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Phenolic composition and magnitude of copigmentation in young and shortly aged red wines made from the cultivars, Cabernet Sauvignon, Cencibel, and Syrah

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Abstract

Young and shortly aged red wines made from single cultivar grapes grown in the warm climate region of La Mancha (Spain), were examined for their phenolic composition and effects of copigmentation on wine colour. In addition to anthocyanins, flavonol and hydroxycinnamic acid profiles allowed the varietal differentiation of these wines. Characteristic flavonols found were principally glycosides, the main ones, being: myricetin 3-glucoside, quercetin 3-glucoside, and quercetin 3-glucuronide. The fraction of red colour due to copigmented anthocyanins ranged from 32% to 43% at the end of the alcoholic fermentation, and this decreased to 20–34% after 3 months of ageing, when a decrease in both monomeric anthocyanin and flavonol concentrations was also observed . Cencibel wines showed the greatest loss in flavonols after 3 months, but these were apparently replaced, in the copigmentation complexes, by other abundant cofactors, such as hydroxycinnamic acids and (−)-epicatechin. Thereafter, Cencibel wines showed proportionally the lowest decrease in this copigmentation effect at this ageing time. After 9 months, the losses of monomeric anthocyanins and cofactors still continued, and only Syrah wines showed a little enhancement of the red colour intensity. At this ageing time, the amounts of monomeric anthocyanins were sufficient for the formation of copigmentation complexes, but the molar ratio of cofactors to anthocyanins was possibly too low for detecting significant effects on colour, especially in Cabernet Sauvignon and Cencibel wines. A second observed effect of copigmentation was the bathochromic shift to bluish hues for Cencibel and Syrah wines, and it only was maintained in the latter wine during all the ageing times checked.

Keywords: Anthocyanins; Cabernet Sauvignon; Cencibel/Tempranillo; Copigmentation; Flavonols; Hydroxycinnamic acids; Red wine colour; Syrah/Shiraz

1. Introduction

The relationship between the colour of red wine and its phenolic composition is well known. Anthocyanins, in their flavilium cation forms, are the pigments which give the red colour to red wine. In red wine grapes, anthocyanins exist exclusively as monomers. However, it is difficult to predict the intensity and the hue of the colour of a young red wine taking into account only the anthocyanin composition of the starting grapes. As early as the first steps of the winemaking process, monomeric anthocyanins are implicated, in both loose associations (copigmentation complexes) and in chemical reactions (oxidation, covalent linking to another phenolic compounds and/or secondary yeast metabolites). The

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copigmentation complexes disappear in the next few months of ageing, due to the transformation of monomeric anthocyanins into polymeric pigments. This conversion leads to an increase in the colour stability against both bisulphite bleaching and pH changes.

Copigmentation is a widespread phenomenon observed in plant tissues containing anthocyanins. Copigmentation effects are normally a combination of enhancement of the red colour intensity and a bathochromic shift from reddish to bluish hues (Boulton, 2001). Although copigmentation is a typical aqueous phenomenon, and it is partially destroyed in the presence of organic cosolvents (Brouillard, Wigand, Dangles, & Cheminat, 1991), such as ethanol, its importance in young red wines mainly determines their characteristic, intense, red-purple colour (Hermosín Gutiérrez, 2003). It has been suggested that copigmentation is the first step for subsequent and more stable covalent linking (Brouillard & Dangles, 1994), leading to the formation of polymeric anthocyanins that are the main kind of pigments present in aged red wines. This evolution, from monomeric to polymeric anthocyanins, means a loss in the red colour intensity and a change of the colour hue from purple-red to red, and then to yellow-red tonalities.

From a molecular point of view, copigmentation is a hydrophobic interaction between monomeric anthocyanins and other phenolic compounds present in red wine, known as copigments or copigmentation cofactors, many of them being colourless. The effectiveness of this interaction depends largely on the planar disposition that can be adopted by cofactors, because they need to stack with the (also planar) flavilium forms of monomeric anthocyanins (Boulton, 2001). Studies with model solutions have shown that flavonols (yellow flavonoid pigments) are the best cofactors present in the phenolic composition of red wine (Baranac, Petranovic, & Dimitric-Markovich, 1996, 1997a, 1997b). However, the importance of flavonols in relation to the colour of red wine has been little evaluated directly in red wines.

Other cofactors found in red wine are less effective than flavonols, but they can account for higher amounts. The flavan-3-ols are the most abundant phenolics in red wine, but they are considered comparatively poor cofactors, except (−)-epicatechin, due to its predominance in aqueous media in a quasi-planar disposition that facilitates its stacking with anthocyanins, so leading to the formation of copigmentation complexes (Liao, Cai, & Haslam, 1992). Hydroxycinnamic acids (caffeic, *p*-coumaric, and ferulic acids), the hydroxycinnamoyltartaric acids (caftaric, coutaric, and fertaric acids), and the *p*-coumaroyl and caffeoyl derivatives of anthocyanins, can also act as cofactors. The study of model systems has demonstrated that one or more cinnamic acid esters stabilise acylated anthocyanins; however, it is necessary to have at least two sugar molecules between the anthocyanidin and the cinnamic acid, in order to allow the molecule to fold in such a way that the cinnamic acid can reach and protect the anthocyanidin from hydration and be involved in an intramolecular copigmentation complex (Dangles, Saito, & Brouillard, 1993; Eiro & Heinonen, 2002; Malien-Aubert, Dangles, & Amiot, 2001). To our knowledge, no similar effect has been reported in relation to grape and wine acylated anthocyanins, which have only one sugar molecule between the anthocyanidin and the cinnamic acid. On the other hand, the addition of caffeic and *p*-coumaric acids in the pre-fermentative step of the winemaking process enhances the intermolecular copigmentation observed in the final red wines (Bloonfield, Heatherbell, & Pour Nikfardjam, 2003; Darias-Martín, Martín-Luís, Carrillo-López, Lamuela-Raventós, Díaz-Romero, & Boulton, 2001, 2002).

In this work, wine from three different cultivars of red wine grapes that were grown in the winemaking zone of La Mancha (middle-southeast Spain, warm climate), has been made. The cultivars assayed were Cencibel (Tempranillo), traditionally used in this zone, and the recently introduced Cabernet Sauvignon and Syrah (Shiraz). The contribution of copigmentation to the total colour of the latter red wines has been measured, and the relationship between the degree of copigmentation and the phenolic composition of the wines has been evaluated. Special attention has been paid to the content of several kinds of phenolic compounds, such as the monomeric anthocyanins implicated in the formation of copigmentation complexes, and the expected copigmentation cofactors, such as flavonols, hydroxycinnamic acids and their derivatives, and monomeric flavan-3-ols. In addition, the changes in wine phenolic composition and copigmentation degree have been checked over several months of ageing.

2. Materials and methods

2.1. Winemaking

Red wine grapes from three cultivars grown in Ciudad Real (region of Castilla-La Mancha, middle-southeast of Spain), harvested in their optimal ripening stage (23–24° Brix; pH 3.4–3.6) and in good sanitary conditions, were used for winemaking. Three batches of grapes (25 kg each) of the cultivars Cabernet Sauvignon and Cencibel (Tempranillo), and four batches of grapes (25 kg each) of the cultivar Syrah, were elaborated in vats of 25 l, with skin maceration until the alcoholic fermentation finished.

Winemaking conditions were: addition of 100 ppm of SO_2, as $K_2S_2O_7$, after stemming and crushing, inocula-

Table 1
Conventional analytical data[a] of the red wines at the moment of bottling

Analytical data	Cabernet Sauvignon ($n = 3$)	Cencibel ($n = 3$)	Syrah ($n = 4$)
Ethanol (% V/V)	13.2	13.1	13.3
pH	3.82	4.12	3.96
Total acidity (g/l as tartaric acid)	5.36	5.24	5.35
Volatile acidity (g/l as acetic acid)	0.38	0.45	0.43
Glucose (g/l)	0.11	0.16	0.01
Fructose (g/l)	0.05	0.09	0.09
Malic acid (g/l)	ND	ND	ND
L-Lactic acid (g/l)	1.53	1.58	1.87
D-Lactic acid (g/l)	0.27	0.42	0.38
Succinic acid (g/l)	0.53	0.78	0.69
Citric acid (g/l)	0.05	0.02	0.03

[a] Average values of replicates; ND, not detected.

tion with *Saccharomyces cerevisiae* selected yeasts (UCLM S325, Fould-Springer), and fermentation temperature kept at 24 °C. Manual punching down was done twice a day. Separation of the wines from solids was performed when relative density reached a constant value. Subsequently, the malolactic fermentation was induced by inoculation with *Oenococcus oeni* lactic acid bacteria (Lactobacter SP1; Laffort); this second fermentation finished in 2–3 weeks, as confirmed by TLC, and then the wines were racked. After 1 month, the wines were racked again, filtered through 1.2 μm membranes (Millipore, Bedford, MA, USA), bottled, and stored in a conditioned room kept at 16–18 °C. The conventional analytical data of these wines at the moment of bottling are shown in Table 1.

2.2. Copigmented and polymerised anthocyanins

The contribution of copigmented anthocyanins to the total wine colour at pH 3.6 (%copigmentation), and the degree of anthocyanin polymerisation (%polymerisation), were determined, following the method proposed by Boulton (1996), as described by Hermosín Gutiérrez (2003). While the selected pH value may not be the pH conditions for each particular wine, it provides the only rational basis on which to compare colour components, which are independent of pH effects, across all wines. There are pH effects on the free monomeric anthocyanin ionisation, the colour of the copigmented form, and the coloured polymers.

2.3. Chromatic characteristics in the space CIELAB

Measures were made after filtration of the samples through 0.45 μm nylon membranes (Millipore). The values of the CIELAB parameters L^*, C^*, and h^*, were calculated from the wine absorbances measured at 450, 520, 570, and 630 nm, according to a proposed simplified method (Ayala, Echávarri, & Negueruela, 1999; Pérez-Caballero, Ayala, Echávarri, & Negueruela, 2003). Colorimetric differences (ΔE^*) between every pair of wines from different cultivars (wine from cultivar 1, and wine from cultivar 2) were calculated as follows:

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta C^*)^2 + (\Delta H^*)^2]^{1/2},$$

$$\Delta H^* = 2(C_1^* C_2^*)^{1/2} \sin(\Delta h^*/2).$$

2.4. Phenolic compounds by HPLC

The separation method employed by Vaadia (1997) was used, that is a modification of a method previously described (Lamuela-Raventós & Waterhouse, 1994). The wine samples, after centrifugation (2500 g) and filtration (0.45 μm nylon membranes, Millipore), were directly injected (10 μl) into a liquid chromatograph (Waters 2690), fitted with a photodiode array detector (Waters 996). The column was Spherisorb C18 (250 mm × 4.6 mm i.d.; 5 μm of particle size), kept at 40 °C. The solvents were A (50 mM $NH_4H_2PO_4$, pH 2.6), B (20% A + 80% acetonitrile), and C (200 mM H_3PO_4, pH 1.5), and the elution gradient used was: 100% A (min 0); 100% A (min 2); 92% A + 8% B (min 5); 14% B + 86% C (min 17); 18% B + 82% C (min 22); 21.5% B + 78.5% C (min 32); 43% B + 57% C (min 62); 100% A (min 70); and 100% A (min 75). From every injection four chromatograms were obtained, extracting the collected data at the wavelength values of 280 nm (gallic acid and monomeric flavan-3-ols), 320 nm (hydroxycinnamic acid derivatives), 360 nm (flavonols), and 520 nm (anthocyanins). Peak identification was performed using standards (gallic acid from Merck; (+)-catechin, (−)-epicatechin, caffeic acid, *p*-coumaric acid, myricetin, and quercetin, from Sigma–Aldrich; kaempferol, isorhamnetin, and rutin from Fluka; quercetin 3-glucoside, kaempferol 3-glucoside, isorhamnetin 3-glucoside, and malvidin 3-monoglucoside chlorhydrate, from Extrasynthese), and by comparison of their chromatographic and UV–Vis spectral characteristics with those found in the literature (Cantos, Espín, & Tomás-Barberán, 2002; Cheynier & Rigaud, 1986; Hermosín Gutiérrez & García-Romero, 2004; Schieber, Berardini, & Carle, 2003; Vaadia, 1997). To confirm the peak assignation made for flavonols, an extract of flavonols from Syrah grape skins (Andrade, Mendes, Falco, Valentão, & Seabra, 2001) was injected and compared with standards. For quantification, calibration curves were obtained from solutions of the corresponding standard when it was commercially available, whereas, for the other compounds, the concentrations were expressed as mg/l of the more similar standard found: caffeic acid for caftaric acid; *p*-coumaric acid for coutaric acid; malvidin

3-monoglucoside for monomeric anthocyanins; quercetin 3-glucoside for myricetin 3-glucoside and quercetin 3-glucuronide; and rutin for flavonol 3-diglycosides.

2.5. Statistical analysis

The data corresponding to the wines made from different cultivars, and at different ageing times, were analysed using the test of Student–Newman–Keuls (SPSS 10.0, SPSS Inc., license UCLM 7876875), searching for significant differences.

3. Results and discussion

3.1. Phenolic composition and its evolution during ageing

Monomeric anthocyanin profiles of all the wines were the characteristic ones, according to the cultivars of provenance (Hermosín Gutiérrez et al., 2004, and references cited therein). Anthocyanin profiles of Cabernet Sauvignon and Syrah grapes and wines are well known, and the anthocyanin profile of a Cencibel wine is shown in Fig. 1. The wine chromatograms contained extra peaks, in addition to those attributable to monomeric anthocyanins, due to the formation of anthocyanin derivatives during the winemaking process and the subsequent ageing. Some of these anthocyanin derivatives showed UV–Vis absorption maxima (271 and 495 nm for the peak eluting just before acetylated petunidin 3-monoglucoside, 279 and 500 nm, together with a shoulder at 305 nm, for the peak eluting just after *p*-coumarylated petunidin 3-monoglucoside) which were very similar to those reported for the anthocyanin derivatives known as pyranoanthocyanins (Håkanson, Pardon, Hayasaka, de Sa, & Herderich, 2003; Mateus et al., 2003; Schwarz, Wabnitz, & Winterhalter, 2003, 2003). To guarantee the peak assignation of monomeric anthocyanins, the wine chromatograms were compared to those corresponding to grape skin extracts of the same cultivar. The grape skins only contained monomeric anthocyanins, which were easily identified by their characteristic and well known UV–Vis spectra. Only monomeric anthocyanins were quantified in wine chromatograms because we could not prove the identity of the other peaks only on the basis of their UV–Vis spectra.

Syrah wines had the highest contents of total monomeric anthocyanins at the end of the alcoholic fermentation, whereas Cabernet Sauvignon and Cencibel wines had similar amounts (Table 2 and Fig. 2). An expected loss of monomeric anthocyanins during ageing was shown by all the wines studied. The loss was faster in the first 3 months of ageing for Cabernet Sauvignon and Syrah wines, whereas Cencibel wines lost their monomeric anthocyanins at an almost constant rate. After 9 months of ageing, the amounts of remaining monomeric anthocyanins were 40% and 38% for Syrah and Cencibel wines, respectively, whereas Cabernet Sauvignon wines only retained 32% of the monomeric anthocyanins initially found at the end of alcoholic fermentation. All the individual monomeric anthocyanins diminished their concentrations, the percentage of diminution being almost equal for the main anthocyanins. Therefore, the characteristic anthocyanin profiles of the wines remained differentiable (Table 2).

The hydroxycinnamic acids found in our wines were caffeic and *p*-coumaric acids, and no peak was found that could be attributable to ferulic acid (Fig. 3(b)), as could be guaranteed by comparison with authentic

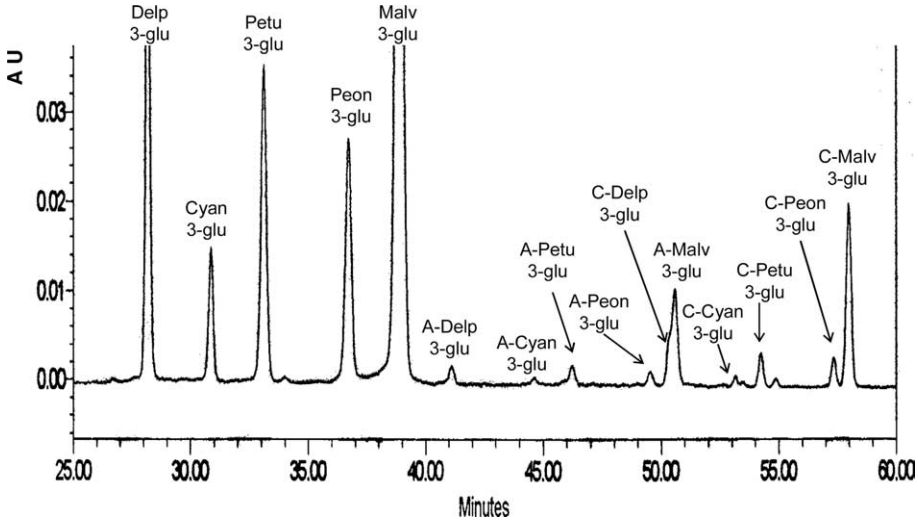

Fig. 1. Chromatographic profile obtained at 520 nm for a Cencibel wine, showing its fraction of monomeric anthocyanins, just at the end of the alcoholic fermentation. Peak assignation: Delp, Cyan, Petu, Peon, and Malv are the anthocyanidins delphinidin, cyanidin, petunidin, peonidin, and malvidin, respectively; 3-glu means 3-monoglucoside; A- and C- are the acetylated and *p*-coumarylated derivatives of anthocyanins, respectively.

Table 2
Monomeric anthocyanin content (mg/l as malvidin 3-monoglucoside)[A], for the single cultivar red wines at the end of alcoholic fermentation (EAF) and after ageing

Monomeric anthocyanin	Ageing time	Cabernet Sauvignon ($n = 3$)		Cencibel ($n = 3$)		Syrah ($n = 4$)	
		MV	SD	MV	SD	MV	SD
Delphinidin 3-monoglucoside	EAF	34.6	6.8	31.3	3.5	26.5	2.6
	3 months	20.3	1.4	19.4	1.7	13.2	8.1
	9 months	10.9	0.6	11.5	1.4	11.3	0.7
Cyanidin 3-monoglucoside	EAF	0.8[a]	0.1	3.4[b]	0.4	2.8[b]	0.8
	3 months	1.0	0.1	1.5	0.7	1.4	1.0
	9 months	0.4	0.0	1.1	0.4	0.9	0.3
Petunidin 3-monoglucoside	EAF	35.3[a]	5.2	62.9[b]	4.6	50.8[c]	4.5
	3 months	23.0[a]	1.4	45.3[b]	2.8	37.7[c]	3.7
	9 months	11.9[a]	1.3	24.4[b]	2.3	22.2[b]	2.0
Peonidin 3-monoglucoside	EAF	16.1[a]	4.6	12.5[a]	1.0	24.0[b]	1.9
	3 months	9.1	1.0	6.3	3.5	13.5	3.0
	9 months	5.2[a]	1.9	4.0[a]	1.4	11.1[b]	2.5
Malvidin 3-monoglucoside	EAF	316[a]	44.7	441[b]	20.5	437[b]	22.7
	3 months	239[a]	21.8	363[b]	10.4	335[b]	26.0
	9 months	122[a]	18.8	191[b]	16.2	197[b]	20.4
Delphinidin 3-monoglucoside–Ac[B]	EAF	16.3[a]	4.4	5.7[b]	1.0	14.1[a]	2.2
	3 months	8.7[a]	0.5	3.3[b]	0.1	9.1[a]	1.5
	9 months	3.6[a]	0.2	1.8[b]	0.2	5.1[c]	0.5
Cyanidin 3-monoglucoside–Ac[B]	EAF	4.9[a]	0.7	1.8[b]	0.2	6.4[c]	0.7
	3 months	3.0[a]	0.1	1.2[b]	0.1	3.9[c]	0.5
	9 months	1.2[a,b]	0.6	0.2[b]	0.3	2.9[a]	1.3
Petunidin 3-monoglucoside–Ac[B]	EAF	14.6[a]	2.3	5.1[b]	0.3	22.2[c]	2.5
	3 months	9.9[a]	0.3	3.9[b]	0.1	15.5[c]	2.2
	9 months	4.0[a]	0.1	1.9[b]	0.3	8.4[c]	1.0
Peonidin 3-monoglucoside–Ac[B]	EAF	14.5[a]	1.6	2.7[b]	0.1	26.3[c]	3.4
	3 months	10.4[a]	1.8	1.6[b]	0.3	17.2[c]	2.6
	9 months	3.9[a]	0.1	0.8[b]	0.2	9.7[c]	1.1
Malvidin 3-monoglucoside–Ac[B]	EAF	159[a]	18.9	24.7[b]	0.3	187[c]	13.2
	3 months	118[a]	13.0	28.1[b]	4.4	147[c]	10.7
	9 months	49.9[a]	2.0	11.8[b]	1.5	78.3[c]	7.2
Delphinidin 3-monoglucoside–Cm[C]	EAF	19.6[a]	3.3	22.9[b,c]	1.9	26.8[c]	3.4
	3 months	4.5	0.8	8.2	4.3	6.9	1.9
	9 months	2.1[a]	0.1	3.5[b]	0.5	3.3[b]	0.3
Cyanidin 3-monoglucoside–Cm[C]	EAF	2.5[a]	0.3	3.9[b]	0.5	2.7[a]	0.4
	3 months	1.4[a]	0.1	2.2[b]	0.1	0.6[c]	0.3
	9 months	ND		0.2	0.4	ND	
Petunidin 3-monoglucoside–Cm[C]	EAF	3.0[a]	0.9	12.2[b]	1.0	8.7[c]	0.8
	3 months	2.2[a]	0.3	8.5[b]	0.4	6.2[c]	0.4
	9 months	ND		1.5	1.1	0.8	0.1
Peonidin 3-monoglucoside–Cm[C]	EAF	6.6[a]	1.4	7.5[a]	0.8	14.8[b]	1.8
	3 months	4.1[a]	0.9	5.0[a]	0.6	8.7[b]	1.0
	9 months	ND		0.6[a]	0.6	1.1[b]	0.1
Malvidin 3-monoglucoside–Cm[C]	EAF	44.1	8.8	53.0	1.8	45.2	3.9
	3 months	28.9[a]	5.3	43.4[b]	1.6	29.4[a]	3.3
	9 months	2.8	0.1	7.6	4.7	6.0	0.1

[a,b,c] Different superscripts in the same row mean significant differences ($\alpha = 0.05$) according to the test of Student–Newman–Keuls. ND, not detected.
[A] Mean values (MV) and standard deviations (SD).
[B] Ac, acetyl derivative.
[C] Cm, *p*-coumaroyl derivative.

standards. These hydroxycinnamic acids appear in wine as a consequence of the hydrolysis of their respective hydroxycinnamoyltartaric acids present in grapes, the so called caftaric, coutaric, and fertaric acids. Caftaric and coutaric acids, as their predominant *trans* isomers, were also detectable in our wine chromatograms (Fig.

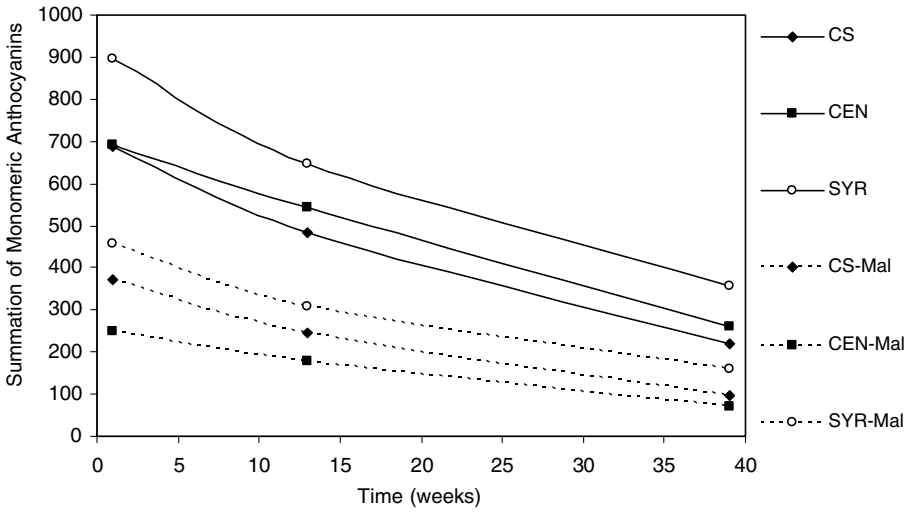

Fig. 2. Evolution during ageing of the total content of monomeric anthocyanins (mg/l as malvidin 3-monoglucoside) for Cabernet Sauvignon (CS), Cencibel (CEN), and Syrah (SYR) wines. Dotted lines correspond to the total monomeric anthocyanins of these wines, excluding malvidin 3-monoglucoside (-Mal).

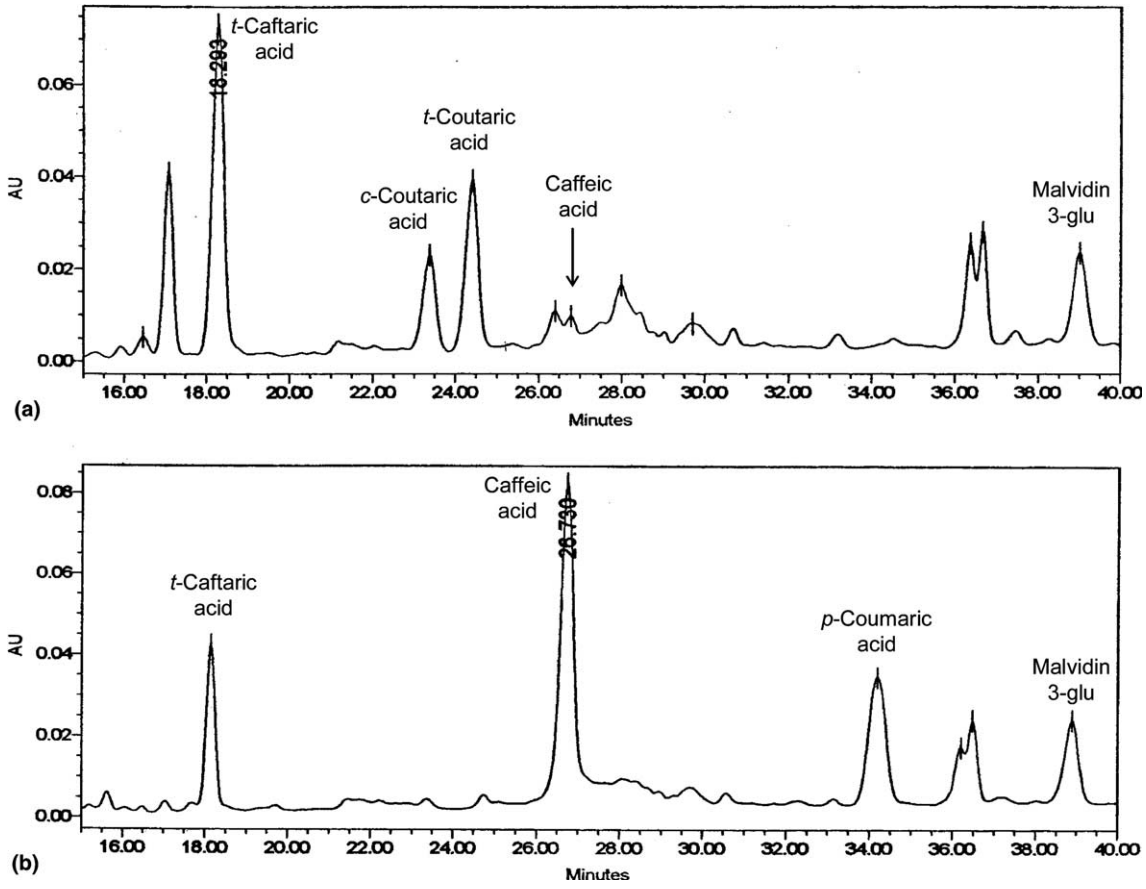

Fig. 3. Chromatographic profile obtained at 320 nm for a Cencibel wine, showing its fraction of hydroxycinnamic acid derivatives: at the end of alcoholic fermentation (a), and after 3 months of ageing (b). Also shown is the presence of the peak corresponding to the major anthocyanin, malvidin 3-monoglucoside.

3(a)). Under the chromatographic conditions used, *t*-caftaric and *t*-coutaric acids eluted first and then came caffeic and *p*-coumaric acids (Vaadia, 1997). The peak assignation for these hydroxycinnamoyltartaric acids was made on the basis of their UV spectra (maximum at 326 nm and shoulder at 296 nm for *t*-caftaric acid;

maximum at 311 nm and shoulder at 295 nm for *t*-coutaric acid), which were very close to those obtained for their corresponding hydroxycinnamic acids (maximum at 322 nm and shoulder at 295 nm for caffeic acid; maximum at 308 nm and shoulder at 295 nm for *p*-coumaric acid). Some evidence indicated the presence of the *cis* isomer of coutaric acid, eluting just before its *trans* isomer, but the accuracy of this assignation was poor because of the partial co-elution of (+)-catechin that dominated the UV spectrum of this peak.

The main cinnamic derivative present in all the wines, just after the alcoholic fermentation, was *t*-caftaric acid (Table 3). This acid was found in similar amounts in the three different wines. In contrast, the amounts of *t*-coutaric acid were very variable for the three types of wines, allowing their differentiation by cultivar. The amounts of the latter acid were always lower than those found for *t*-caftaric acid, although for Cencibel wines, the two amounts were comparable. During ageing, the hydrolysis of caftaric and coutaric acids continued, generating caffeic and *p*-coumaric acids, respectively, as can be noted when compared Fig. 3(a) and (b). This hydrolysis process has already been described as a slow process that occurs during ageing (Karagiannis, Economou, & Lanaridis, 2000; Somers, Vérette, & Pocock, 1987). However, we have found that the hydrolysis of hydroxycinnamoyltartaric acids in our wines was almost complete after 3 months of ageing, and only Cabernet Sauvignon wines retained a small amount of *t*-caftaric acid after 9 months of ageing. A possible explanation for the acceleration of this hydrolytic process could be the relatively high temperature of storage of wines during ageing, around 16–18 °C. As a consequence of this hydrolysis, caffeic acid was the main hydroxycinnamic acid found in the aged wines, and the variable amounts of *p*-coumaric acid allowed the differentiation of the wines according to their cultivar, at least after 3 months of ageing. Longer ageing times led to losses in caffeic and *p*-coumaric acids, although, for Syrah wines, only *p*-coumaric acid seemed to be suffer a significant loss.

At the end of alcoholic fermentation, (+)-catechin was the main monomeric flavan-3-ol found in Cabernet Sauvignon wines, whereas (−)-epicatechin was in Syrah wines (Table 3). Cencibel wines showed similar amounts of both monomeric flavan-3-ols. The peak assignation of the above mentioned compounds were made by comparison with authentic standards and the quantification of (+)-catechin was reasonably accurate because the interference of the co-eluting *c*-coutaric acid could be avoided (Fig. 4). During ageing, the amounts of these compounds normally decreased, little in the first 3 months, but much more in the next months. However,

Table 3
Contents (mg/l)[A] of gallic acid, monomeric flavan-3-ols, and hydroxycinnamic acid derivatives, for the single cultivar red wines at the end of alcoholic fermentation (EAF) and after ageing

Phenolic compound	Ageing time	Cabernet Sauvignon ($n = 3$)		Cencibel ($n = 3$)		Syrah ($n = 4$)	
		MV	SD	MV	SD	MV	SD
Gallic acid	EAF	8.0	1.7	7.9	4.0	14.2	2.2
	3 months	11.6	4.5	14.0	0.5	13.1	4.3
	9 months	18.6	6.1	15.9	1.4	17.2	2.1
(+)-Catechin	EAF	96.6^{a}	35.3	29.9^{b}	0.3	53.1^{b}	6.6
	3 months	97.3^{a}	35.8	21.6^{b}	8.3	30.0^{b}	6.3
	9 months	38.6	8.9	27.1	15.3	43.0	8.3
(−)-Epicatechin	EAF	39.3^{a}	11.9	30.3^{a}	1.5	87.9^{b}	10.0
	3 months	34.9	3.4	54.3	3.6	74.3	30.9
	9 months	16.7	3.9	54.2	20.8	51.9	22.6
t-Caftaric acid[B]	EAF	34.2	7.5	27.1	0.7	30.2	2.1
	3 months	6.1^{a}	1.0	2.2^{b}	0.0	2.7^{b}	0.5
	9 months	1.4	0.7	ND		ND	
t-Coutaric acid[C]	EAF	7.9^{a}	1.8	24.0^{b}	1.7	14.6^{c}	1.6
	3 months	1.4^{a}	0.0	0.2^{b}	0.0	0.2^{b}	0.1
	9 months	ND		ND		ND	
Caffeic acid	EAF	ND		ND		ND	
	3 months	25.6^{a,b}	0.9	27.6^{b,c}	0.2	29.6^{c}	1.7
	9 months	14.0^{a}	1.1	17.5^{a}	5.5	27.0^{b}	2.2
p-Coumaric acid	EAF	ND		ND		ND	
	3 months	3.8^{a}	0.0	16.8^{b}	0.7	12.5^{c}	1.1
	9 months	1.8^{a}	0.0	4.2^{a,b}	2.0	6.4^{b}	1.1

^{a,b,c} Different superscripts in the same row mean significant differences ($\alpha = 0.05$) according to the test of Student–Newman–Keuls. ND, not detected.
[A] Mean values (MV) and standard deviations (SD).
[B] As caffeic acid.
[C] As *p*-coumaric acid.

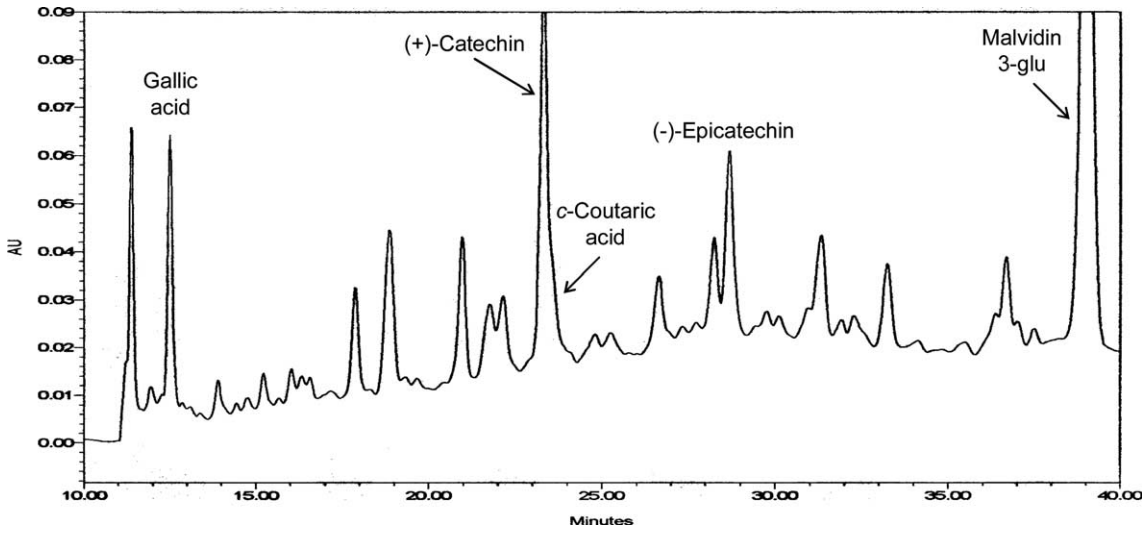

Fig. 4. Chromatographic profile obtained at 280 nm for a Cencibel wine, showing gallic acid and the flavan-3-ols monomers, (+)-catechin and (−)-epicatechin. Also shown are the peaks assigned as malvidin 3-monoglucoside and *c*-coutaric acid.

an increase in the concentration of (−)-epicatechin after 3 months was observed for Cencibel wines, that was maintained after 9 months of ageing. A recuperation of the initially decreased concentration of (+)-catechin was also observed for Syrah wines. This diversity in the evolution of the concentration of monomeric flavan-3-ols can be attributable to different causes: on one hand, the concentration of monomeric flavan-3-ols tends to a diminution because they are involved in oxidation reactions, as well as in tannin–tannin and anthocyanin–tannin (or tannin–anthocyanin) polymerisation reactions; on the other hand, an increase in the concentration of monomeric flavan-3-ols is expected because they can be released from their galloylated precursors (Singleton & Trousdale, 1983), as shown by the observed increase of gallic acid after ageing, for all the wines studied.

The chromatographic pattern shown by the flavonol fraction of our wines (Fig. 5) was very close to that previously reported (Cheynier et al., 1986). All the standards used for identification and quantification were found in our samples, with the exception of the flavonol aglycones, kaempferol and isorhamnetin. Five unknown peaks with visible absorption maxima in the range 346–356 nm were assigned as flavonol glycosides (Table 4), according to the assignations given by Cheynier et al. (1986): the UV–Vis spectra of three of them led to their consideration as quercetin derivatives; one of them was assigned as myricetin 3-glucoside, because it showed the lowest retention time of the flavonol peaks; and the last one seemed to be a kaempferol derivative because its visible absorption maximum (346 nm) was very close to that of kaempferol 3-glucoside (347 nm). The majority of the flavonols found were glycosides, being, in the main, the expected myricetin 3-glucoside, quercetin 3-glucuronide, and quercetin 3-glucoside (Table 5). Cabernet Sauvignon wines showed the highest contents of flavonols at the end of alcoholic fermentation (114 mg/l in total), mainly quercetin 3-glucuronide and quercetin 3-glucoside. At this stage Cencibel wines had the lowest amounts of flavonols (59.1 mg/l in total) with myricetin 3-glucoside as the main compound, whereas Syrah wines had an intermediate content of flavonols (85.0 mg/l in total), characterised by high amounts of the 3-glucosides of both myricetin and quercetin. The little and frequently partial data for flavonol content in red wine show that flavonols ranged from traces to 60 mg/l, as the aglycone quercetin (Burns et al., 2000; McDonald et al., 1998; Singleton, 1988; Vuorinen, Määttä, & Törrönen, 2000). In contrast, the flavonol content of red grapes can be as high as 600 mg/kg, as the glycoside forms (Andrade et al., 2001).

During ageing, a decrease in the concentration of flavonol glycosides was noted (Table 5 and Fig. 6). The decrease was moderate for Cabernet Sauvignon and Syrah wines after 3 months (87% and 79% of the initial amounts still remained, respectively), but it was strong for Cencibel wines (39% of the initial amount remained). The amounts of flavonol glycosides dramatically decreased after 9 months of ageing for Cabernet Sauvignon and Cencibel wines (only 22% and 19% of the initial amounts remained, respectively), and decreased to one half of the initial amount for Syrah wines. These losses of flavonol glycosides cannot be explained only by the hydrolysis of glycosidic linkages, because no parallel increase in the amounts of the corresponding aglycones was observed, at least to the expected extent. It is necessary to invoke other kinds of reactions (condensation, oxidation reactions) involving flavonol glyco-

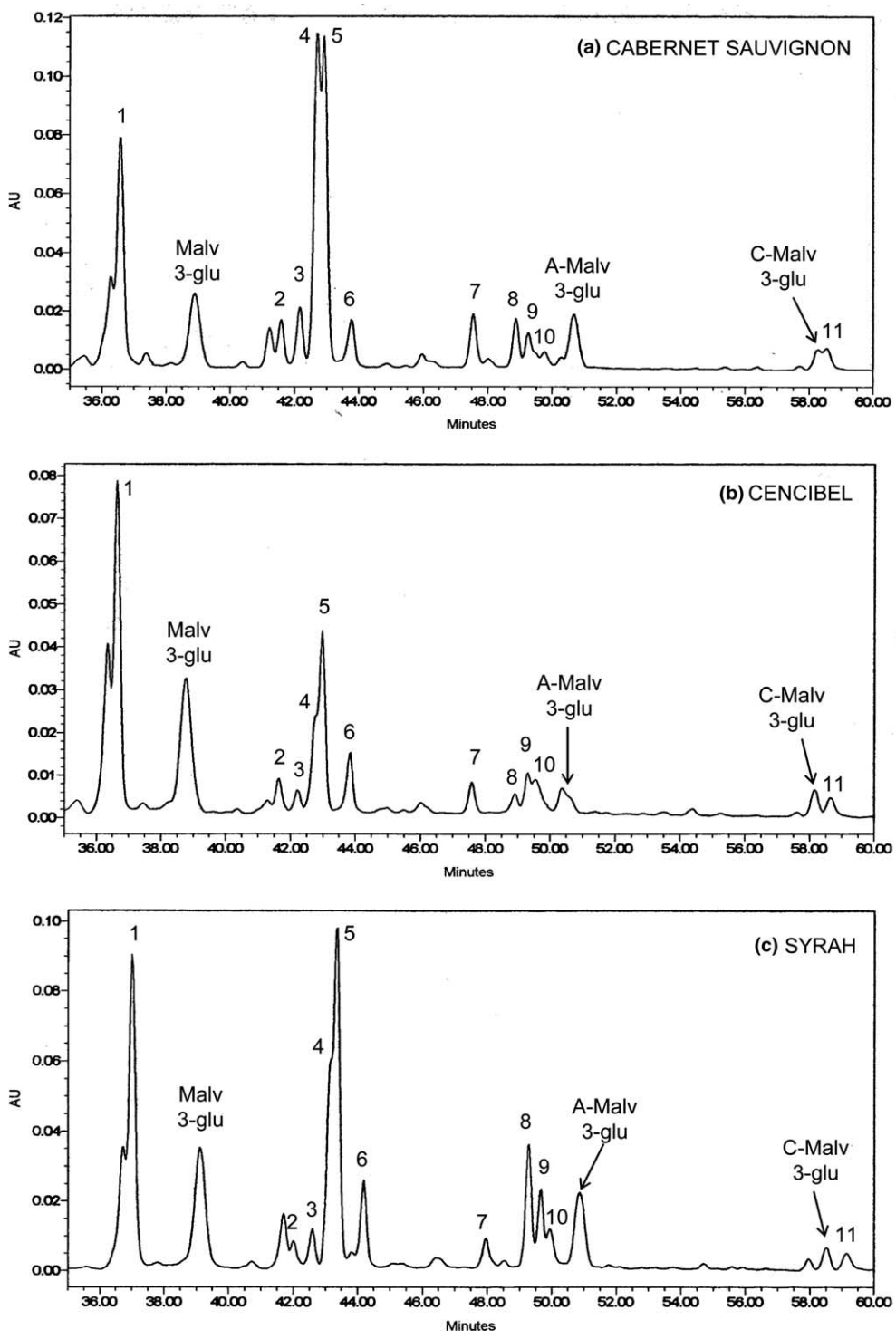

Fig. 5. Chromatographic profiles obtained at 360 nm for the three different single cultivar wines, just at the end of alcoholic fermentation: Cabernet Sauvignon (a), Cencibel (b), and Syrah (c). Flavonol peaks (1–11) are assigned as in Table 4. Also shown are the peaks assigned to the derivatives of the main anthocyanins (peak assignation as in Fig. 1).

sides or even their corresponding aglycones after hydrolysis.

The proportions of the three more abundant flavonol glycosides changed during ageing, decreasing for quercetin 3-glucuronide and 3-glucoside, and increasing for myricetin 3-glucoside (Table 6). However, it was possible to differentiate our wines according to their cultivar on the basis of their flavonol profiles, within the time of ageing checked. Two of the three more abundant flavonol glycosides found, myricetin 3-glucoside and quercetin

Table 4
Peak assignation, average of the retention times, and absorption maxima in the UV–Vis region, for the flavonol peaks found in the chromatograms obtained at 360 nm, and the same data corresponding to standards

Peak number	Flavonol assignation	Retention time (min)	Absorption maxima[a] (nm) of separated peaks found in samples	Absorption maxima[a] (nm) of standards under HPLC conditions
1	Myricetin 3-glucoside	36.73	256 (brd), 300 (sh), 353	NCA
2	Rutin	41.73	254, 265 (sh), 300 (sh), 354	255, 263 (sh), 292 (sh), 352
3	Quercetin 3-glucosylgalactoside	42.31	254, 265 (sh), 300 (sh), 355	NCA
4	Quercetin 3-glucuronide	42.91	255, 263 (sh), 293 (sh), 352	NCA
5	Quercetin 3-glucoside	43.06	255, 263 (sh), 293 (sh), 352	255, 263 (sh), 293 (sh), 352
6	Quercetin 3-glucosylxyloside	43.92	253, 265 (sh), 293 (sh), 356	NCA
7	Kaempferol 3-glucosylarabinoside	47.69	263, 290 (sh), 346	NCA
8	Kaempferol 3-glucoside	49.01	263, 290 (sh), 347	263, 293 (sh), 345
9	Isorhamnetin 3-glucoside	49.40	253, 264 (sh), 295 (sh), 352	253, 265 (sh), 293 (sh), 352
10	Myricetin	49.54	250, 265 (sh), 300 (sh), 370	252, 265 (sh), 302 (sh), 371
11	Quercetin	58.73	254, 265 (sh), 297 (sh), 369	254, 265 (sh), 300 (sh), 369
	Kaempferol	64.70	ND	263, 293 (sh), 318 (sh), 364
	Isorhamnetin	65.85	ND	253, 265 (sh), 298 (sh), 369

[a] brd, broad maximum; sh, shoulder over a maximum; ND, not detected; NCA, not commercially available.

3-glucuronide, could always distinguish the single cultivar wines considered. The third more abundant flavonol glycoside, quercetin 3-glucoside, was found in higher proportions in the French cultivar wines (Cabernet Sauvignon and Syrah) than in the Spanish cultivar wine (Cencibel), and all the three wines could be distinguished just at the end of the alcoholic fermentation. Other minority flavonols contributing to cultivar differentiation in these wines were: kaempferol 3-glucoside, isorhamnetin 3-glucoside, and the aglycone myricetin, only at the end of alcoholic fermentation; and the aglycone quercetin up to 3 months of ageing. Recent studies have demonstrated that flavonol profiles serve as a differentiation tool for table grape cultivars (Cantos et al., 2002) and characterisation of some flavonols found in different red wine grape cultivars has also been achieved (Andrade et al., 2001), although former studies did not find consistent differences between cultivars of red wine grapes (Di Stefano, Foti, & Borsa, 1993). To our knowledge, this is the first time that the complete flavonol profile of Cencibel wines has been described, and we have not found many studies where the flavonol profiles have been used as a differentiating characteristic for red wines according to either their grape cultivar or their geographical origin (Dugo, Giuffrida, Salvo, Alfa, & Pellicano, 2002; McDonald et al., 1998; Rodríguez Delgado, González Hernández, Conde González, & Pérez Trujillo, 2002). The above results suggest that flavonol profiles could be used as a general chemical indicator for single cultivar wines, as is well established for the anthocyanins profiles, but many more wine samples, of a wider diversity of cultivars, need to be analysed to confirm such a supposition.

3.2. Copigmentation effects on wine colour

At the end of the alcoholic fermentation, the degrees of anthocyanin polymerisation (%polymerisation) were similar for the three kinds of wines (Table 7). In contrast, the contribution of copigmented anthocyanins to the total wine colour (%copigmentation) was quite variable. Percentage copigmentation is a measure of the effect of copigmentation on the enhancement of the red colour intensity of the wine. Before making an attempt at establishing a relationship between %copigmentation and the content of monomeric anthocyanins of a red wine, it must be bore in mind that the content of the main monomeric anthocyanin found in grapes of *Vitis vinifera* and their wines, malvidin 3-monoglucoside, surprisingly seemed to have a poor correlation with the level of copigmentation measured in varietal wines from the Napa Valley (including Cabernet Sauvignon and Syrah wines), and a better correlation was found when this single anthocyanin was subtracted from the total monomeric anthocyanin content (Vaadia, 1997).

The highest values for %copigmentation, at the end of alcoholic fermentation, corresponded to Syrah and Cabernet Sauvignon wines. In the first case, these wines also showed the highest contents of monomeric anthocyanins, even when the content of malvidin 3-monoglucoside was not considered (Fig. 2, dotted lines). In contrast, the lower content of monomeric anthocyanins for Cabernet Sauvignon wines looked to be balanced by the highest content of flavonols (Table 5), the best kind of copigmentation cofactors (Baranac et al., 1996, 1997a, 1997b). Cencibel wines had similar contents of monomeric anthocyanins to Cabernet Sauvignon wines, but the total of monomeric anthocyanins, excluding malvidin 3-monoglucoside, was lower for the former wine (250 vs. 372 mg/l, Fig. 2); in addition, Cencibel wines showed the lowest content of flavonols (Fig. 6). Both circumstances could explain why Cencibel wines had the lowest values for %copigmentation at this time.

During ageing, an increase in %polymerisation and a decrease in %copigmentation were observed for all the wines, the latter being dramatic after 9 months of ageing

Table 5
Contents of flavonols (mg/l)[A], for the single cultivar red wines at the end of alcoholic fermentation (EAF) and after ageing

Flavonol	Ageing time	Cabernet Sauvignon ($n = 3$)		Cencibel ($n = 3$)		Syrah ($n = 4$)	
		MV	SD	MV	SD	MV	SD
Myricetin 3-glucoside[B]	EAF	19.8	3.0	21.6	1.1	20.3	2.3
	3 months	17.8[a]	0.5	7.7[b]	0.5	17.3[a]	1.9
	9 months	5.6[a]	0.8	4.8[a]	0.1	11.6[b]	0.8
Rutin	EAF	6.3[a]	0.4	3.5[b]	0.6	3.8[b]	0.2
	3 months	4.6[a]	1.0	0.5[b]	0.2	1.3[b]	1.5
	9 months	2.2[a]	1.2	0.2[b]	0.2	0.2[b]	0.0
Quercetin 3-glucosylgalactoside[C]	EAF	8.4[a]	0.4	2.4[b]	0.3	4.0[c]	0.7
	3 months	6.9[a]	0.1	1.0[b]	0.1	2.9[c]	0.6
	9 months	1.2[a]	0.3	0.3[b]	0.0	1.6[a]	0.3
Quercetin 3-glucuronide[B]	EAF	25.6[a]	0.7	5.2[b]	0.6	9.8[c]	1.4
	3 months	19.3[a]	1.2	1.4[b]	0.1	5.9[c]	0.8
	9 months	4.1[a]	0.5	0.5[b]	0.1	4.4[a]	0.7
Quercetin 3-glucoside	EAF	25.6[a]	1.7	10.3[b]	0.3	18.4[c]	2.4
	3 months	24.6[a]	2.2	3.7[b]	0.2	16.8[c]	2.6
	9 months	5.1[a]	0.7	2.0[b]	0.1	8.0[c]	1.2
Quercetin 3-glucosylxyloside[C]	EAF	8.1[a,b]	0.8	6.6[a]	0.4	9.1[b]	1.0
	3 months	7.4[a]	0.2	2.7[b]	0.2	7.9[a]	0.6
	9 months	2.0[a]	0.2	1.7[a]	0.0	6.3[b]	0.7
Kaempferol 3-glucosylarabinoside[C]	EAF	8.5[a]	0.6	3.1[b]	0.2	3.1[b]	0.5
	3 months	5.5[a]	3.1	1.3[b]	0.1	2.0[b]	0.6
	9 months	1.4[a]	0.2	0.5[b]	0.0	1.1[a]	0.3
Kaempferol 3-glucoside	EAF	5.0[a]	0.5	1.3[b]	0.1	9.1[c]	1.5
	3 months	5.0[a]	0.4	0.6[b]	0.0	5.9[a]	1.7
	9 months	1.2[a]	0.1	0.6[a]	0.4	4.5[b]	0.5
Isorhamnetin 3-glucoside	EAF	1.9[a]	0.2	2.1[a]	0.2	4.5[b]	0.7
	3 months	2.4[a]	0.5	1.5[a]	0.1	5.0[b]	1.0
	9 months	1.0[a]	0.1	0.6[a]	0.1	3.3[b]	0.3
Myricetin	EAF	0.5[a]	0.1	1.6[b]	0.1	1.4[b]	0.2
	3 months	0.2	0.1	0.2	0.1	1.1	1.1
	9 months	0.1[a]	0.0	0.0[b]	0.0	0.3[c]	0.0
Quercetin	EAF	4.3[a]	1.2	1.4[b]	0.2	1.4[b]	0.2
	3 months	5.4[a]	0.6	2.7[b]	0.3	1.0[c]	0.1
	9 months	0.0[a]	0.0	0.0[a]	0.0	0.1[b]	0.0

[a,b,c] Different superscripts in the same row mean significant differences ($\alpha = 0.05$) according to the test of Student–Newman–Keuls.
[A] Mean values (MV) and standard deviations (SD).
[B] As quercetin 3-glucoside.
[C] As rutin.

(Table 7). The increase in %polymerisation is in agreement with the already observed losses of monomeric anthocyanins (Fig. 2). It is well known that the main reaction involving monomeric anthocyanins is the formation of polymeric red pigments through condensation with flavan-3-ols, and this reaction can be mediated and accelerated by acetaldehyde. Moreover, today the formation of a new kind of anthocyanin-derived pigments, the so-called pyranoanthocyanins, is well recognised. These are resistant to bisulphite bleaching. Pyranoanthocyanins are generated by reaction between monomeric anthocyanins and wine compounds having a polarised double bond, as do some secondary yeast metabolites (pyruvic acid), 4-vinylphenols, 8-vinylflavanols, and hydroxycinnamic acids (Fulcrand, Benabdeljalil, Rigaud, Cheynier, & Moutounet, 1998; Fulcrand, Cameira dos Santos, Sarni-Manchado, Cheynier, & Favre-Bonvin, 1996; Hayasaka & Asenstorfer, 2002; Mateus et al., 2003; Schwarz et al., 2003; Schwarz, Jerz, & Winterhalter, 2003). As has been previously outlined in this discussion, some evidence of the presence of pyranoanthocyanins was found in the wine chromatograms (Fig. 1). The decrease in %copigmentation is also in agreement with the parallel losses of monomeric anthocyanins, due to their unique ability in forming copigmentation complexes.

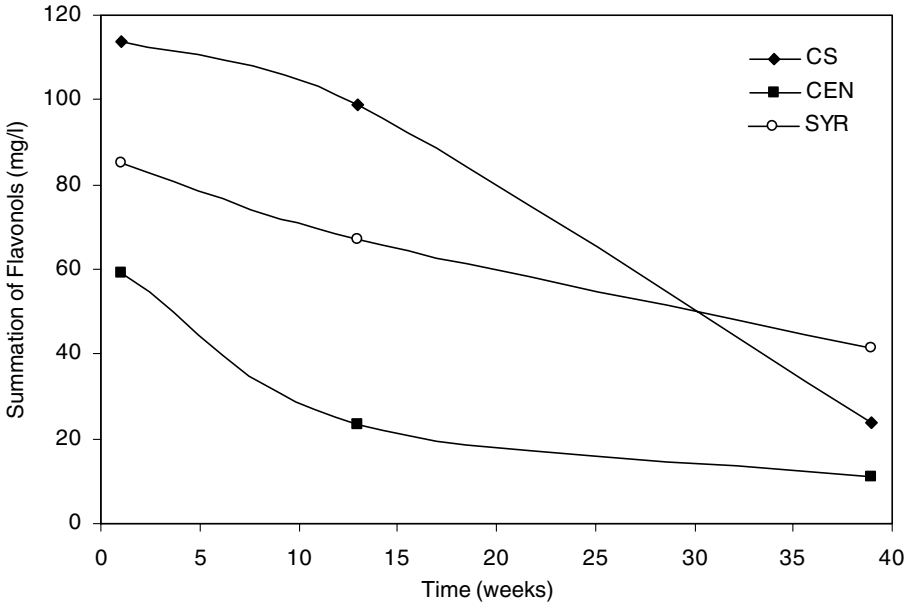

Fig. 6. Evolution during ageing of the total content of flavonols for Cabernet Sauvignon (CS), Cencibel (CEN), and Syrah (SYR) wines.

Although Cencibel wines suffered proportionally the smallest loss of monomeric anthocyanins after 3 months of ageing (78% of monomeric anthocyanins remained, Fig. 2), they also suffered proportionally the greatest loss of flavonols (only 39% of the initial flavonols remained, Fig. 6), and so they showed the lowest values for %copigmentation at that time. The decrease of %copigmentation for cabernet sauvignon wines after 3 months of ageing (from 43% to 25%, the percentage of decrease of %copigmentation was 42%) was proportionally more important than those for cencibel wines (from 32% to 20%, the percentage of decrease of %copigmentation was 38%), and even more when compared to syrah wines (from 44% to 34%, the percentage of decrease of %copigmentation was 23%). An explanation could be suggested attending to the other copigmentation cofactors present in the wines studied, that may act as a remaining pool for replacing the disappeared flavonols in the copigmentation complexes after 3 months of ageing. *p*-Coumaric acid was found in lower amounts in Cabernet Sauvignon wines than in Syrah wines, and the amounts found in Cencibel wines were even higher (Table 3). On the other hand, Cencibel wines showed an increase in the content of (−)-epicatechin (Table 3). The enhancement of %copigmentation in red wines has been reported when *p*-coumaric acid was added at the pre-fermentative step of the winemaking process (Bloonfield et al., 2003) and (−)-epicatechin has been considered as a good copigmentation cofactor, in contrast to the rest of the flavan-3-ols (Liao et al., 1992).

After 9 months of ageing, the effect of copigmentation on colour intensity (%copigmentation) was only observable in Syrah wines (Table 7). This wine maintained (at this time) the highest contents of both monomeric anthocyanins and flavonols (Figs. 2 and 6). It has been suggested that a minimum concentration of monomeric anthocyanins (around 35 mM, that is 18.5 mg/l as malvidin 3-monoglucoside) is necessary for detection of copigmentation effects (Asen & Jurd, 1967; Jurd & Asen, 1966). The amounts of monomeric anthocyanins, after this time of ageing, were clearly higher than this minimum value in all the wines, even when malvidin 3-monoglucoside was not considered in the summations of monomeric anthocyanins (Fig. 2, dotted lines). The reason for the lack of copigmentation in Cabernet Sauvignon and Cencibel wines at this time might be the dramatic decreases in their contents of copigmentation cofactors (Tables 3 and 5). As the copigmentation effects largely depend on the molar ratio of cofactor to pigment, it seems that the low amounts of copigmentation cofactors, especially those of flavonols, could be insufficient for detecting copigmentation effects at this ageing time.

In addition to the enhancement of the red colour intensity, copigmentation normally also induces a bathochromic shift in young red wine colour, leading to a more bluish hue. The CIELAB parameter h^* is also known as the hue angle and takes values from 0 to 360: the h^* values of 0 and 360 are equivalent and correspond to purple-red colour; h^* values higher than 0 (up to approximately 15) mean that the bluish hue (purple) of the purple-red colour gradually decreases and the colour changes to red; h^* values lower than 360 (up to approximately 345) mean that the bluish hue of the purple-red colour increases and the

Table 6
Characteristic flavonol profiles (molar percentage of every individual flavonol, calculated from the amounts shown in Table 5)[A] of the single cultivar red wines, at the end of alcoholic fermentation (EAF) and after ageing

Flavonol	Ageing time	Cabernet Sauvignon ($n = 3$)		Cencibel ($n = 3$)		Syrah ($n = 4$)	
		MV	SD	MV	SD	MV	SD
Myricetin 3-glucoside	EAF	17.5[a]	1.60	37.4[b]	0.68	23.7[c]	0.44
	3 months	18.2[a]	0.10	32.5[b]	1.34	26.0[c]	1.08
	9 months	23.9[a]	1.56	45.0[b]	1.98	28.1[c]	1.01
Rutin	EAF	4.26	0.55	4.58	0.71	3.45	0.42
	3 months	3.57	0.68	1.56	0.56	1.52	1.86
	9 months	7.23[a]	4.47	1.09[b]	1.10	0.45[b]	0.05
Quercetin 3-glucosylgalactoside	EAF	5.70[a]	0.19	3.10[b]	0.25	3.52[c]	0.19
	3 months	5.33[a]	0.09	3.15[b]	0.12	3.33[b]	0.35
	9 months	3.82[a]	0.61	2.33[b]	0.55	2.99[a,b]	0.28
Quercetin 3-glucuronide	EAF	22.9[a]	1.96	9.06[b]	0.66	11.4[b]	1.41
	3 months	19.7[a]	1.83	5.90[b]	0.36	8.81[c]	0.86
	9 months	17.4[a]	0.82	4.49[b]	0.63	10.7[c]	1.24
Quercetin 3-glucoside	EAF	22.8[a]	0.17	18.0[b]	0.38	21.5[c]	0.59
	3 months	25.0[a]	1.42	15.4[b]	0.57	25.1[a]	1.76
	9 months	21.8	1.51	18.7	1.97	19.2	1.05
Quercetin 3-glucosylxyloside	EAF	5.47[a]	0.20	8.67[b]	0.39	8.13[b]	0.32
	3 months	5.71[a]	0.05	8.58[b]	0.29	9.04[b]	0.50
	9 months	6.38[a]	0.15	11.6[b]	0.75	11.6[b]	0.28
Kaempferol 3-glucosylarabinoside	EAF	5.77[a]	0.09	4.15[b]	0.18	2.76[c]	0.18
	3 months	4.23	2.26	4.09	0.18	2.30	0.60
	9 months	4.55[a]	0.34	3.69[a]	0.71	2.00[b]	0.37
Kaempferol 3-glucoside	EAF	4.57[a]	0.19	2.42[b]	0.13	11.0[c]	0.54
	3 months	5.32[a,b]	0.56	2.71[a]	0.20	9.32[b]	2.93
	9 months	5.19[a]	0.22	4.41[a]	3.01	11.4[b]	0.38
Isorhamnetin 3-glucoside	EAF	1.63[a]	0.07	3.54[b]	0.20	5.14[c]	0.24
	3 months	2.34[a]	0.52	6.04[b]	0.22	7.29[b]	0.83
	9 months	4.17[a]	0.03	5.25[a]	0.70	7.76[b]	0.59
Myricetin	EAF	0.65[a]	0.06	4.07[b]	0.38	2.46[c]	0.21
	3 months	0.35	0.08	1.37	0.41	2.25	2.14
	9 months	0.66[a]	0.04	0.52[a]	0.29	1.14[b]	0.14
Quercetin	EAF	5.81[a]	1.21	3.67[b]	0.20	2.48[c]	0.12
	3 months	8.42[a]	1.24	17.3[b]	1.51	2.42[c]	0.43
	9 months	0.19	0.01	0.33	0.25	0.29	0.04

[a,b,c] Different superscripts in the same row mean significant differences ($\alpha = 0.05$) according to the test of Student–Newman–Keuls.
[A] Mean values (MV) and standard deviations (SD).

colour changes to purple. The values found for the colorimetric parameter h^* indicated that such a bathochromic shift was clearly appreciable for Cencibel and Syrah wines (Table 7), and was weaker for Cabernet Sauvignon wines. The increase in anthocyanin polymerisation during ageing, and the subsequent decrease in the degree of copigmentation, led to clearer wines (higher values of L^*), with less colour purity (lower values of C^*), and with less bluish hues, although Syrah wines could still be described as purple-red. The differences in the colorimetric parameters C^* and h^*, allow differentiation of the wines according to the cultivar employed, at the end of the alcoholic fermentation (Table 7). The colorimetric differences (ΔE^*) calculated for every pair of wines from different cultivars, ranged for 10–20 CIELAB units at this time. These colorimetric differences can be considered as visually detectable, because these values are higher than the estimation of 3.0 CIELAB units as the acceptable tolerance for the human eye in distinguishing the colour of red wines, when trained tasters use standardised wine-tasting glasses (Martınez, Melgosa, Pérez, Hita, & Negueruela, 2001). These colorimetric differences remained almost unchanged after 3 months of ageing ($\Delta E^* = 5$–11) and after 9 months of ageing ($\Delta E^* = 8$–11), but the values of C^* and h^* were unable to totally differentiate wines from different cultivars at those ageing times.

Table 7
Contribution to the total wine colour at pH 3.6 of copigmented anthocyanins (%copigmentation), degree of polymerised anthocyanins (%polymerisation), and chromatic characteristics (CIELAB) at wine pH, for the single cultivar red wines at the end of alcoholic fermentation (EAF) and during ageing

Colour characteristic[A]	Ageing time	Cabernet Sauvignon ($n = 3$)		Cencibel ($n = 3$)		Syrah ($n = 4$)	
		MV	SD	MV	SD	MV	SD
%Copigmentation	EAF	42.6[a]	3.1	32.0[b]	1.2	44.2[a]	1.3
	3 months	24.5[a]	1.6	20.3[b]	1.5	34.3[c]	1.9
	9 months	ND		ND		5.0	5.4
%Polymerisation	EAF	20.3	0.8	19.9	0.5	19.9	0.6
	3 months	42.4[a]	3.1	27.6[b]	1.0	26.3[b]	1.9
	9 months	44.4[a]	2.2	32.5[b]	2.7	30.4[b]	5.0
L^*	EAF	48.00[a]	1.97	55.98[b]	1.76	46.53[a]	3.11
	3 months	53.40	2.97	54.97	0.23	49.60	5.34
	9 months	55.72	3.04	62.00	1.97	57.78	2.59
C^*	EAF	53.28[a]	1.08	43.91[b]	1.56	61.12[c]	1.77
	3 months	40.43[a]	1.48	37.32[a]	1.47	45.80[b]	3.33
	9 months	38.15[a]	0.70	33.19[b]	1.00	22.72[a]	3.24
h^*	EAF	0.53[a]	0.79	348.10[b]	0.30	354.00[c]	1.70
	3 months	4.43[a]	4.96	358.33[b]	0.55	353.87[b]	1.76
	9 months	18.74[a]	3.63	22.72[a]	3.24	7.61[b]	5.42

[a,b,c] Different superscripts in the same row mean significant differences ($\alpha = 0.05$) according to the test of Student–Newman–Keuls. ND = not detected.
[A] Mean values (MV) and standard deviations (SD).

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