ELSEVIER

Journal of Chromatography A, 915 (2001) 53–60

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Value of high-performance liquid chromatographic analysis of anthocyanins in the differentiation of red grape cultivars and red wines made from them ☆

Eugenio Revilla[a,*], Eva García-Beneytez[b], Félix Cabello[b], Guillermo Martín-Ortega[a], José-María Ryan[1,a]

[a]*Departamento de Química Agrícola, Geología y Geoquímica, Universidad Autónoma de Madrid, 28049 Madrid, Spain*
[b]*Departamento de Agroalimentación, Instituto Madrileño de Investigación Agraria y Alimentaria, 28800 Alcalá de Henares, Spain*

Received 20 November 2000; received in revised form 30 January 2001; accepted 5 February 2001

Abstract

A HPLC method that allows the separation of several anthocyanins present in red grapes and red wines, using a linear gradient of acetonitrile in water at pH 1.3, using perchloric acid as an acid modifier, is described. Data clearly show that the anthocyanins profile of red grapes may be complex, but quite different for each cultivar studied. Thus, those molecules may be used as chemotaxonomic markers for classifying red grape cultivars. However, the anthocyanin profile of red wines clearly differs from that presented by grapes employed in making it, because red wine contains a higher relative amount of malvidin-3-*O*-glucoside than grapes, and the relative amount of other anthocyanins in wines is usually lower than in grapes. Therefore, the use of anthocyanins present in wines to determine the grape cultivar used for winemaking needs a careful evaluation of the influence of different technological procedures on the anthocyanins fingerprint.

Keywords: Grapes; Wine; Food analysis; Anthocyanins; Polyphenols; Phenols

1. Introduction

The importance of polyphenols in viticulture and enology is well known, and dozens of different substances of this type have been successfully described in grapes and wines [1]. Among them, anthocyanins have been postulated as chemical markers to differentiate grape cultivars and also red wines made with different grape cultivars [2–7]. However, this tool should be used with care. Different clones have been identified in many *Vitis vinifera* cultivars, and some data suggest that the anthocyanin composition of some clones belonging to the same cultivar may be significantly different [8]. Furthermore, the content of anthocyanins in grapes changes during grape maturation, and their biosynthesis is affected by several environmental conditions, such as light intensity and temperature [9]. Nevertheless, the

☆Presented at the 29th Scientific Meeting of the Spanish Group of Chromatography and Related Techniques, Alcalá de Henares (Madrid), 12–14 July 2000.

*Corresponding author. Fax: +34-91-3974-826.
E-mail address: eugenio.revilla@uam.es (E. Revilla).
[1]Present address: Compañía Vinícola del Norte de España, 26200 Haro, Spain.

0021-9673/01/$ – see front matter
PII: S0021-9673(01)00635-5

analysis of anthocyanins in wines has been proposed as a useful tool to check out the authenticity of wines, especially if grape cultivar is mentioned in bottle labels, or if legal standards prescribe the cultivar identity of certain wines [10].

A number of different procedures have been described in the literature to extract anthocyanins from grapes, and to analyze these substances in grapes and wines by high-performance liquid chromatography (HPLC) [2,6,8,11–14].To our knowledge, only in the recent years the anthocyanin fingerprint of grapes and wines made with them has been studied, but with different methods [15]. In some cases, extraction procedures to analyze anthocyanins in grapes use acid solvents that may cause the partial hydrolysis of acetylated anthocyanins, as it has been demonstrated by several authors [6,13,16], leading to analytical results that would not be valid for chemotaxonomic purposes. On the other hand, some HPLC procedures which use an organic acid as acid modifier may cause the formation of laboratory artifacts, as those described when formic acid was used for that purpose [2].

In this paper, a HPLC method that allows the separation of acylated and non-acylated anthocyanidins in red grapes and wines, using perchloric acid as an acid modifier, is described. This method has been successfully applied to the analysis of those substances in six grape cultivars (Cabernet Sauvignon, Garnacha, Graciano, Mencia, Merlot, and Tempranillo) and in red wines made with them, and it allows to estimate differences among cultivars based on their chromatographic fingerprints related with anthocyanins. Furthermore, the procedure has been applied to examine if the ageing of Tempranillo red wines in oak barrels or in stainless steel tanks affects the relative amount of eight different anthocyanins, because to our knowledge, there are not enough data on the effect of different types of ageing on the anthocyanin composition of red wines.

2. Experimental

2.1. Reagents and standards

Acetonitrile of HPLC-gradient grade was obtained from Merck (Darmstadt, Germany). Perchloric acid of analytical-reagent grade was obtained from Scharlau (Barcelona, Spain). All other chemicals (analytical-reagent grade) were obtained from Panreac (Mollet del Valles, Spain). Water was purified in a Milli-Q water purification system (Millipore, Bedford, MA, USA). Standards of several anthocyanins were prepared from red grape skins as previously described in the literature [2].

2.2. Samples

Grape samples of six cultivars (Cabernet Sauvignon, Garnacha, Graciano, Mencia, Merlot, and Tempranillo), which are the most commonly used in Spain for making premium red wines aged in oak barrels, were collected at El Encin Ampelographic Collection, Alcalá de Henares, Spain, in 1999. Six corresponding experimental wines were made from them, and conserved in glass bottles after the end of malolactic fermentation. Extraction of phenolic compounds from grape skins was carried out following the procedure described by Bourzeix et al. [17]. Additionally, samples of four Tempranillo wines of AOC Rioja, made in 1999 at industrial scale (about 20 000 l), were monitored during their ageing during 8 months in oak barrels (225 l) and in stainless steel tanks (5000 l). These samples were kindly supplied by Bodegas CVNE, Haro, Spain. Grape extracts and wines were filtered through a 0.45-μm nylon membrane (Cole Palmer, USA) prior to their analysis.

2.3. Equipment

Analyses were performed with a liquid chromatograph that consisted of Waters (Milford, MA, USA) M510 and M501 pumps, a Waters 680 gradient controller, a Rheodyne 7725 injection valve furnished with a 20-μl loop, a Waters 996 photodiode array detector and a Millenium^{32} workstation. Separation was carried out using a Waters Nova-Pak C_{18} steel cartridge, 3.9×150 mm, filled with 5 μm particles, using a Waters Sentry Nova-Pak C_{18} guard cartridge, 20×3.9 mm, both thermostated in a water bath at 32°C.

2.4. Chromatographic conditions

The mobile phase was a linear gradient of water–

Table 1
Linear gradient used for the separation of anthocyanins present in grapes and wines

Time (min)	Solvent A (%)	Solvent B (%)
0	95	5
5	90	10
25	80	20
38	75	25
50	65	35
53	0	100
55	0	100

acetonitrile (40:60) adjusted to pH 1.3 with perchloric acid (solvent B), in water–acetonitrile (95:5) adjusted to pH 1.3 with perchloric acid (solvent A), at a flow-rate of 1.5 ml/min, as shown in Table 1. Spectra were recorded each second between 250 and 600 nm, with a bandwith of 1.2 nm.

3. Results and discussion

Fig. 1 shows a typical chromatogram of an extract of Cabernet Sauvignon grapes recorded at 520 nm. As can be noted, more than 15 different peaks appear in the chromatogram. Some of them have been assigned to different acylated and non-acylated anthocyanins on the basis of the retention times and UV–Vis spectra, as compared with those of standards isolated from grapes. In Cabernet Sauvignon, malvidin-3-*O*-glucoside is the major component. Other major anthocyanins present in these grapes are two acylated derivatives of that compound (malvidin-3-*O*-acetylglucoside acetate and malvidin-3-*O*-*p*-coumarylglucoside, the former being more abundant than the latter), delphinidin-3-*O*-glucoside, petunidin-3-*O*-glucoside and peonidin-3-*O*-glucoside. To evaluate the reliability of the method, a skin extract of Cabernet Sauvignon grapes was injected 10 times during 3 days. Table 2 shows, for every anthocyanin considered, the repeatabilities of the relative amount (%), absolute retention time (min) and relative retention index related to malvidin-3-*O*-glucoside, and their standard deviations. As it can be noted, standard deviations were too low, and the procedure allowed a good separation of the nine anthocyanins considered. Thus, the chromatographic method may be adequate for the estimation of their relative content in plant products.

Fig. 2 shows a typical chromatogram of Tempranillo grapes recorded at 520 nm. As can be noted, the anthocyanin fingerprint of Tempranillo grapes clearly differs from that observed in Cabernet Sauvignon grapes (Fig. 1). Certainly, malvidin-3-*O*-glucoside is the major anthocyanin in both cultivars, but Cabernet Sauvignon presents a remarkable amount of malvidin-3-*O*-acetylglucoside if compared with Tempranillo On the other hand, Tempranillo contains a remarkable amount of the other four

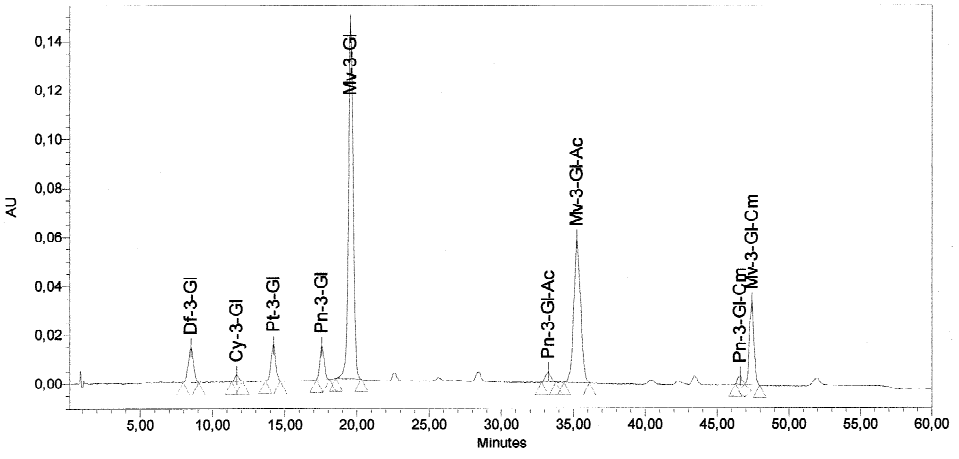

Fig. 1. Chromatogram of a skin extract of Cabernet Sauvignon grapes recorded at 520 nm. Df-3-Gl, Delphinidin-3-*O*-glucoside; Cy-3-Gl, cyanidin-3-*O*-glucoside; Pt-3-Gl, petunidin-3-*O*-glucoside; Pn-3-Gl, peonidin-3-*O*-glucoside; Mv-3-Gl, malvidin-3-*O*-glucoside; Pn-3-Gl-Ac, peonidin-3-*O*-acetylglucoside; Mv-3-Gl-Ac, malvidin-3-*O*-acetylglucoside; Pn-3-Gl-Cm, peonidin-3-*O*-*p*-coumarylglucoside; Mv-3-Gl-Cm, malvidin-3-*O*-*p*-coumarylglucoside.

Table 2
Repeatabilities of relative amount, retention time and retention index (relative to malvidin-3-*O*-glucoside) for nine anthocyanins in skins grapes extracts of Cabernet Sauvignon cultivar ($n=10$)

Compound	Relative amount (%)	Retention time (min)	Retention index
Delphinidin-3-*O*-glucoside	4.80±0.09	8.669±0.185	0.438±0.008
Cyanidin-3-*O*-glucoside	0.72±0.04	11.887±0.153	0.601±0.004
Petunidin-3-*O*-glucoside	4.57±0.07	14.408±0.147	0.728±0.003
Peonidin-3-*O*-glucoside	3.95±0.11	17.784±0.145	0.898±0.002
Malvidin-3-*O*-glucoside	46.30±0.27	19.795±0.154	1.000
Peonidin-3-*O*-acetylglucoside	1.50±0.07	33.416±0.358	1.688±0.016
Malvidin-3-*O*-acetylglucoside	26.49±0.32	35.334±0.402	1.785±0.018
Peonidin-3-*O*-*p*-coumarylglucoside	1.07±0.06	46.548±0.283	2.352±0.016
Malvidin-3-*O*-*p*-coumarylglucoside	10.59±0.14	47.406±0.292	2.395±0.016

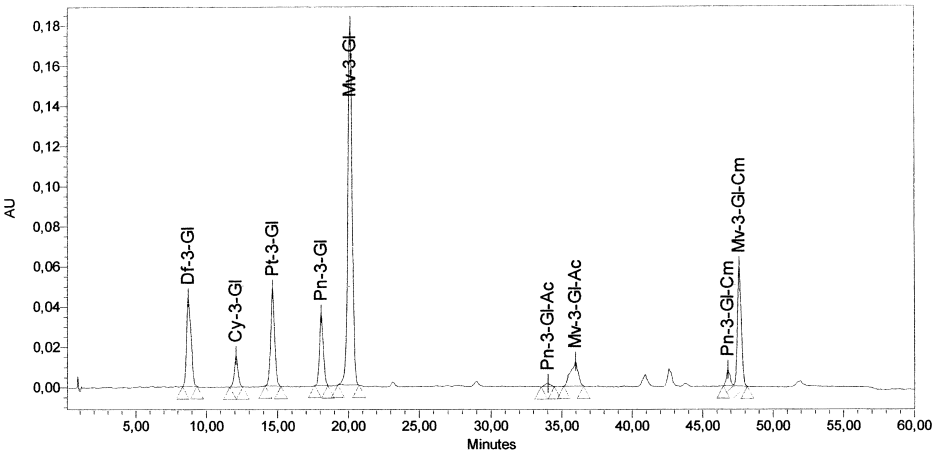

Fig. 2. Chromatogram of a skin extract of Tempranillo grapes recorded at 520 nm. For key to substances, see Fig. 1.

non-acylated anthocyanins (3-*O*-glucosides of delphinidin, cyanidin, petunidin and peonidin) if compared with Cabernet Sauvignon.

The other four grape cultivars studied presented a different anthocyanin fingerprint when compared with Cabernet Sauvignon and Tempranillo, as is indicated in Table 3, that shows the relative amount of nine anthocyanins in the skins of the six grape cultivars studied. Results are mean values of three samples collected in the same vineyard at the end of

Table 3
Relative amount and standard deviation for nine anthocyanins from skins of six different grape cultivars

Cultivar	Relative amount (%)±SD								
	Df-3-Gl	Cy-3-Gl	Pt-3-Gl	Pn-3-Gl	Mv-3-Gl	Pn-3-Gl-Ac	Mv-3-Gl-Ac	Pn-3-Gl-Cm	Mv-3-Gl-Cm
Cabernet Sauvignon	4.67±0.21	0.90±0.04	4.21±0.06	4.87±0.15	41.45±0.18	2.24±0.08	30.35±0.44	1.14±0.03	10.15±0.10
Garnacha	2.26±0.08	1.02±0.12	3.73±0.14	12.69±0.42	64.69±0.41	0.30±0.05	2.52±0.03	2.36±0.03	10.41±0.40
Graciano	6.81±0,49	1.28±0.18	7.21±0.14	12.79±0.56	53.69±0.64	0.89±0.04	6.08±0.25	2.17±0.06	9.12±0.46
Mencia	5.13±0.19	2.15±0.28	6.68±0.18	14.85±1.69	47.40±1.17	2.42±1.17	10.53±0.52	2.18±0.01	7.66±0.79
Merlot	7.53±0.07	5.52±0.10	7.00±0.14	14.27±0.22	35.54±0.25	3.16±0.02	14.99±0.15	2.51±0.03	9.48±0.10
Tempranillo	10.98±0.44	3.26±0.12	11.11±0.07	7.81±0.32	46.35±1.04	0.45±0.03	5.18±0.16	1.74±0.11	13.19±0.28

Results are mean values of three samples collected at harvest in the same vineyard in 1999. For key to substances, see Fig. 1.

grape maturation. A series of experimental wines were made with them to evaluate their anthocyanin fingerprints, as it is discussed below. As it can be noted, malvidin-3-*O*-glucoside is the major anthocyanin in the six cultivars considered, as it could be expected on the basis of literature data [2,3,6], because most red grape cultivars used in winemaking contain a remarkable amount of malvidin-3-*O*-glucoside. Nevertheless, the relative amount of this anthocyanin in the cultivars studied is quite variable, ranging from 35% (Merlot) to more than 64% (Garnacha). In most cases, the relative amount of malvidin-3-*O*-*p*-coumarylglucoside is relatively high (between 7 and 13%), but the relative amount of malvidin-3-*O*-acetylglucoside is quite variable, ranging from 2.5% (Garnacha) to more than 30% (Cabernet Sauvignon). Thus, the relative amount of acylated anthocyanins in the cultivars studied may be quite different, ranging from more than 43% (Cabernet Sauvignon) to 16% (Garnacha). Four cultivars (Garnacha, Graciano, Mencia and Merlot) contain a relatively high amount of peonidin-3-*O*-glucoside (higher than 12%), but the relative amount of the other non-acylated anthocyanins is quite small, except in Tempranillo grapes. Data clearly show that the anthocyanin fingerprint of grape cultivars is quite different at harvest, and this fact may allow the use of this tool to differentiate grape cultivars, as it has been proposed previously [2–4,7].

Table 4 shows the relative content of nine anthocyanins at different stages during the maturation of Tempranillo grapes. As we have observed, relative amount of each anthocyanin was quite similar during grape maturation, despite the sugar content of grapes. Certainly, the relative amount of the three anthocyanins derived from malvidin (3-*O*-glucoside, 3-*O*-acetylglucoside and 3-*O*-*p*-coumarylglucoside) slightly increased during maturation and, in the other hand, the relative amount of other non-acylated anthocyanins slightly decreased during that period of grape development. Probably, this behavior is related to anthocyanin metabolism, as it was suggested by Roggero et al. [8]. However, the changes observed in our research were too small, and they did not modify the anthocyanin pattern of Tempranillo grapes when compared with the other cultivars studied. These results suggest that the anthocyanins fingerprint of Tempranillo grapes is quite constant during the latter stages of grape maturation, showing that HPLC analysis of anthocyanins may be an adequate tool for the characterization of grape cultivars.

Fig. 3 shows typical chromatograms recorded at 520 nm of Cabernet Sauvignon and Tempranillo experimental wines, made with grapes collected at El Encin Ampelographic, 9 months after the end of the alcoholic fermentation. It can be noted that these chromatograms differ from those obtained from grape extracts. Thus, wines contain a higher relative amount of malvidin-3-*O*-glucoside than grapes, and the relative amount of eight other anthocyanins is lower in wines than in grapes. This fact was observed for six red wines made with corresponding six grape cultivars mentioned above, as shown in Table 5. This means that anthocyanins fingerprint of red wines is significantly different from that of grapes used for making them, but each wine present a characteristic anthocyanins fingerprint, as it has been previously reported by other authors [5,7,15]. For a better understanding of this question, and taking into account that many different technological approaches may be used during the making of red wines, the relative amount of eight different anthocyanins in four Tempranillo wines, made in 1999 at industrial scale, using grapes grown in the AOC Rioja, was

Table 4
Relative amount and standard deviation for nine anthocyanins from skins of Tempranillo grapes collected in 1999 at different stages of maturation

Days after veraison	Relative amount (%)±SD								
	Df-3-Gl	Cy-3-Gl	Pt-3-Gl	Pn-3-Gl	Mv-3-Gl	Pn-3-Gl-Ac	Mv-3-Gl-Ac	Pn-3-Gl-Cm	Mv-3-Gl-Cm
20	14.73±0.46	4.10±0.12	13.19±0.22	8.41±0.70	44.50±0.18	0.33±0.01	4.48±0.26	1.35±0.08	9.29±0.52
30	11.47±0.32	2.81±0.11	11.16±0.17	6.57±0.09	45.10±0.61	0.36±0.04	5.39±0.08	1.86±0.02	15.29±0.23
36	10.98±0.44	3.26±0.12	11.11±0.07	7.81±0.32	46.35±1.04	0.45±0.03	5.18±0.16	1.74±0.11	13.19±0.28

For key to substances, see Fig. 1.

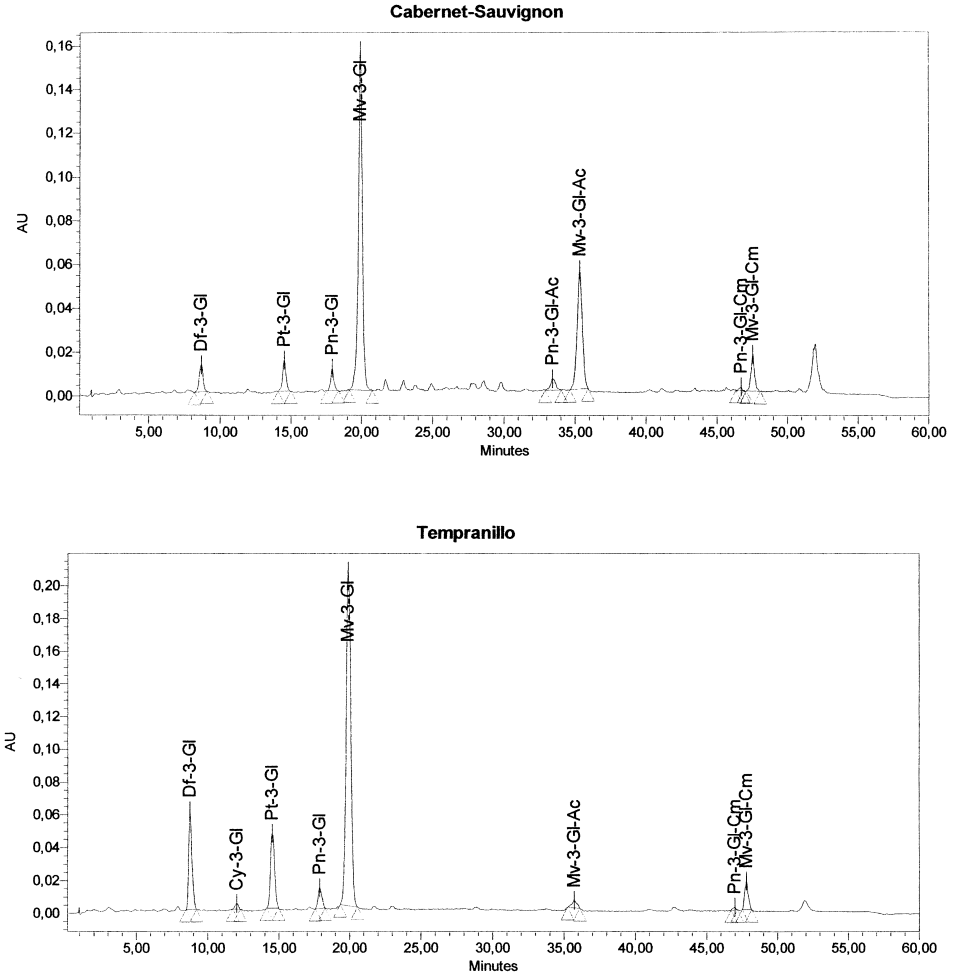

Fig. 3. Chromatograms of Cabernet Sauvignon and Tempranillo wines recorded at 520 nm. For key to substances, see Fig. 1.

Table 5
Relative amount of nine different anthocyanins in experimental wines (vintage 1999) made from six different grape cultivars

Cultivar	Relative amount (%)								
	Df-3-Gl	Cy-3-Gl	Pt-3-Gl	Pn-3-Gl	Mv-3-Gl	Pn-3-Gl-Ac	Mv-3-Gl-Ac	Pn-3-Gl-Cm	Mv-3-Gl-Cm
Cabernet Sauvignon	3.91	0.28	1.36	2.85	55.85	1.62	26.22	0.15	4.76
Garnacha	1.09	0.18	2.15	4.06	86.32	–	2.63	0.62	2.94
Graciano	3.49	0.20	5.29	8.75	65.55	2.05	6.92	2.11	5.65
Mencia	2.86	0.24	6.01	3.93	64.63	3.52	12.18	1.36	5.29
Merlot	5.60	0.58	6.69	6.33	56.49	3.82	15.04	0.45	5.00
Tempranillo	6.33	0.27	10.92	1.59	68.26	0.92	4.23	0.56	6.90

For key to substances, see Fig. 1.

Table 6
Relative amount of eight anthocyanins in four Tempranillo wines (vintage 1999) during their ageing in oak barrels or in stainless steel tanks

Wine	Type of ageing	Days of ageing	Relative amount (%)							
			Df-3-Gl	Cy-3-Gl	Pt-3-Gl	Pn-3-Gl	Mv-3-Gl	Mv-3-Gl-Ac	Pn-3-Gl-Cm	Mv-3-Gl-Cm
1	Oak barrel	55	15.20	1.22	14.32	4.25	56.98	3.10	0.80	4.13
1	Oak barrel	145	15.72	1.29	15.15	4.20	56.29	2.90	0.47	3.98
1	Oak barrel	235	14.82	1.31	14.85	4.16	54.69	3.57	0.99	5.61
1	Stainless steel tank	25	15.09	1.43	13.87	4.52	57.14	2.82	0.74	4.38
1	Stainless steel tank	145	14.92	1.69	13.95	4.23	56.50	3.30	0.76	4.64
1	Stainless steel tank	235	15.27	1.44	14.43	3.96	55.16	3.53	0.69	5.51
2	Oak barrel	55	15.81	1.54	14.56	4.50	56.14	2.86	0.68	3.90
2	Oak barrel	145	15.65	1.60	14.64	4.38	55.73	2.98	0.72	4.29
2	Oak barrel	235	15.16	1.51	15.10	3.72	55.10	3.49	0.67	5.24
3	Oak barrel	55	13.95	1.03	14.26	3.08	59.35	2.79	0.50	5.05
3	Oak barrel	145	14.19	1.03	14.08	2.81	58.62	3.08	0.67	5.51
3	Oak barrel	235	13.57	0.91	13.80	2.67	58.58	3.49	0.79	6.23
3	Stainless steel tank	25	12.81	1.02	13.04	3.03	61.66	3.18	0.61	4.65
3	Stainless steel tank	145	12.87	1.14	13.49	3.08	59.75	3.64	0.64	5.40
3	Stainless steel tank	235	11.59	0.91	13.94	3.18	59.51	3.68	0.72	6.47
4	Oak barrel	55	13.76	1.32	13.91	3.04	60.39	3.15	0.43	4.00
4	Oak barrel	145	13.64	1.09	13.60	3.04	58.97	3.15	0.84	5.51
4	Oak barrel	235	13.85	0.96	13.30	3.22	58.18	3.60	0.91	5.97

For key to substances, see Fig. 1.

monitored by HPLC during their ageing during 8 months in oak barrels (225 l) or in stainless steel tanks (5000 l). Results are shown in Table 6 and, as can be noted, the relative amount of analyzed anthocyanins is quite similar in each wine, despite the type of ageing and its length. The relative amount of peonidin-3-*O*-acetylglucoside cannot be considered, as it was lower than 0.1% in all samples. Certainly, the relative amount of acylated anthocyanins derived from malvidin (3-*O*-acetylglucoside and 3-*O*-*p*-coumarylglucoside) slightly increased during the first 8 months of wine maturation, and, on the other hand, the relative amount of malvidin-3-*O*-glucoside slightly decreased during that period. Nevertheless, the anthocyanin fingerprints of the four Tempranillo wines studied were quite similar, despite the quality of grapes and the different technologies used during winemaking and wine ageing. All these data suggest that the changes in the anthocyanins fingerprint of wines in relation to the anthocyanins fingerprint of grapes probably take place during the alcoholic fermentation, and this fact could be explain by several hypothesis. This could reside in the existing differences: rate of anthocyanin extraction, degradation, or polymerization during alcoholic fermentation, or the different capacity of adsorption of each anthocyanin in yeast cell walls. The answer to all these questions needs further research to understand differences between the anthocyanins fingerprint of grapes and corresponding wines.

Acknowledgements

This work has been carried out with financial support from Instituto Nacional de Investigaciones Agrarias y Alimentarias (project SC00-033) and from Bodegas CVNE (contract No. 006040). The authors also acknowledge the financial support of Grupo Iberdrola (Bilbao, Spain) and of Comunidad de Madrid for two of us (J.-M.R. and E.G.-B, respectively).

References

[1] V. Cheynier, M. Moutounet, P. Sarni-Manchado, in: C. Flanzy (Ed.), Oenologie – Fondements Scientifiques et Techniques, Technique et Documentation Lavoisier, Paris, 1998, p. 123.
[2] J. Bakker, C.F. Timberlake, J. Sci. Food Agric. 36 (1985) 1315.
[3] J.P. Roggero, J.L. Larice, C. Rocheville-Divorne, P. Archier, S. Coen, Rev. Franc. Oenol. (Cah. Sci.) 112 (1988) 42.
[4] R. Eder, S. Wendelin, J. Barna, Mitt. Klosterneuburg 40 (1990) 68.
[5] C. Santos, S.S. Muñoz, Y. Gutierrez, E. Hebrero, J.L. Vicente, P. Galindo, J.C. Rivas, J. Agric. Food Chem. 39 (1991) 1086.
[6] T. Castia, M.A. Franco, F. Mattivi, G. Muggioli, G. Sferlazzo, G. Versini, Sci. Aliments 12 (1992) 239.
[7] B. Holbach, R. Marx, M. Ackermann, Lebensm.-Chem. 51 (1997) 78.
[8] J.P. Roggero, S. Coen, B. Ragonett, Am. J. Enol. Vitic. 37 (1986) 77.
[9] W.M. Kliever, Am. J. Enol. Vitic. 28 (1977) 96.
[10] R. Wittkowski, in: Proceedings of the XXIV World Vine and Wine Congress, Section II, Vol. 1, Mainz, July 1999, p. 118.
[11] L.W. Wulf, C.W. Nagel, Am. J. Enol. Vitic. 29 (1978) 42.
[12] E. Hebrero, C. Santos-Buelga, J.C. Rivas-Gonzalo, Am. J. Enol. Vitic. 39 (1988) 227.
[13] E. Revilla, J.M. Ryan, G. Martin-Ortega, J. Agric. Food Chem. 46 (1998) 4592.
[14] K. Yokotsuka, A. Nagao, K. Nakazawa, M. Sato, Am. J. Enol. Vitic. 50 (1999) 1.
[15] G. Mazza, L. Fukumoto, P. Delaquis, B. Girard, B. Ewert, J. Agric. Food Chem. 47 (1999) 4009.
[16] B.E. Van Wyk, P.J.D. Winter, Biochem. Syst. Ecol. 22 (1994) 813.
[17] M. Bourzeix, D. Weyland, N. Heredia, Bull. OIV 59 (1986) 1171.