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Evolution of Flavanols, Anthocyanins, and Their Derivatives during the Aging of Red Wines Elaborated from Grapes Harvested at Different Stages of Ripening

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Several red wines were elaborated to assess the effect of the degree of grape ripening on wine color and on the levels of flavanol and anthocyanin compounds, which are chiefly responsible for the wine color attributes. Two different cultivars and three different degrees of ripening were studied in two consecutive years. The wines were aged for 1 year in American oak barrels of medium-high char followed by 6 months in the bottle. The results showed that the wines made from more mature grapes had generally higher free anthocyanin content, and during aging the decrease of the free anthocyanins and flavanols took place in conjunction with an increase in the levels of the anthocyanin derivatives or "new pigments", which are responsible for maintaining color intensity and adding violet hues in aged wines. From the results obtained, it seems to be clear that the characteristics and composition of grapes harvested later (7−8 days in this region and for these varieties) than the usual time are quite beneficial to obtaining quality aged wines. The phenolic composition of wines made from the last harvested grapes is mainly responsible for the stability of their color, which is extremely important in product acceptance with a significant increase hence of product quality.

KEYWORDS: Aging; anthocyanins; color; flavanols; red grapes; red wines; ripening

INTRODUCTION

Phenolic compounds are one of the major quality factors in the grape and then in the resulting wine. Many factors may influence the phenolic composition of wines, such as grape variety, edaphoclimatic conditions, and cultural and technological practices.

One of these factors can also be the maturity degree of the grapes used. Habitually, winemakers have used technological parameters such as berry weight, sugar content, and acidity of the grapes to determine the best harvest moment (*1*−*4*). However, these indices are not enough to obtain quality wines because their evolution does not coincide with the trend of some other important compounds (*3*, *5*). Therefore, nowadays more and more winemakers are also considering other grape characteristics such as the content of polyphenols, mainly due to their contribution to wine color and to other sensorial characteristics of wines such as bitterness and astringency (*6*).

Most of the polyphenols are highly unstable and are quickly transformed into several pigments, and various types of reactions occur during the aging process of wines. Among these reactions, oxidation processes that give rise to the formation of orangish yellow pigments, and copigmentation, cycloaddition, and condensation or polymerization reactions that stabilize the red-violet coloring, are especially important. The principal reactions that may take place comprise: (a) condensation reactions of flavanols directly with other flavanols and with anthocyanins (*7*, *8*); (b) acetaldehyde-mediated condensation reactions of flavanols with other flavanols and with anthocyanins, resulting in more stable compounds (*9*−*12*); and (c) condensation reactions of anthocyanins and/or flavanols with other lower molecular weight compounds such as pyruvic acid, vinylphenol, or glyoxylic acid (*13*−*17*) to form what are termed "new pigments", which are much more stable and maintain wine color intensity for longer periods (*12*, *18*). In addition, more recently new kinds of blue anthocyanin pigments were detected in red wines (*19*), which result from the reaction between anthocyanin−pyruvic acid adducts and vinyl-flavanols. All of these reactions not only modify wine color but also bring about changes in such other attributes as astringency and bitter flavors, which decrease and thereby round out or soften the wine.

The development of the phenolic compounds, especially anthocyanins and flavanols, during the ripening period has previously been investigated by several authors (*20*−*24*). However, no papers have been found about the influence of grape maturity degree on the final aged wine quality. Besides, in the experience of some winemakers, more mature grapes generally tend to yield wines that are more stable in color and have more or stronger violet hues. However, there is no published work dealing with these findings in the scientific

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literature. It is assumed that the source of this behavior is ascribable basically to changes in the composition of the phenolic substances present in the grapes as a result of ripening or to changes in cell permeability or similar processes that increase the extraction yields of phenols.

In view of the foregoing, the main object of this study was to assess the effect of the degree of grape ripening on wine composition and quality and on wine behavior during aging. Two sorts of red grape varieties, Tinto Fino and Cabernet Sauvignon, were used to obtain red varietal wines made from grapes at three different degrees of ripening. The study was performed in two consecutive years and focused on wine color, which is clearly and directly related to wine quality, and on the levels of flavanols, anthocyanins, and derivatives of both types of compounds, which are chiefly responsible for the wine color attributes. Wines were assayed immediately after fermentation and at different times during aging for 1 year in American oak barrels followed by 6 months in the bottle.

MATERIALS AND METHODS

Samples. Wines were made from the grapes of two different red varieties, Tinto fino (TF) and Cabernet Sauvignon (CS), grown on adjacent plots on a vineyard registered with and operating under the "Ribera del Duero" Designation of Origin located within the limits of the municipality of Peñafiel (Valladolid, Spain).

The grapes were harvested at three different stages of ripening. The first stage was the conventional harvest time based mainly on sugar content, also taking into account acidity and grape sanitary condition. In the studied region, acidity level should be monitored to avoid problems related to low acidity values, whereas content of sugar is not usually a limiting factor. The wines made from grapes harvested at this first ripening stage have been designated TF-1 and CS-1, respectively, according to the grape variety. The other two harvest dates were 1 week and 2 weeks after the conventional date, and the wine batches were designated TF-2, CS-2, TF-3, and CS-3, respectively. During this time the grapes were observed to remain turgid, and senescence did not set in. In addition, during the further 2-week period there was no significant rainfall and temperature and light levels were suitable for continued ripening.

The harvest mode was as follows: the whole plots of every studied variety were divided in three parts, and one of them was collected at every harvest date.

Because the vegetative cycles of the two grape varieties employed differed, the harvest dates for each of the varieties were different. In the studied area the native variety (TF) ripens earlier than the French variety (CS), with habitual differences of 2−3 weeks.

All of the wines, for each variety at each ripening stage, were made in duplicate batches in stainless steel vats with a capacity of 1200 kg using traditional red winemaking methods. After being harvested by hand and sorted to remove damaged grapes, the grapes were destemmed and transferred to the vats for controlled fermentation at a temperature of between 25 and 28 °C with the addition of a small amount of SO_2 (0.04 g/L). The total polyphenol index (TPI as measured by absorbance at 280 nm) and changes in density were monitored daily during fermentation. The wines were drawn off at the peak TPI level or, more precisely, when a continuous drop in the TPI was recorded, which coincided with nearly complete consumption of the reducing sugars (at a density value of ~1000). The maceration time was ~14 days, with slight differences in time for each wine (variety, maturity degree, and vintage). Alcoholic fermentation was deemed to be over when the sugar level was <3 g/L, at which point the wines were racked and transferred into barrels where malolactic fermentation and wood aging were carried out. During this phase racking and transfer of wine from one barrel to another was carried out periodically (approximately every 4 months).

The wines were kept in barrels made from new medium-high char American oak for 12 months. After 1 year in the wood, the wines were bottled and allowed to continue aging in the bottle in dark cellars at controlled temperature and humidity levels during 6 months.

This study was carried out using the grapes from 1999 and 2000 vintages.

Analytical Methods. All of the analyses described below were carried out in duplicate, and the ones related to wine were carried out periodically during wine aging. Samples were taken at 0, 2, 8, and 12 months after being in wood barrels and the last one after 18 months of aging (12 months in wood and 6 months in bottle).

Sampling, Skin Extraction, and Analyses of Grapes and/or Musts. Representative samples of grapes (~2 kg) were collected at random from several vines and from different parts of various clusters. The total weight of 100 berries and the weight of the seeds and skins of them were obtained, and the solids/juice ratio was calculated. This coefficient correlated the weight of the solid part of the grapes (skins and seeds) with the volume of must obtained by pressing the pulp.

Two samples of 50 berries were used for the skin extraction process, following the method proposed by Izcara and González-Sanjosé (*25*). Total phenols were analyzed in these extracts following the methods set out in Paronetto (*26*).

Sugar content, pH, and titratable acidity were measured in the must obtained after the rest of the grapes had been crushed, and they were determined using the OIV methods (*27*).

HPLC-DAD and HPLC-MS Analyses of Anthocyanins. The content of the individual anthocyanins was determined by directly injecting wine filtered through filters with a pore size of 0.45 μm (Millipore, Bedford, MA) on a Beckman HPLC with System Gold software. The chromatographic separation was carried out on a Spherisorb ODS2 column (150 mm × 4.6 mm i.d., 5 μm particle size) and a guard column of the same material. The solvents were (A) water/formic acid (9:1) and (B) methanol/water/formic acid (45:45:10). The gradient was linear at a flow rate of 0.8 mL/min from 35 to 95% solvent B for 20 min, from 95 to 100% B for 5 min, and then isocratic for 5 min. Detection was carried out at two different wavelengths—530 and 313 nm. Peak identification was carried out by using the elution times, the absorbance relationship 530/313 (*28*), and a Hewlett-Packard model HP 1100 LC-MS liquid chromatograph applying the conditions established by Revilla et al. (*15*).

Because standards for all of these compounds were not available, all anthocyanin compounds have been expressed as malvidin 3-glucoside equivalents (mg/L of Mv-3-glu), the primary pigment in both grape varieties. The calibration line was constructed using Mv-3-glu from Extrasynthèse (Lyon, France).

Fractionation and HPLC-DAD and HPLC-MS Analyses of Flavanols and Their Derivatives. Because of the large number of phenolic compounds present in wines and the low levels of some of them, the determination of the flavan-3-ol monomers and polymers required preliminary separation and concentration steps. Column separation was performed on Amberlite XAD-2 resin (Sigma-Aldrich, St. Louis, MO) with a particle size of 150−250 μm according to the method of DiStefano and Cravero (*29*) with certain slight modifications detailed in a previous work (*30*). The flavanol monomers were identified in the ether fraction, whereas the flavan-3-ol derivatives (with higher molecular weights) were extracted using ethyl acetate. These two obtained fractions were evaporated to dryness in a rotary vacuum evaporator (T < 35 °C) and immediately redissolved in a known volume of methanol (2 mL). The extracts obtained were analyzed using the Beckman HPLC for peak quantification and the HP 1100 LC-MS for mass detection according to a slightly modified version of the method described by Pérez-Magariño et al. (*31*) designed to improve component separation. A Spherisorb ODS2 column (250 mm × 4.6 mm i.d., 3 μm particle size) and a guard column of the same material were used, and the solvents were 4.5% formic acid in water (solvent A) and solvent A/acetonitrile (9:1) (solvent B). The gradient was linear at a flow rate of 0.7 mL/min, from 0 to 50% solvent B for 40 min; from 50 to 100% B for 50 min; and isocratic conditions for a further 20 min.

Flavan-3-ols, catechin, and epicatechin were extracted mainly in the first fraction; small amounts also were present in the second one. These components were expressed as milligrams per liter of catechin and epicatechin, respectively, applying their calibration line constructed using standards from Extrasynthèse (Lyon, France). The flavan-3-ol

Table 1. Parameters of the Two Grape Cultivars in the Three Harvest Stages and from the Two Vintages

	1999 vintage[a]					
parameter	TF-1	TF-2	TF-3	CS-1	CS-2	CS-3
wt of 100 berries (g)	130	147	153	88	100	105
solids/juice ratio	0.48	0.37	0.47	0.60	0.54	0.74
sugar content (g/L)	225	240	242	225	238	240
TAc[b] (g/L tartaric acid)	6.2	5.6	4.6	7.8	7.6	6.4
pH	3.55	3.77	3.79	3.36	3.36	3.47
TPhe[c] (mg/100 berries)	234	245	246	190	205	206

	2000 vintage[a]					
parameter	TF-1	TF-2	TF-3	CS-1	CS-2	CS-3
wt of 100 berries (g)	135	145	151	91	97	100
solids/juice ratio	0.50	0.38	0.45	0.63	0.53	0.76
sugar content (g/L)	233	245	249	231	232	244
TAc[b] (g/L tartaric acid)	5.7	5.9	4.4	7.8	7.4	6.6
pH	3.62	3.73	3.74	3.47	3.50	3.51
TPhe[c] (mg/100 berries)	221	242	243	192	202	200

[a] TF, Tinto Fino; CS, Cabernet Sauvignon; 1, 2, and 3, harvest stage. [b] Titratable acidity. [c] Total polyphenols in the skin.

derivatives, catechin and/or epicatechin dimers, trimers, and tetramers, coumaric acid derivatives, and galloylated derivatives were expressed as catechin equivalents (milligrams per liter).

Milli-Q water, formic acid (Merck, Darmstadt, Germany), and methanol and acetonitrile (Lab-Scan, Dublin, Ireland) were used in all HPLC measurements.

Color Measurements. The wine color assays were performed by calculating the Glories color index parameters (*32*), which are widely used by wineries and readily interpretable. The parameters are color intensity, tonality, percentage yellow, percentage red, and percentage blue. Spectrophotometric absorbance readings were taken at 420, 520, and 620 nm using a Beckman model DU-650 diode array spectrophotometer (Las Rozas, Spain) with quartz cuvettes with a path length of 1 mm.

Statistical Analyses. Statistical analysis of the data was carried out using analysis of variance (ANOVA) and the least significant difference (LSD) test to determine statistically different values at a significance level of $\alpha = 0.05$.

All statistical analyses were performed using the Statgraphics Plus 4.0 statistical package for Windows (*33*).

RESULTS AND DISCUSSION

Table 1 shows the data of the two grape varieties studied at different harvest dates selected and from the two vintages considered. It was observed that the weight of berries increased gradually in the last 2 weeks, especially during the first additional week. This fact was directly correlated with the increase of grape volume and of sugar levels in the pulp.

During the last 2 weeks, acidity decreased, mainly during the second additional week, giving levels below the recommendation levels for wine quality in TF grapes. Accumulation of skin phenols continued at least during the first additional week in both studied varieties.

An HPLC chromatogram of aged wine flavanol monomers, polymers, and other derivatives detected at 280 nm is shown in **Figure 1**. The catechin was isolated in the first column separation fraction, whereas epicatechin was distributed between the first and second column separation fractions.

Figure 2 shows the HPLC chromatogram recorded at 530 nm of the individual anthocyanins of aged wine. Nineteen compounds were identified: five anthocyanin glucosides (peaks 1, 3, 4, 6, and 7), five acetylated anthocyanins (peaks 9 and 11−14), five cinnamylated anthocyanins (peaks 15−19), and four newly formed anthocyanin derivatives (peaks 2, 5, 8, and 10).

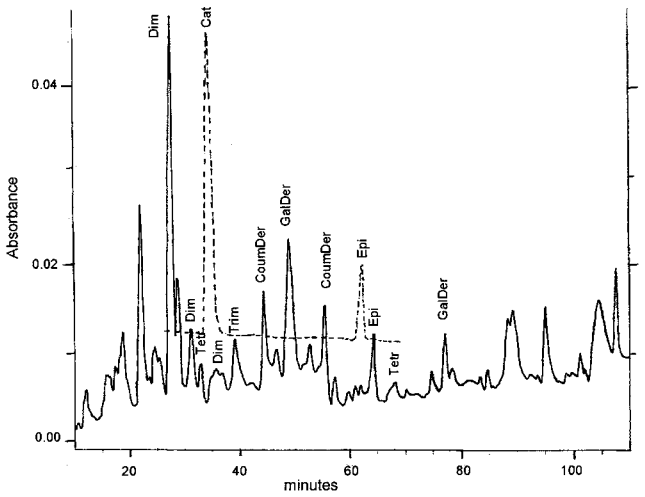

Figure 1. Chromatogram of aged wine flavanols detected at 280 nm: (- - -) first fraction; (—) second fraction. Cat, catechin; Epi, epicatechin; Dim, dimers; Trim, trimers; Tetr, tetramers; CoumDer, coumaric acid derivatives; GalDer, galloylated derivatives.

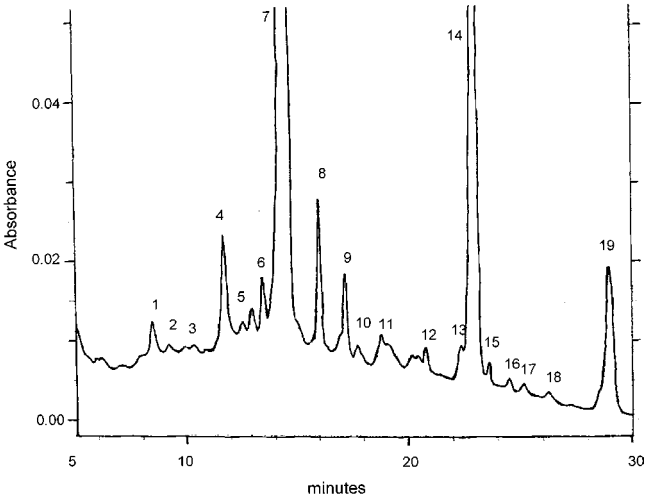

Figure 2. Chromatogram of aged wine anthocyanins recorded at 530 nm: peaks 1, 3, 4, 6, and 7, delphinidin, cyanidin, petunidin, peonidin, and malvidin-3-glucosides; peaks 9 and 11–14, delphinidin, cyanidin, petunidin, peonidin, and malvidin acetyl-3-glucosides; peaks 15 and 17–19, delphinidin, petunidin, peonidin, and malvidin coumaroyl-3-glucosides; peak 16, malvidin caffeoyl-3-glucoside; peaks 2, 5, 8, and 10, pyruvic acid derivatives of delphinidin, petunidin, and malvidin 3-glucoside and vitisin B, respectively.

Table 2 shows the mean values of four replications (two for each vintage). Standard deviation and α values of ANOVA results of unaged wines (before wood aging) showed that vintage effect was not detected, with the exception of percentage of red in TF-1. Therefore, to simplify presentation and interpretation of the results, it was decided to consider the wines from the two years as replications.

The ANOVA and LSD test results showed that "maturity date" or "ripening stages" effects were detected, but these are different for each individual component as well as for each of the two grape varieties studied (**Table 2**).

Results note that the CS wines were much richer in catechin and epicatechin than the TF wines. This finding was not observed for the other derivatives with the exception of the galloylated derivatives. These results could be attributable to several possible factors, principally, first, the content of these

Table 2. Mean Values and Standard Deviation of Unaged Wines[a]

compound		TF-1[b]	TF-2[b]	TF-3[b]	CS-1[b]	CS-2[b]	CS-3[b]
catechin	mean (σ)	c 22.23 (0.17)	b 17.32 (0.25)	a 11.85 (0.13)	c 101.13 (0.52)	b 93.95 (0.48)	a 89.48 (1.91)
(mg/L)	α value[b]	0.9475	0.3178	0.2886	0.2831	0.9820	0.2960
epicatechin	mean (σ)	a 18.24 (0.74)	b 23.44 (1.32)	b 22.95 (0.35)	a 50.01 (1.04)	b 56.65 (1.26)	b 58.20 (0.49)
(mg/L)	α value	0.2887	0.2701	0.5860	0.2980	0.2725	0.3160
dimers	mean (σ)	a 10.89 (0.43)	b 13.14 (0.29)	a 10.60 (0.78)	a 14.84 (0.24)	a 15.34 (0.40)	b 16.22 (0.33)
(mg/L)	α value	0.5442	0.5297	0.2959	0.6056	0.4356	0.6877
trimers	mean (σ)	a 0.60 (0.05)	b 0.75 (0.03)	c 1.02 (0.01)	a 0.67 (0.03)	b 0.80 (0.04)	c 1.01 (0.04)
(mg/L)	α value	0.3044	0.3018	0.4665	0.3023	0.2703	0.2864
tetramers	mean (σ)	a 3.59 (0.49)	a 4.13 (0.49)	a 3.97 (0.08)	a 3.85 (0.40)	a 3.34 (0.10)	a 3.35 (0.04)
(mg/L)	α value	0.1800	0.2136	0.3398	0.1865	0.3222	0.4748
galloylated Der[c]	mean (σ)	a 2.10 (0.09)	a 2.14 (0.12)	b 2.71 (0.08)	a 4.29 (0.08)	b 5.41 (0.21)	c 6.13 (0.09)
(mg/L)	α value	0.5469	0.5552	0.7423	0.6616	0.1228	0.5151
coumaric Der[c]	mean (σ)	b 2.08 (0.17)	a 1.73 (0.15)	b 2.13 (0.11)	a 1.72 (0.13)	b 2.29 (0.40)	a, b 1.95 (0.19)
(mg/L)	α value	0.3659	0.3418	0.3008	0.2109	0.1740	0.3814
glucosides Acy[c]	mean (σ)	a 303.81 (4.52)	c 357.53 (4.77)	b 322.74 (3.78)	a 375.23 (1.48)	c 401.68 (1.75)	b 396.67 (1.33)
(mg/L)	α value	0.2748	0.2787	0.2684	0.3946	0.3187	0.7724
acetylated Acy[c]	mean (σ)	a 15.85 (0.13)	c 22.96 (2.23)	b 19.77 (0.40)	a 152.89 (2.12)	a 151.04 (1.97)	b 159.42 (1.27)
(mg/L)	α value	0.5150	0.1478	0.3783	0.2758	0.2523	0.3036
cinnamylated Acy[c]	mean (σ)	b 23.32 (0.48)	a 20.17 (1.68)	c 25.05 (0.41)	b 33.82 (0.23)	a 24.62 (0.55)	c 36.55 (0.51)
(mg/L)	α value	0.5104	0.2793	0.3747	0.5611	0.3041	0.3268
derivative Acy[c]	mean (σ)	a 0.52 (0.03)	b 1.17 (0.28)	b 1.20 (0.05)	a 1.98 (0.01)	b 2.92 (0.13)	a 2.05 (0.10)
(mg/L)	α value	0.3018	0.2687	0.2725	0.7987	0.3020	0.3197

parameter		TF-1[b]	TF-2[b]	TF-3[b]	CS-1[b]	CS-2[b]	CS-3[b]
color intensity	mean (σ)	c 1.733 (0.026)	b 1.623 (0.009)	a 1.422 (0.006)	a 2.590 (0.026)	c 2.754 (0.004)	b 2.660 (0.003)
	α value[b]	0.1552	0.2649	0.2965	0.2712	0.7935	0.2929
tonality	mean (σ)	a 0.422 (0.001)	c 0.447 (0.001)	b 0.429 (0.001)	a 0.358 (0.006)	b 0.392 (0.003)	a 0.360 (0.001)
	α value	0.5528	0.5528	0.6985	0.3932	0.2965	0.5528
% yellow	mean (σ)	a 26.33 (0.01)	c 27.67 (0.03)	b 27.04 (0.01)	a 24.20 (0.13)	b 25.74 (0.11)	a 24.29 (0.04)
	α value	0.9990	0.1548	0.5528	0.1755	0.1948	0.2999
% red	mean (σ)	b 62.33 (0.11)	a 61.98 (0.09)	c 63.12 (0.06)	b 67.55 (0.38)	a 65.68 (0.16)	b 67.60 (0.06)
	α value	0.0342	0.4053	0.4477	0.1256	0.2588	0.2965
% blue	mean (σ)	c 11.35 (0.11)	b 10.36 (0.09)	a 9.85 (0.06)	a 8.26 (0.31)	a 8.59 (0.11)	a 8.12 (0.07)
	α value	0.0565	0.7905	0.5337	0.2837	0.7644	0.8378

[a] Values with the same letter for each grape variety were not significantly different. [b] TF, Tinto fino; CS, Cabernet Sauvignon; 1, 2, and 3, harvest stage. [c] Der, derivatives; Acy, anthocyanins.

derivatives being higher in the skins and seeds of the CS variety and/or, second, the higher concentrations being due to a higher ratio of solids (skins plus seeds) to liquid (pulp or juice) of this variety. Data reported in previous studies (*34*) did not indicate that the solid parts of CS grapes were richer in flavan components than TF grapes; hence, the first possible factor would not appear to be responsible. In contrast, the second possible factor is supported by the fact that CS berries are smaller than TF berries, producing a higher solids-to-juice ratio (**Table 1**). The similar levels of dimers, trimers, and tetramers between the two varieties can be due to the fact that these compounds are continuously changing and transforming into one another.

The wines made from the least ripe grapes collected on the first harvest date were generally richer in catechin than those made from the ripest grapes collected on the last harvest date. These results are in agreement with published results (*23*, *35*), suggesting that flavan-3-ol monomers underwent a decrease in the latter stages of grape ripening. However, epicatechin did not show this tendency.

The dimer and trimer flavan-3-ol derivatives tended to reach higher levels in the wines made from the grapes collected on the later harvest dates, a finding in consonance with results reported previously (*22*), which indicated that the degree of flavanol polymerization increased with the degree of grape ripening. The more or less constant levels of the tetramers in all of the wines made from both varieties irrespective of harvest date (no statistically significant differences were observed) may be explained on the basis of the lower solubility or extractability of these components, which thus limits their presence in young wines.

In general, the higher levels of galloylated derivatives were recorded in the wines made from the grapes harvested latest. This effect was most clearly observable in the wines made from the CS grapes. The differences in the coumaric acid derivatives were statistically significant, but quantitatively the variations were not particularly large and there was no clear trend in the levels of these components with harvest date.

In the interest of concision and simplicity in presenting the results, the anthocyanin contents have been grouped together as total of glucosides (GlsAcy), acetylated (AcAcy), and cinnamylated anthocyanins (CinAcy) and as anthocyanin derivatives (DerAcy), which includes vitisin B and the pyruvic acid derivatives of the anthocyanins malvidin 3-glucoside, petunidin 3-glucoside, and delphinidin 3-glucoside.

The wines made from the CS variety grapes had higher anthocyanin contents than the wines made from the TF variety grapes irrespective of the degree of ripening. This may be caused by the difference in berry size of these two grape varieties, which gives a higher solids-to-juice ratio in the former variety (**Table 1**). In addition, the data showed the well-known fact that CS grapes are richer in acetylated anthocyanins than TF grapes.

The wines made from more mature grapes (second and third harvest dates) were generally richer in free anthocyanin levels. Hardly any differences in anthocyanin derivative levels were observed. However, within the TF wines, the highest levels were recorded in the wines made from the ripest grapes (TF-2 and TF-3), whereas within the CS wines, the wines richest in these pigments were made from the grapes collected on the second harvest date (CS-2), and the differences between the wines made from the grapes collected on the first and third harvest dates

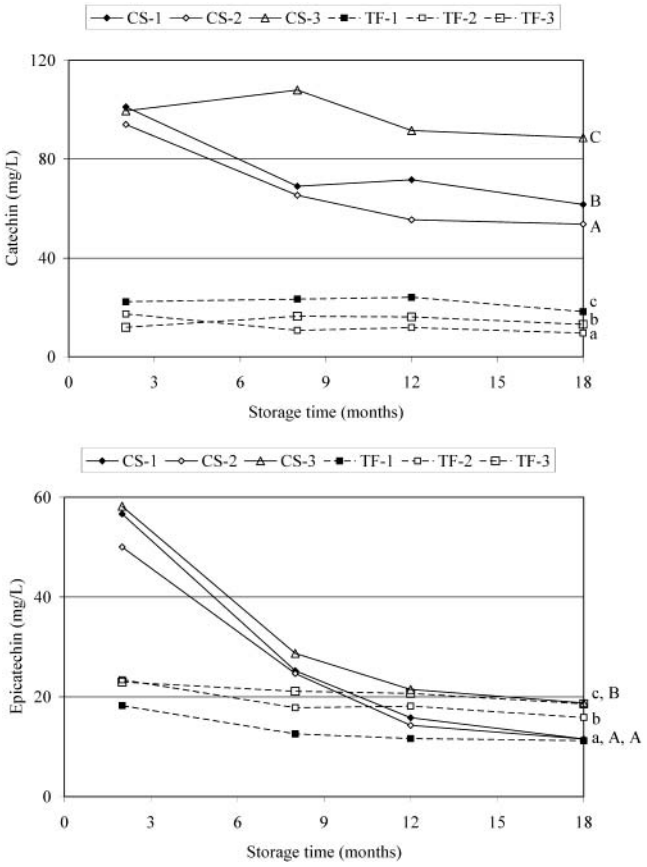

Figure 3. Evolution of catechin and epicatechin of the wines by different harvest date (1–3) during their aging process. Parameters with the same letter in each variety were not significantly different at 18 months. Lower case letters compare TF (Tinto Fino) wines, and upper case letters compare CS (Cabernet Sauvignon) wines.

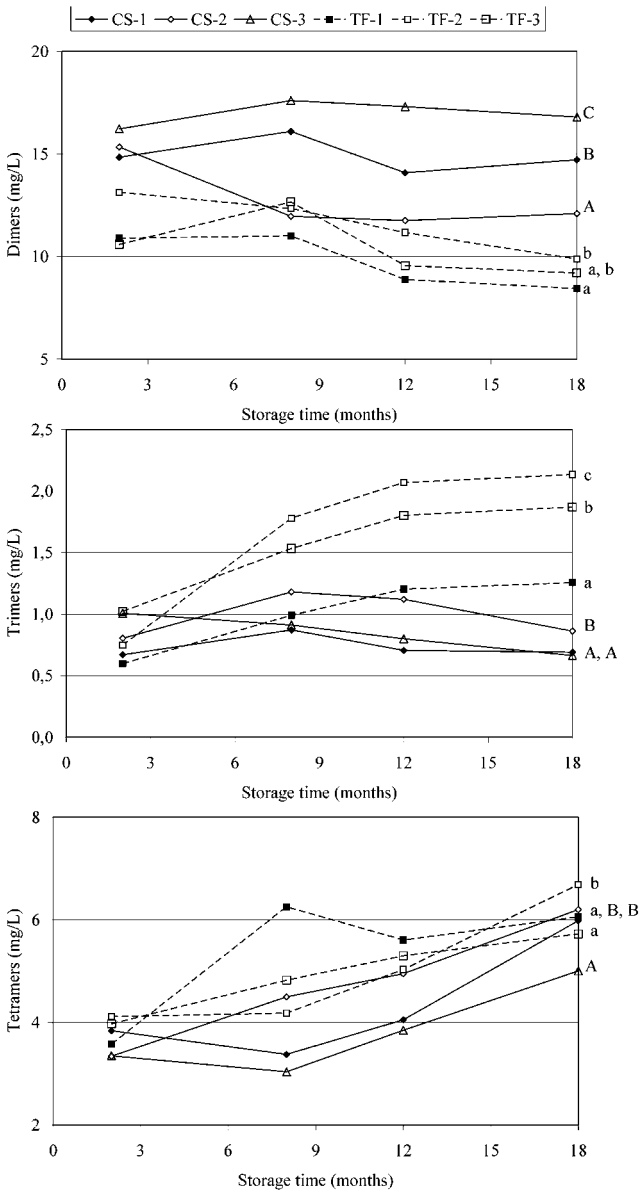

Figure 4. Evolution of dimers, trimers, and tetramers of the wines by different harvest date (1–3) during their aging process. Parameters with the same letter in each variety were not significantly different at 18 months. Lower case letters compare TF (Tinto Fino) wines, and upper case letters compare CS (Cabernet Sauvignon) wines.

were not significant. The results for the anthocyanin derivatives were in agreement with the findings reported in prior studies (*13*, *15*).

The CS wines had higher color intensity values than did the TF wines, together with lower tonality values, both directly related to the higher percentage red and lower percentage yellow values in the CS wines. These wines also had a lower violet intensity (lower percentage of blue). All of these findings are consistent with the generally higher phenolics content in the CS wines compared with the TF wines. Their higher anthocyanin richness increased the percentage red while lowering the tonality value.

No clear trends with grape ripening were observed, but once again the two varieties displayed differences. The TF wines with the highest intensity values were the wines made from the grapes collected on the first harvest date, and these wines also had the highest percentage blue and the lowest tonality value. In contrast, the CS wines with the highest color intensity values were the wines made from the grapes collected on the second harvest date, and these wines also had the highest tonality and percentage yellow values. The TF wines made from the grapes collected on the third harvest date had the highest percentage red but the lowest color intensity values. The CS wines made from the grapes collected on the first harvest date had the lowest color intensity value and a high percentage red value. These results show that the highest percentage red is not always paired with the highest color intensity, which attests to the importance of color modifiers shifting the predominant color, red, toward other hues, basically blue. These findings are in consonance with the results reported in previous studies (*36*−*38*).

Really, it is the wines aged for 18 months (12 months in the wood and 6 months in the bottle) that are released into the marketplace for sale, so the following shows the evolution of these wines during aging.

The monomeric flavanol, catechin, and epicatechin contents decreased substantially during aging, particularly in the CS wines, which initially had considerably higher levels (**Figure 3**). Levels of the dimeric derivatives also fell, although not as much. At the same time, there was an increase in the more highly polymerized derivatives, for example, the trimeric and above all tetrameric derivatives (**Figure 4**). These results indicate that polymerization and condensation of the phenolics increase during aging, which is in agreement with the decrease of monomeric flavanol observed and with the results obtained in previous work (*39*). The galloylated derivatives increased during aging, whereas the coumaric derivatives showed different

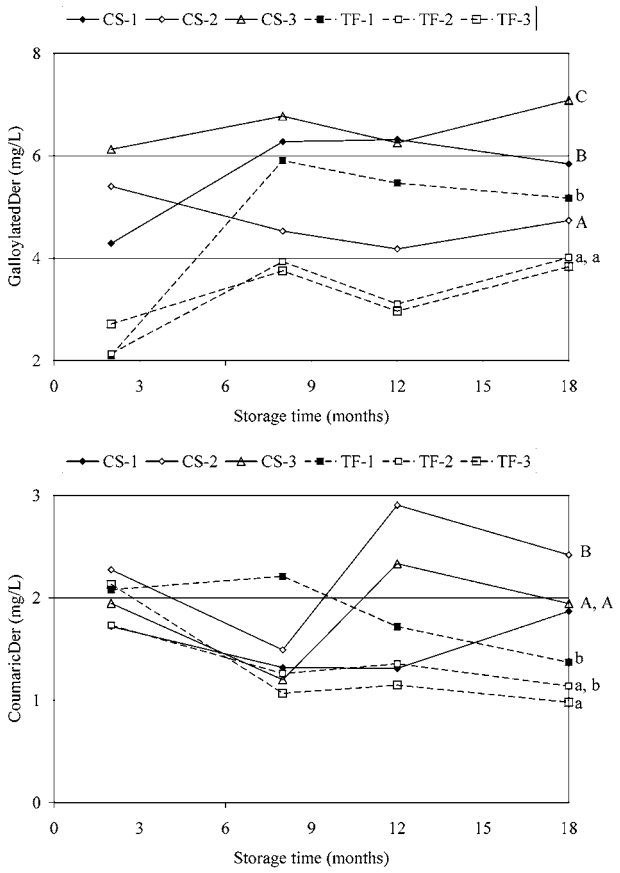

Figure 5. Evolution of galloylated (GalloylatedDer) and coumaric derivatives (CoumaricDer) of the wines by different harvest date (1–3) during their aging process. Parameters with the same letter in each variety were not significantly different at 18 months. Lower case letters compare TF (Tinto Fino) wines, and upper case letters compare CS (Cabernet Sauvignon) wines.

trends in the two grape varieties, decreasing especially in TF wines (**Figure 5**).

In general, the differences found among the wines elaborated from grapes harvested at different ripening degree after aging were maintained or increased. The wines made from more mature grapes had higher levels of flavanols and their derivatives.

The free anthocyanin content decreased sharply during aging, the greatest losses taking place in the first months of aging and in the wines made from the variety richer in these compounds (the CS wines). Then the differences observed in the initial wines ceased to be detectable. Despite the decrease in the anthocyanins, the TF-2 and CS-3 wines still exhibited the highest levels of free anthocyanins (**Figure 6**).

The loss of free anthocyanins was at least partially the outcome of condensation of these components, mainly with flavanols, a process that may or may not be mediated by acetaldehyde. It was also caused by cycloaddition reactions, chiefly with pyruvic acid. This finding was consistent with the results obtained, which indicate that the decrease in the levels of the free anthocyanins and flavanols took place in conjunction with an increase in the levels of the anthocyanin derivatives, this process being particularly pronounced in the TF wines. A number of researchers have reported these "new pigments" to be responsible for maintaining and stabilizing color intensity in aged wines (*12*, *18*), which could account for the substantial increase in the percentage blue value in the aged wines as compared to the young wines, a finding which again was especially pronounced in the TF wines.

The wines made from more mature grapes showed higher levels in these new pigments, in both TF and CS wines, but the differences were not statistically significant between wines from the second and third harvests.

These new pigments identified show a shift in the dominant wavelength toward the orange (*13*), having a maximum between 513 and 520 nm. However, other kinds of new anthocyanins such as the malvidin−catechin condensates (*15*, *40*) and anthocyanin-derived pigments resulting from the reaction between anthocyanin−pyruvic acid adducts and vinyl-flavanols (*19*), show maximum absorptions at 545 and 575 nm, respectively, which also increase during the aging process (*19*, *38*). These blue anthocyanins can be identified by LC-MS (*15*, *19*), although their quantification without a previous separation is difficult. Then, it will be necessary to fractionate the wine sample to an adequate detection and quantification of these new pigments formed during the aging process, as was recently demonstrated by some authors (*41*−*43*). These authors have also reported that wines with higher levels of pyruvic derivative anthocyanins also presented higher contents of new blue pigments, showing higher blue tones in the wines analyzed. Then although we can only quantify the pyruvic anthocyanin derivatives, the color of these wines indicates the presence of some new blue pigments.

Figure 7 shows the evolution of different chromatic parameters, and, as expected, there was a decrease in color intensity and red color and, therefore, an increase in tonality and yellow tones. Despite this, color intensity values remained high, particularly in the CS wines, whereas tonality levels were relatively low for wines that had been aged for 18 months. All of the wines exhibited uniform trends, but aging accentuated the "harvest date effects". Therefore, the wines made from the grapes collected on the second and third harvest dates had higher values for percentage blue, which contrasted completely with the findings for the wines at time 0 (unaged wines). This finding is in all likelihood directly correlated with the substantial increase in levels of pigment derivatives of anthocyanins that took place during aging.

Summarizing, it seems to be clear that delaying the harvest date between 1 and 2 weeks for these varieties and in this region produced grapes with better composition and characteristics in order to obtain a "better" aged wine. These wines showed good color stability, which is an extremely important factor in product acceptance and hence product quality. The significant increase in anthocyanin derivative levels contributes to color stability by maintaining color intensity and increasing the blue component.

At the same time, the results showed that the amount of time the grapes are left on the vines needs to be carefully limited, because if allowed to ripen too much, some of the beneficial effects noted above are lost. This fact is demonstrated by the wines made from the grapes collected on the third harvest date not exhibiting better quality characteristics than the wines made from the grapes collected on the second harvest date.

Obviously, the amount of time that the grape should be left on the vine must be considered for each vineyard individually, taking into account the climatic conditions of each region and year, the variety, and the cultural practices.

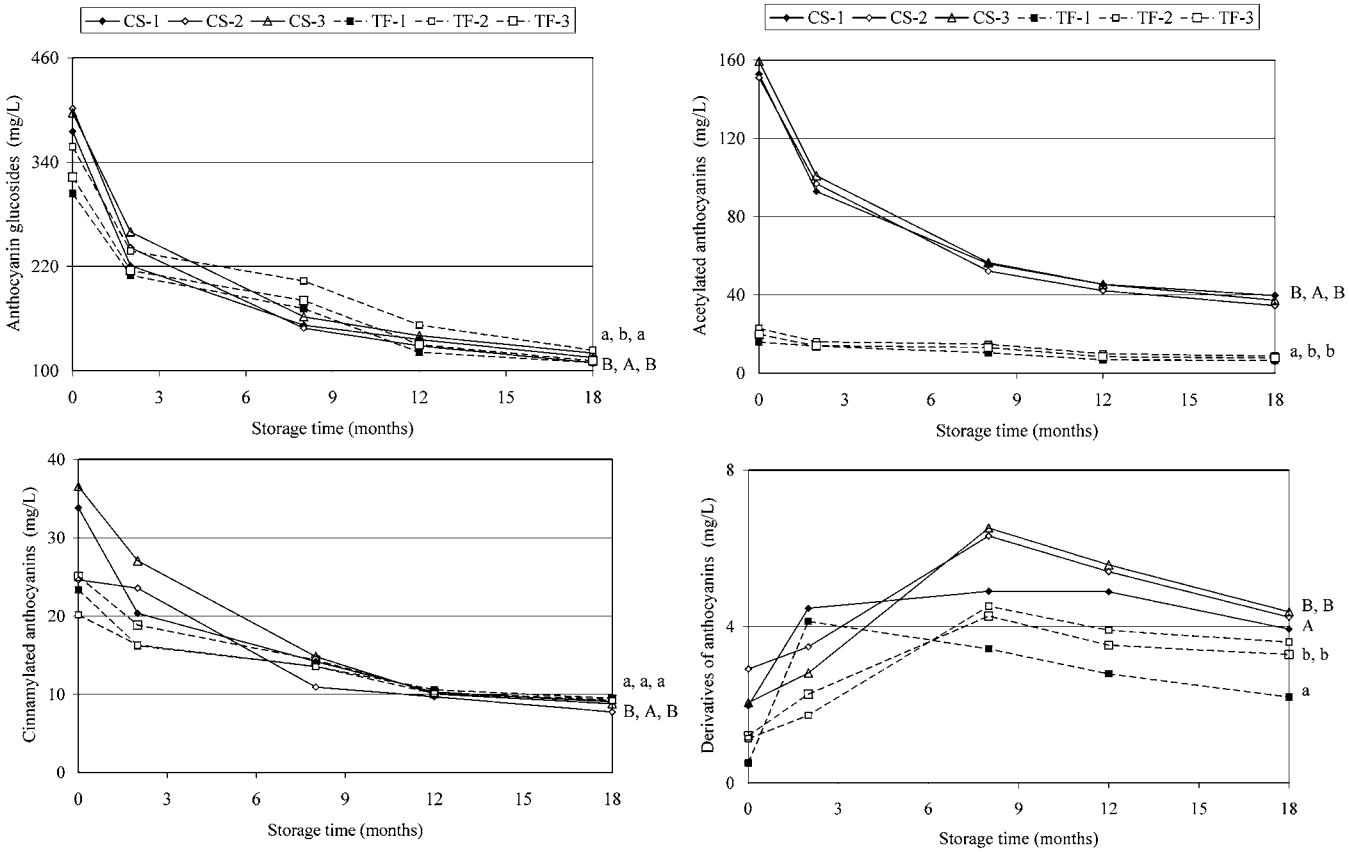

Figure 6. Evolution of individual anthocyanins of the wines by different harvest date (1–3) during their aging process. Parameters with the same letter in each variety were not significantly different at 18 months Lower case letters compare TF (Tinto Fino) wines, and upper case letters compare CS (Cabernet Sauvignon) wines.

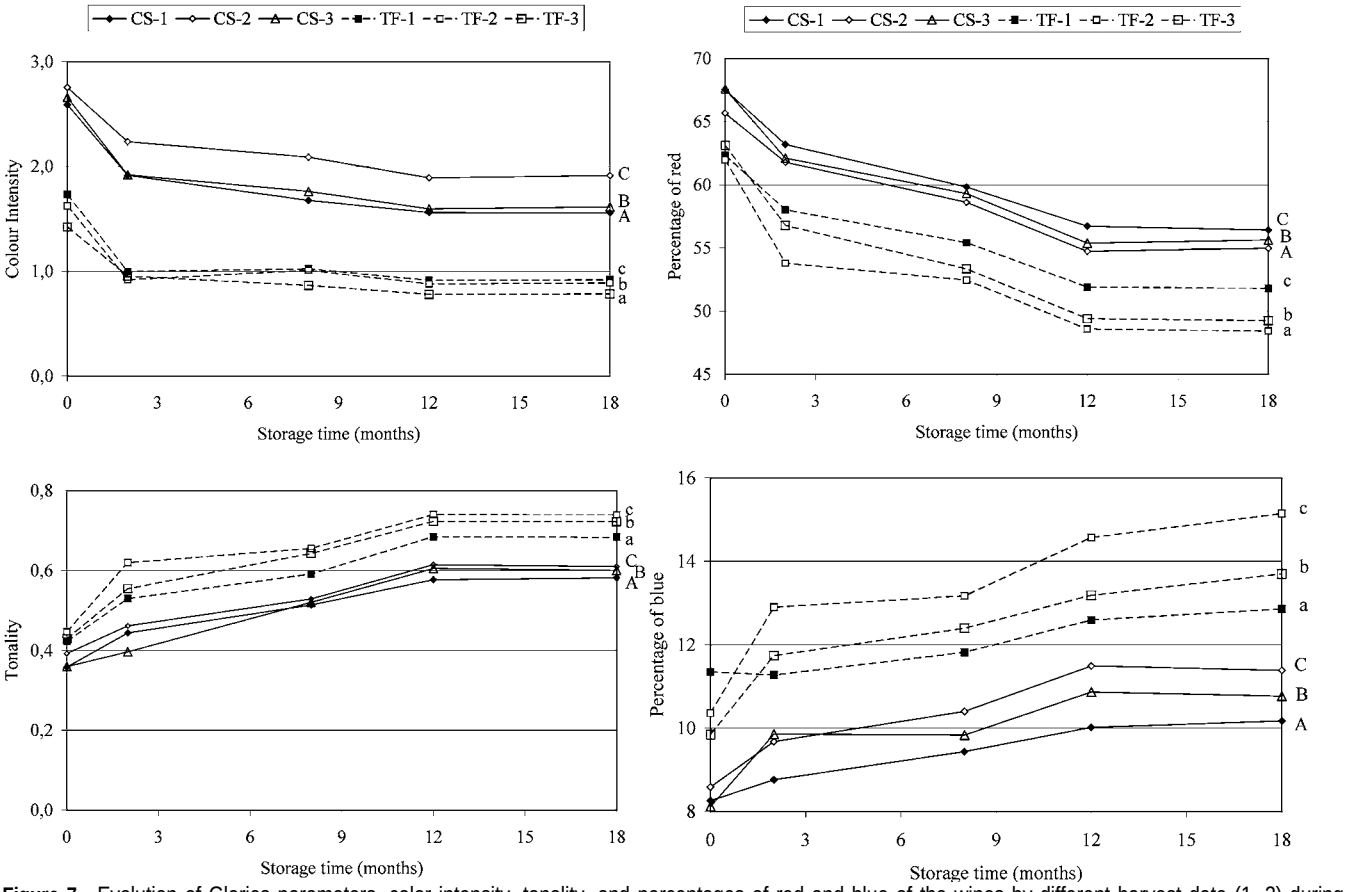

Figure 7. Evolution of Glories parameters, color intensity, tonality, and percentages of red and blue of the wines by different harvest date (1–3) during their aging process. Parameters with the same letter in each variety were not significantly different at 18 months Lower case letters compare TF (Tinto Fino) wines, and upper case letters compare CS (Cabernet Sauvignon) wines.

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