alicante Bouschet wines (14%–18%). In Mencia wines, acetylated anthocyanins were more abundant than *p*-coumarylated anthocyanins (10%–16% and 5%–11%, respectively). On the other hand, the relative amount of *p*-coumarylated anthocyanins was higher than the relative amount of acetylated anthocyanins in Alicante Bouschet grapes (9%–11% and 5%–7%, respectively). Similar results have been reported previously for Mencia and Alicante Bouschet wines [30], and are closely related to the different anthocyanin fingerprint of those grape cultivars.

Results obtained by HPLC-DAD analysis of anthocyanins to obtain the anthocyanin fingerprint of wines (14 variables) were submitted to PCA. Table 8 displays the weights in the principal components 1, 2, and 3 of the variables determined by HPLC-DAD to obtain the anthocyanin fingerprint of wines. Those three principal components, with eigenvalues higher than 1, explained 84.66% of total variance; each of them was affected mainly by several variables. Thus, component 1 (which explained 55.20% of total variance) was affected mainly by seven variables with weight >0.3 or <−0.3; component 2 (which explained 17.05% of total variance), by five variables with weight >0.3 or <−0.3, and component 3 (which explained 12.41% of total variance) by three variables with weight >0.3.

Table 8. Weight of the 14 variables determined by HPLC-DAD to obtain the anthocyanin fingerprint of Mencia and Alicante Bouschet wines in the principal components 1–3. For key to substances, see text.

Variable	Principal Component 1	Principal Component 2	Principal Component 3
DpGl	−0.018052	0.474169	0.376404
CyGl	0.342602	0.108723	0.059271
PtGl	−0.276536	0.308831	0.251433
PnGl	0.355876	0.022064	−0.053677
MvGl	−0.347133	−0.033895	−0.077616
DpGlAc	−0.327862	0.111526	−0.010015
PtGlAc	−0.343434	0.036475	0.140569
PnGlAc	−0.109280	−0.496591	0.147264
MvGlAc	−0.334181	−0.109008	0.063837
DpGlCm	0.255941	0.177460	0.165177
PtGlCm	0.136000	−0.317206	0.488207
PnGlCm	0.320346	−0.115650	−0.178406
MvGlCm	−0.106560	−0.465310	−0.075131
MvGlCf	0.107887	−0.179100	0.658755

Some relationships among variables, probably related to the biosynthetic pathway of anthocyanins, can be observed. Thus, CyGl and PnGl, which are the cyanidin-derived monoglucosides [31], had a positive weight for principal components 1 and 2; on the other hand, the delphinidin-derived monoglucosides (DpGl, PtGl and MvGl) had a negative weight for principal component 1. Moreover, acetylated anthocyanins had a negative weight for principal component 1, whereas *p*-cumarylated derivatives (except MvGlCm) and MvGlCf had a positive weight for principal component 1. In addition, acylated derivatives of PnGl and MvGl had a negative weight for component 2.

The plot of principal components 1 and 2 obtained by PCA is displayed in Figure 2. Taking into account the weights of variables for principal components 1 and 2, Alicante Bouschet wines, which contained higher amounts of CyGl, PnGl and *p*-coumarylated anthocyanins than Mencia wines, are located in the right side of the plot. Meanwhile, Mencia wines, richer in delphinidin-derived monoglucosides and in acetylated derivatives, are placed in the left side of the plot. In addition, LDA was performed for data of the anthocyanin fingerprint of wines. This analysis showed clearly that all wines were correctly classified in two different classes (Mencia and Alicante Bouschet); the standardized coefficients of the discriminant function had an absolute value higher than 25 for DpGl, PnGl, MvGl, and MvGlAc. These results are in agreement with those obtained previously for single variety wines of different cultivars grown in Spain, including Mencia and Alicante Bouschet [30], and suggest that the anthocyanin fingerprint of young red wines made in the area of AOC Valdeorras should be an adequate analytical tool to assess the grape cultivar used in winemaking.

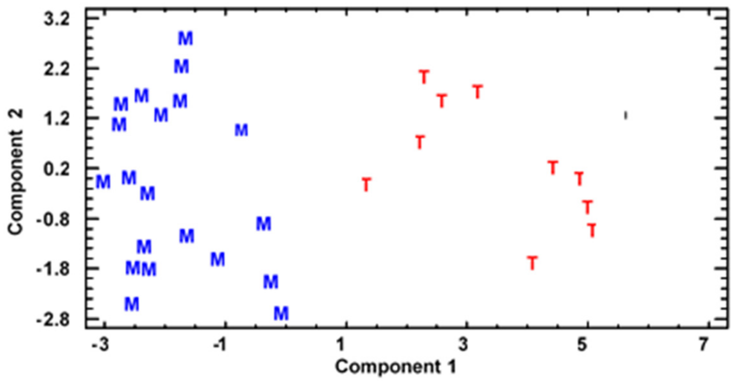

Figure 2. Dispersion plot of principal components 1 and 2 for HPLC data obtained for Mencia (M) and Alicante Bouschet (T) wines.

4. Conclusions

Young red wines of Mencia and Alicante Bouschet grapes made in AOC Valdeorras show important differences in color and in their general phenolic composition. Usually, Alicante Bouschet wines presented a more intense color than Mencia wines, usually associated with higher amounts of total anthocyans, total anthocyanins, and total tannins. Nevertheless, the general phenolic composition of Mencia wines was more homogeneous than that showed by Alicante Bouschet. This fact probably reflects that winemaking of Alicante Bouschet wines is managed to obtain wines with various phenolic profiles, capable to be used in blending with Mencia wines, depending on the style of wines required by cellars.

Data obtained by HPLC reveal that Alicante Bouschet and Mencia wines presented different anthocyanin profiles, and suggest that the anthocyanin fingerprint of single cultivar young red wines made in the area of AOC Valdeorras should be an adequate analytical tool to assess the grape cultivar used in winemaking for regulatory purposes.

Acknowledgments: Authors thank to Adega Cooperativa Virxen das Viñas (A Rua, Ourense, Spain) and Adega Cooperativa Jesus Nazareno (O Barco de Valdeorras, Ourense, Spain) for suppling samples of young red wines. The work has been supported by Universidad Autonoma de Madrid (Funds for preparing B.Sc. dissertations).

Author Contributions: Eugenio Revilla and Manuel M. Losada conceived and designed the experiments; Encina Gutierrez performed the experiments; Eugenio Revilla, Manuel M. Losada and Encina Gutierrez analyzed the data; Eugenio Revilla wrote the paper.

Conflicts of Interest: The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

References

1. Singleton, V.L.; Esau, P. Phenolic substances in grapes and wines, and their significance. *Adv. Food Res. Suppl.* **1969**, *1*, 1–261. [PubMed]
2. He, F.; Niang, N.; Mu, L.; Pan, Q.; Wang, J.; Reeves, M.J.; Duan, C. Anthocyanins and their variation in red wines I. Monomeric anthocyanins and their color expression. *Molecules* **2012**, *17*, 1571–1601. [CrossRef] [PubMed]
3. He, F.; Niang, N.; Mu, L.; Pan, Q.; Wang, J.; Reeves, M.J.; Duan, C. Anthocyanins and their variation in red wines II. Anthocyanin derived pigments and their color evolution. *Molecules* **2012**, *17*, 1483–1519. [CrossRef] [PubMed]
4. Jackson, R.S. *Wine Science: Principles and Applications*, 4th ed.; Elsevier-Academic Press: London, UK, 2014; pp. 368–379.
5. Valdeorras, a nai do Viño. Available online: http://www.dovaldeorras.tv (accessed on 3 May 2016).

6. Castro-Voces, A. Antecedentes históricos de la Valdeorras vitivinícola. In *Godello de Valdeorras, Emblema de Una Tierra*; Castro-Voces, A., Losada-Arias, M.M., Rodriguez-Rodriguez, C., Eds.; Consello Regulador da Denominación de Orixe Valdeorras: O Barco de Valdeorras, Spain, 2006; pp. 31–57.
7. Garcia-Falcon, M.S.; Perez-Lamela, C.; Martinez-Carballo, E.; Simal-Gandara, J. Determination of phenolic compounds in wines: Influence of bottle storage of young red wines on their evolution. *Food Chem.* **2007**, *105*, 248–259. [CrossRef]
8. Letaief, H.; Rolle, L.; Zeppa, G.; Orriols, I.; Gerbi, V. Phenolic chracaterization of grapevine cultivars from Galicia (Spain): Brancellao, Merenzao and Mencia (*Vitis vinifera* L.). *Ital. J. Food Sci.* **2007**, *19*, 101–109.
9. Soto-Vazquez, E.; Rio-Segade, S.; Orriols-Fernandez, I. Effect of the winemaking technique on phenolic composition and chromatic characteristics of young red wines. *Eur. Food Res. Technol.* **2010**, *231*, 789–802. [CrossRef]
10. Vilanova, M.; Rodriguez, I.; Canosa, P.; Otero, I.; Gamero, E.; Moreno, D.; Talaverano, I.; Valdes, E. Variability in chemical composition of *Vitis vinífera* cv Mencía from different geographic areas and vintages in Ribeira Sacra (NW Spain). *Food Chem.* **2015**, *169*, 187–196. [CrossRef] [PubMed]
11. Castillo-Muñoz, N.; Fernandez-Gonzalez, M.; Gomez-Alonso, S.; Garcia-Romero, E.; Hermosin-Gutierrez, I. Red-color related phenolic composition of Garnacha Tintorera (*Vitis vinífera* L.) grapes and red wines. *J. Agric. Food Chem.* **2009**, *57*, 7883–7891. [CrossRef] [PubMed]
12. Figueirido-Gonzalez, M.; Cancho-Grande, B.; Simal-Gandara, J. Garnacha Tintorera-based sweet wines: Chromatic properties and global phenolic composition by means of UV-Vis spectrophotometry. *Food Chem.* **2013**, *140*, 217–224. [CrossRef] [PubMed]
13. Figueirido-Gonzalez, M.; Regueiro, J.; Cancho-Grande, B.; Simal-Gandara, J. Garnacha Tintorera-based sweet wines: Detailed phenolic composition by HPLC/DAD-ESI/MS analysis. *Food Chem.* **2014**, *143*, 282–292. [CrossRef] [PubMed]
14. Bakker, J.; Timberlake, C.F. The distribution of anthocyanins in grape skin extracts of Port wine cultivars as determined by high performance liquid chromatography. *J. Sci. Food Agric.* **1985**, *36*, 1315–1324. [CrossRef]
15. García-Beneytez, F.; Cabello, F.; Revilla, E. Analysis of grape and wine anthocyanins by HPLC-MS. *J. Agric. Food Chem.* **2003**, *51*, 5622–5629. [CrossRef] [PubMed]
16. International Organization of Vine and Wine. *Recueil des Méthodes Internationales d'Analyse des Vins et des Môuts*; OIV: Paris, France, 2008.
17. Zoecklein, B.W.; Fugelsang, K.C.; Gump, B.H.; Nury, F.S. *Wine Analysis and Production*; Chapman and Hall: New York, NY, USA, 1995; pp. 146–151.
18. Somers, T.C.; Evans, M.E. Spectral evaluation of young red wines: Anthocyanin equilibria, total phenolics, free and molecular SO_2, "chemical age". *J. Sci. Food Agric.* **1977**, *28*, 279–287. [CrossRef]
19. Glories, Y. La coleur des vins rouges. 2. Mesure, origine et interpretation. *Connaiss. Vigne Vin* **1984**, *18*, 253–271.
20. Niketic-Aleksic, G.K.; Hrazdina, G. Quantitative analysis of the anthocyanin content in grape juices and wines. *Lebensm. Wiss. Technol.* **1972**, *5*, 163–165.
21. Sarneckis, C.J.; Dambergs, R.G.; Jones, P.; Mercurio, M.; Herderich, M.J.; Smith, P.A. Quantification of condensed tannins by precipitation with methylcellulose: Development and validation of an optimized tool for grape and wine analysis. *Aust. J. Grape Wine Res.* **2006**, *12*, 39–49. [CrossRef]
22. Revilla, E.; Carrasco, D.; Benito, A.; Arroyo-Garcia, R. Anthocyanin composition of several wild grape accessions. *Am. J. Enol. Vitic.* **2010**, *61*, 636–642. [CrossRef]
23. Boulton, R. The copigmentation of anthocyanins and its role in the color of red wine: A critical review. *Am. J. Enol. Vitic.* **2001**, *52*, 67–87.
24. Ramis, G.; Garcia, M.C. *Quimiometría*; Editorial Sintesis: Madrid, Spain, 2001; pp. 157–181.
25. Blouin, J.; Guimberteau, G. *Maduración y Madurez de la Uva*; Ediciones Mundi-Prensa: Madrid, Spain, 2002; pp. 72–77.
26. Downey, M.O.; Harvey, J.D.; Robinson, S.P. Analysis of tannins in seeds and skins of Shiraz grapes throughout berry development. *Aust. J. Grape Wine Res.* **2003**, *9*, 15–27. [CrossRef]
27. Smith, P.A.; Mcrae, J.M.; Bindon, K.A. Impact of winemaking practices on the concentration and composition of tannins in red wine. *Aust. J. Grape Wine Res.* **2015**, *21*, 601–614. [CrossRef]

28. Berente, B.; De la Calle Garcia, D.; Reichenbächer, M.; Danzer, K. Method development for the determination of anthocyanins in red wines by high performance liquid chromatography and classification of German red wines by means of multivariate statistical methods. *J. Chromatogr. A* **2000**, *871*, 95–103. [CrossRef]
29. Otteneder, H.; Marx, R.; Zimmer, M. Analysis of the anthocyanin composition of Cabernet Sauvignon and Portugieser wuines provide an objective assessment of the grape varieties. *Aust. J. Grape Wine Res.* **2004**, *10*, 3–7. [CrossRef]
30. Revilla, E.; Garcia-Beneytez, E.; Lopez, J.F.; Cabello, F. Anthocyanin pattern of several red grape cultivars and single-cultivar young wines. *Acta Aliment.* **2013**, *42*, 23–35. [CrossRef]
31. He, F.; Mu, L.; Yan, G.L.; Liang, N.N.; Pan, Q.H.; Wang, J.; Reeves, M.J.; Duan, C.Q. Biosynthesis of anthocyanins and their regulation in colored grapes. *Molecules* **2010**, *15*, 9057–9091. [CrossRef] [PubMed]