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Aging Effect on the Pigment Composition and Color of *Vitis vinifera* L. Cv. Tannat Wines. Contribution of the Main Pigment Families to Wine Color

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Red wines made from *Vitis vinifera* L. cv. Tannat grapes are known to possess high contents of tannins and intense color, features that are responsible for the originality of these wines. This work aimed to study the evolution of the pigment composition and CIELAB color parameters as Tannat wines become older, as well as to establish the contribution to wine color of the main pigment families. Tannat wines produced in Uruguay from grapes of the same vineyard in six consecutive vintages (1998−2003) and Tannat grapes of the 2003 harvest were analyzed by means of HPLC-DAD-MS and UV−vis spectrometric techniques. The correlations between the different pigment families and the CIELAB parameters revealed the importance of the variations of the percentage, found in anthocyanins and flavanol−anthocyanin acetaldehyde-mediated condensation products (decrease) and pyranoanthocyanins and direct condensation products (increase), in the modification of the color from purple-red hues to more orange-red ones. The color suffered qualitative rather than quantitative changes, that is, the hue (h^*_{ab}) increased, whereas the chroma (C^*_{ab}) and lightness (L^*) did not show a defined trend with time.

KEYWORDS: Tannat; color; anthocyanins; anthocyanin-derived pigments; pyranoanthocyanins; flavanol−anthocyanin direct condensation products

INTRODUCTION

The color of wine is one of the first features perceived by consumers that can influence its acceptance and inform about its age, conditions of storage, etc. Anthocyanins are the pigments responsible for grape color, which are extracted to the must during winemaking, providing it the characteristic purple-red hue of young red wines. Once extracted and from the end of the fermentation, its concentration begins to decline probably due to, in the first steps of winemaking, their adsorption by yeasts; later, the reactions of condensation, polymerization, oxidation, and precipitation could be involved in anthocyanin disappearance. Some of these reactions imply degradation of the anthocyanin, whereas others yield products that can provide, in turn, different hues to the wine. Thus, as a result, the color of the wine evolves, as the wine becomes older, to orange-brick hues. Despite the numerous studies in wine color, the contribution of each pigment family during aging has still to be determined.

Seventy-eight percent of the grapes produced in Uruguay in 2004 were *Vitis vinifera* L., and among them, the Tannat cultivar was the second most cultivated in this country (*1*), Uruguay being, in addition to France, its place of origin, almost the only country in which it is nowadays cultivated. Nevertheless, in the international wine market there is an increasing demand for Tannat wines because they are considered to be original and quality wines. In fact, one of the most remarkable features of these wines is their intense color, and some studies have shown that Tannat wines, when compared to red wines made from other grape varieties, possessed the highest pigment contents (*2*−*4*). These wines are also characterized by high tannin contents (*4*), which lend structure to the wine. Thus, the phenolic fraction of this variety is, nowadays, beginning to be the aim of different studies. The phenolic potential, main anthocyanins, total pigment content, and their variations with vintage and vineyard treatments have also been reported for Tannat grapes (*4*−*6*) as well as the main anthocyanins, anthocyanin and flavanol contents, and antioxidant capacity of Tannat wines at different ages (*3*, *4*, *6*, *7*). More recently, the detailed anthocyanin composition of a young Tannat wine has also been reported (*2*). Nevertheless, as far as we know, there are no studies on the evolution of Tannat wine pigments and the correspondence with color changes observed during aging. On the contrary, the total anthocyanin content and traditional color parameters have been determined in wines made from other *V. vinifera* cultivars.

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However, these studies have been focused on the evolution of anthocyanin levels during certain stages of winemaking in order to see the influence of different affecting factors, such as vintage, grape variety, winemaking protocol, and aging in oak barrels or in stainless steel tanks, on anthocyanin contents (*8*−*10*). Others have also studied the evolution of anthocyanin derivatives either in commercial or in experimental wines, most of them considering fixed stages of winemaking and/or aging (*11*, *12*) or only monitoring one compound or one family of anthocyanin derivatives (*13*−*15*). In these cases, the same wine samples were monitored throughout all of the study periods, which have conditioned their lengths. An experimental approach that might overcome this problem is to analyze simultaneously wines made from grapes of the same variety grown in the same vineyard in consecutive vintages, each sample representing a given moment in wine life. It would be possible, therefore, to study the evolutions of different pigment families during long periods and to establish the correspondence between composition and color evolution during aging. It is well-known that the vintage can affect the pigment content, the pigment profile being hardly modified. Thus, the conclusions obtained from such a study should refer to the evolution trend of the different pigment families and to their relative contents rather than to the particular values obtained for each sample.

This study aimed to establish the pigment composition of six Tannat wines produced in Uruguay in consecutive vintages (1998−2003), as well as that of Tannat grapes collected in the 2003 vintage, in order to determine the qualitative and quantitative changes produced in the main pigment families (anthocyanins, pyranoanthocyanins, direct and acetaldehyde-meditated flavanol−anthocyanin condensation products) as wine became older. The determination of the color properties of the samples (by calculation of the CIELAB parameters) was also considered in this study in order to monitor the color evolution during this 6-year aging period and to establish the contribution to wine color of the different pigment families at different stages of wine life.

MATERIALS AND METHODS

Grape Skin Extract Samples. *V. vinifera* L. cv. Tannat grapes were collected at harvest in the northern region of Uruguay (Cerro Chapeu), in the 2003 vintage, and kept at −18 °C until extraction.

A set of 50 g of berries was randomly sampled, and the skins were separated from pulp and seeds. The skin was macerated with 100 mL of methanol/10% formic acid (95:5) for 24 h, favored by applying ultrasound for 5 min. The liquid was separated from the solid and concentrated to 50 mL by rotavapor apparatus at 35 °C.

The extract was diluted 1/5 with acidified water (HCl at pH 0.5) and filtered through a 0.45 μm Millex syringe-driven filter unit (Millipore Corp., Bedford, MA) before injection.

Wine Samples. Wine samples were produced in the 1998, 1999, 2000, 2001, 2002, and 2003 vintages from batches of 10 tonnes of fresh grapes (*V. vinifera* L. cv. Tannat) sourced from the same vineyard (Cerro Chapeu, Uruguay) in all of the vintages, and for all of them the same winemaking steps were followed: The grapes were destemmed and crushed, and a subsample was analyzed for sugar content, total acidity, and pH. SO_2 was added to the must (50 mg/L), which was then inoculated with reactivated dry yeast (*Saccharomyces cerevisiae*, strain D 254; Lallemand, Rexdale, ON, Canada). Fermentation was carried out at 20−22 °C in open stainless steel tanks, and when the must density reached 1000 g/L, the wine was separated from the skins. The skins were then pressed and both wine fractions combined and sent to 225 L French oak barrels (50% new wood), where fermentation was completed. Upon completion of alcoholic fermentation, malolactic fermentation (MLF) was activated by inoculation with *Oenoccocus oeni* strain DSM 7008 (Viniflora, Chr. Hansen's, Horsholm, Denmark) at 16 °C. MLF was followed by determining the concentrations of malic and lactic acid by thin-layer chromatography (TLC) (*16*).

Once MLF was finished, the wines were treated with 50 mg/L SO_2. Barrels were maintained for 18 months at 16 °C, and free SO_2 was corrected at 30 mg/L periodically. Then, samples were taken from 10 barrels and stabilized at 4 °C for 10 days and sterile-filtered (0.45 μm cellulose acetate membrane). Free SO_2 content was adjusted to 35 mg/L, and finally, the samples were bottled and maintained at 15 °C until the moment of the HPLC-DAD-MS analysis. (**Table 1** shows the age of the samples at the moment of analysis and the maturation and aging time spent by each, in barrels and in bottles.)

Table 1. Wine Samples Analyzed

vintage	age of sample (months)	time in barrel (months)	time in bottle (months)
2003	4	4	
2002	16	16	
2001	28	18	10
2000	40	18	22
1999	52	18	34
1998	64	18	46

The six samples were analyzed, respectively, after 64, 52, 40, 28, 16, and 4 months from their harvests. Prior to the HPLC-DAD-MS analyses, the samples were diluted 1/5 with acidified water (HCl at pH 0.5) and filtered through a 0.45 μm Millex syringe-driven filter unit (Millipore Corp.) before injection. No treatment of the samples was carried out prior to the spectrophotometric measurements.

HPLC-DAD Analysis. HPLC-DAD analysis was performed in a Hewlett-Packard 1100 series liquid chromatograph, and detection was carried out using a photodiode detector. An Aqua C18 reverse phase, 5 μm, 150 mm × 4.6 mm column (Phenomenex, Torrance, CA) thermostated at 35 °C was used.

The solvents used were (A) an aqueous solution (0.1%) of trifluoroacetic acid (TFA) and (B) 100% HPLC-grade acetonitrile, establishing the following gradient: isocratic 10% B for 5 min, from 10 to 15% B for 15 min, isocratic 15% B for 5 min, from 15 to 18% B for 5 min, and from 18 to 35% B for 20 min, at a flow rate of 0.5 mL min^{-1}. Detection was carried out at 520 nm as the preferred wavelength. Spectra were recorded from 220 to 600 nm.

HPLC-MS Analysis. HPLC-MS analyses were performed using a Finnigan LCQ MS detector (Thermoquest, San Jose, CA) equipped with an API source, using an electrospray ionization (ESI) interface. The LC system was connected to the probe of the mass spectrometer via the UV cell outlet. Nitrogen was used as sheath and auxiliary gas. The sheath gas flow was 1.2 L min^{-1} and the auxiliary gas flow, 6 L min^{-1}. The capillary voltage was 4 V and the capillary temperature, 195 °C. Spectra were recorded in positive ion mode between *m*/*z* 120 and 1500. The mass spectrometer was programmed to do a series of three consecutive scans: a full mass, an MS^2 scan of the most abundant ion in the full mass, and an MS^3 of the most abundant ion in the MS^2. The normalized energy of collision was 45%.

Colorimetric Measurements. Absorption spectra (190−1100 nm) were recorded using a Hewlett-Packard UV−vis HP3853 spectrophotometer in 2 mm path length quartz cells. The analysis of color was made only from the visible spectra (380−770 nm) data, using the CIE 1964 standard observer (10° visual field) and the CIE standard illuminant D65 as references. CIELAB parameters were calculated using the software ChromaLab (*17*) without transformation of the optical path.

Statistical Analysis. Data from the six wine samples were assembled, and linear correlation between the different groups of pigments and color parameters was performed using Minitab 14.1 software (*18*).

RESULTS AND DISCUSSION

Qualitative Analysis. The identification of the different detected pigments was carried out considering the data obtained in the HPLC-DAD-MS analyses. The chromatographic and spectral (UV−vis and MS) features of each detected compound and its presence or absence in the different samples are listed

in **Table 2**. Peak numbers correspond to those shown in the chromatograms, recorded at 520 nm (**Figure 1**), of the grape and wine samples.

Anthocyanins. The data obtained for compounds **7**, **10**, **12**, **15**, and **18** (retention time, UV−vis spectra, molecular ions, and fragmentation pattern) were in agreement with those corresponding to the 3-monoglucosides of delphinidin, cyanidin, petunidin, peonidin, and malvidin, respectively, reported in young Tannat wines (*2*). All of these compounds were detected in grape and in all of the wine samples considered.

Similarly, compounds **22**, **28**, **31**, **40**, and **41** were identified as the acetyl derivatives of the corresponding monoglucosides. Those compounds were detected in all of the samples, but in the oldest wines, the low levels of some of them (cyanidin- and peonidin-3-acetylglucosides) did not allow their quantification.

Compounds **42**, **49**, **51**, **61**, and **62** presented a shoulder in the UV−vis spectra within the range of 309−313 nm and fragmentation patterns characteristic of the compounds acylated with *p*-coumaric acid, thus allowing their identification as the *p*-coumaroyl derivatives of the five anthocyanin monoglucosides. These compounds presented an important decrease in the aged Tannat wines, and in the 1998 sample, only malvidin-3-(*p*-coumaroyl)glucoside was detected.

The molecular ions of compounds **36**, **43**, **44**, **53**, and **55** showed the same *m*/*z* ratio and the same fragmentation patterns as those of compounds **42**, **49**, **51**, **61**, and **62**, respectively. Compounds **53** and **55** were identified as the *cis* isomers of peonidin- and malvidin-3-(*p*-coumaroyl)glucosides, as has already been reported for young Tannat wines (*2*). The presence of these two compounds has also been reported in Tempranillo, Graciano, and Cabernet Sauvignon grapes (*19*) and wines (*20*) and in wines produced in Uruguay from different grape varieties (Caladoc, Marselan, Marzemino, and Cheveñasco) (*2*). Similarly, compounds **36**, **43**, and **44**, not reported yet in either Tannat wines or grapes, were identified as the *cis* isomers of delphinidin-, cyanidin-, and petunidn-3-(*p*-coumaroyl)glucosides, which eluted before their corresponding *trans* isomers as already reported in Tempranillo wines (*11*). These compounds were found only in grapes and in young wines (**Table 2**).

The UV−vis spectrum of compound **47** showed a shoulder at 326 nn, which was indicative of the acylation of the molecule with caffeic acid, and its retention time and data obtained in the MS analysis were helpful in confirming the identity of this compound as malvidin-3-caffeoylglucoside. This compound has been found only in the grape sample and in the youngest wines.

Pyranoanthocyanins. The spectral features of compounds **4**, **8**, **9**, **11**, **13**, **14**, **17**, **19**, **21**, **23**, **27**, and **39** corresponded to those of pyranoanthocyanins originated in the cycloaddition of pyruvic acid to different anthocyanins (A-type vitisins), showing, some of them, UV−vis spectra with absorbance maxima at 503−510 nm. Compounds **4**, **11**, **14**, and **17** have already been identified in young Tannat wines (*2*) and corresponded to the A-type vitisins of delphinidin-, petunidin-, peonidin-, and malvidin-3-glucosides. Compound **9**, corresponding to the pyruvic adduct of cyanidin-3-glucoside, was not previously reported in Tannat wines. Nevertheless, from a quantitative point of view, this compound was not relevant.

The presence of acylation with acetic acid in compounds **8**, **13**, **21**, and **23** was detected by the fragmentation of their molecular ions in the MS^2 analyses and were identified as the A-type vitisins of delphinidin-, petunidin-, peonidin-, and malvidin-3-acetylglucosides, respectively. Compounds **8** and **21** were not previously reported in Tannat wines.

Similarly, compounds **19**, **27**, and **39** were identified as the A-type vitisins of delphinidin-, peonidin-, and malvidin-3-(*p*-coumaroyl)glucosides respectively. These compounds were present in all of the samples, except in the samples of 52 and 64 months, from which delphinidin-3-(*p*-coumaroyl)glucoside was absent.

Compounds **16**, **24**, **34**, and **56** showed UV−vis spectra similar to those of the pyruvic adducts of the anthocyanins, but with the absorption maximum hypsochromically shifted toward 490−494 nm, which correspond to B-type vitisins or vinyl adducts (originated in the cycloaddition between acetaldehyde and anthocyanins). Compounds **16** and **24** were identified as the B-type vitisins of petunidin- and malvidin-3-glucosides, respectively, the former not being detected in the oldest samples. Compounds **34** and **56** corresponded to the B-type vitisins of malvidin-3-acetylglucoside and malvidin-3-(*p*-coumaroyl)glucoside, respectively. Except the petunidin-3-glucoside derivative, all the other B-type vitisins were also found in the grape sample. Although vitisin B has been reported in grape pomace from a Sicilian *V. vinifera* L. cultivar (*21*) and in juices made from Salvador grapes (hybrid from *V. vinifera* and *Vitis rupestris* Scheele) (*22*), this is the first time that the acetyl and *p*-coumaroyl derivatives of this compound have been detected and identified in grapes.

The UV−vis spectra and fragmentation patterns of compounds **45**, **58**, **63**, **64**, **68**, and **71** were typical of the compounds originated in the reaction between the different anthocyanins and 4-vinylphenol (*23*−*29*) for which different mechanisms have been proposed (*28*, *30*). They were identified as follows: compounds **45**, **58**, **63**, and **64** were the 4-vinylphenol derivatives of the 3-glucosides of delphinidin, petunidin, peonidin, and malvidin, respectively, whereas compounds **68** and **71** corresponded to the 4-vinylphenol derivatives of malvidin-3-acetylglucoside and malvidin-3-(*p*-coumaroyl)glucoside. These compounds were present in all of the Tannat wine samples analyzed.

The UV−vis spectra along with the retention times, *m*/*z* ratios, and fragmentation patterns of compounds **65**, **67**,and **70** allowed their identification as the vinylguaiacol adducts of peonidin-3-glucoside, malvidin-3-glucoside, and malvidin-3-acetylglucoside, respectively. The presence of compound **65**, even when not relevant in quantitative terms in grapes and wines, is here reported for the first time in Tannat wines.

Other compounds related to the vinylphenol and vinylguaiacol adducts are the vinylcatechol adducts of the anthocyanins. In the Tannat samples analyzed the vinylcatechol adducts of petunidin- and malvidin-3-glucosides, malvidin-3-acetylglucoside, and malvidin-3-(*p*-coumaroyl)glucoside (compounds **48**, **60**, **66**, and **69**, respectively) have been detected and identified by taking into account their chromatographic and spectral features (**Table 2**).

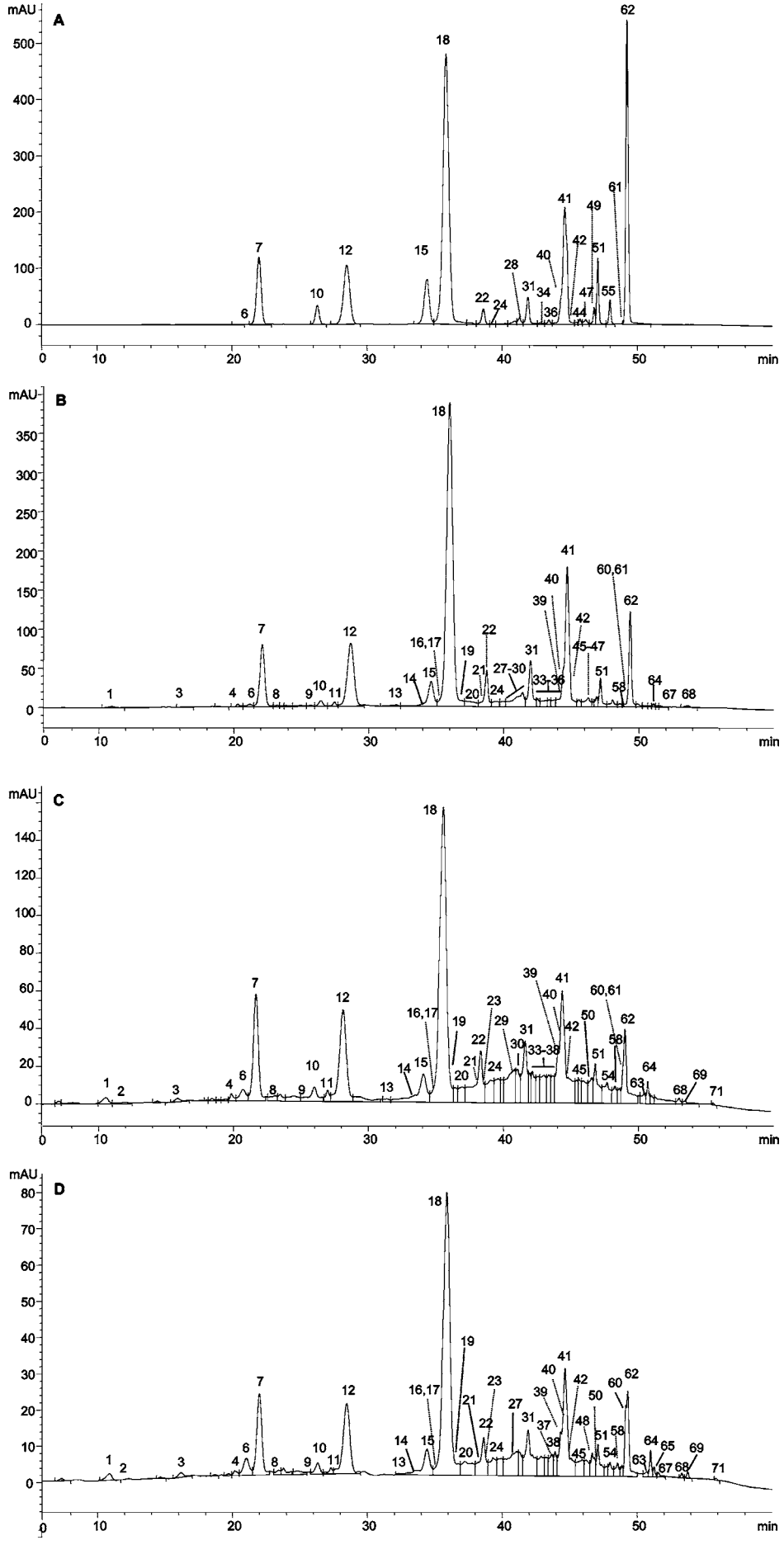
The vinylphenol derivatives were more abundant than the vinylguaiacol and vinylcatechol derivatives, which could be explained by the different decarboxylase activities (*31*) during fermentation toward the hydroxycinnamic acids to give a product that might react rapidly with anthocyanins and by the different content of these acids.

The molecular ion corresponding to compound **50** possessed the same *m*/*z* ratio (805) as that of compound **59**. These compounds have already been reported in young Tannat wines (*2*) and were identified as the products resulting from the cycloaddition reaction between the monoglucoside of malvidin and vinylcatechin or vinylepicatechin, respectively. These two

Table 2. Chromatographic and Spectral (UV–Vis and MS) Features, Identities Proposed, and Semiquantification of All Compounds Detected in the Grape and Wine Samples[a]

peak	t_R (min)	M^+ (*m/z*)	MS^2 fragments	MS^3 fragments	λ_{max} (nm)	peak identity	GRA	W03	W02	W01	W00	W99	W98
						Anthocyanins							
7	22.1	465	303	303, 150	277, 346, 524	delphinidin-3-glucoside	**	**	**	**	**	*	*
10	26.4	449	287	287	280, 328, 516	cyanidin-3-glucoside	**	**	*	*	*	*	tr
12	28.7	479	317	317, 302	276, 346, 525	petunidin-3-glucoside	***	***	**	**	**	**	*
15	34.6	463	301	286, 301	280, 328, 516	peonidin-3-glucoside	***	**	**	**	**	*	*
18	36.0	493	331	331, 316, 295, 270	278, 347, 527	malvidin-3-glucoside	***	***	***	***	***	**	**
22	38.7	507	303	303, 275, 257	276, 349, 527	delphinidin-3-acetylglucoside	**	**	**	*	*	*	tr
28	41.4	491	287	287	280, 330, 521	cyanidin-3-acetylglucoside	**	*	*	*	*	tr	nd
31	42.0	521	317	317, 302	278, 347, 529	petunidin-3-acetylglucoside	**	**	**	**	**	*	tr
36	43.6	611	303, 286	303	280, 301, 535	delphinidin-3-(*p*-coumaroyl)glucoside (*cis*)	*	tr	nd	nd	nd	nd	nd
40	44.4	505	301	301	280, 330, 522	peonidin-3-acetylglucoside	**	**	*	*	*	*	nd
41	44.7	535	331	331, 315, 399	278, 349, 530	malvidin-3-acetylglucoside	***	***	**	**	**	**	*
42	44.9	611	303, 286	303	282, 313, 532	delphinidin-3-(*p*-coumaroyl)glucoside	**	**	*	*	*	*	nd
43	45.6	595	287	287		cyanidin-3-(*p*-coumaroyl)glucoside (*cis*)	*	nd	nd	nd	nd	nd	nd
44	45.9	625	317	317, 302	280, 300, 536	petunidin-3-(*p*-coumaroyl)glucoside (*cis*)	**	*	nd	nd	nd	nd	nd
47	46.2	655	331	331, 316, 299, 270	282, 326, 436, 532	malvidin-3-caffeoylglucoside	**	*	tr	nd	nd	nd	nd
49	46.9	595	287	287	283, 312, 366, 522	cyanidin-3-(*p*-coumaroyl)glucoside	**	*	*	nd	nd	nd	nd
51	47.2	625	317	317, 302	282, 311, 532	petunidin-3-(*p*-coumaroyl)glucoside	**	**	**	*	*	*	nd
53	48.1	609	301	301	280, 303, 532	peonidin-3-(*p*-coumaroyl)glucoside (*cis*)	*	nd	nd	nd	nd	nd	nd
55	48.1	639	331	331, 316, 299	281, 303, 535	malvidin-3-(*p*-coumaroyl)glucoside (*cis*)	**	*	*	nd	nd	nd	nd
61	49.3	609	301	301	282, 313, 526	peonidin-3-(*p*-coumaroyl)glucoside	**	**	*	*	*	nd	nd
62	49.4	639	331	331, 316, 299	283, 313, 532	malvidin-3-(*p*-coumaroyl)glucoside	***	***	**	**	**	*	*
						Pyranoanthocyanins							
4	20.2	533	371	371, 325, 281, 268	297, 368, 507	A-type vitisin of Dp-3-glc	nd	*	*	tr	*	tr	tr
8	23.1	575	371	371, 343, 326		A-type vitisin of Dp-3-acetylglc	nd	*	tr	tr	tr	nd	nd
9	25.2	517			234, 352, 503	A-type vitisin of Cy-3-glc	nd	tr	nd	nd	nd	nd	nd
11	27.4	547	385	370, 385, 353, 342	299, 371, 508	A-type vitisin of Pt-3-glc	nd	*	*	*	*	*	*
13	32.0	589	385	385, 370		A-type vitisin of Pt-3-acetylglc	nd	*	*	tr	tr	tr	tr
14	34.0	531			503	A-type vitisin of Pn-3-glc	nd	*	*	tr	*	tr	tr
16	34.9	503			492	B-type vitisin of Pt-3-glc	nd	tr	tr	tr	nd	nd	nd
17	35.6	561	399	399, 383, 366, 338	299, 372, 510	vitisin A	nd	**	**	*	**	*	*
19	36.8	679	371	371, 281, 298, 325		A-type vitisin of Dp-3-(*p*-coumaroyl)glc	nd	*	tr	nd	tr	tr	tr
21	38.4	573				A-type vitisin of Pn-3-acetylglc	nd	*	tr	tr	tr	tr	tr
23	38.7	603	399	399		A-type vitisin of Mv-3-acetylglc	nd	**	*	*	*	*	tr
24	39.8	517	355	355, 339, 294, 266, 322, 202	294, 358, 490	vitisin B	*	*	*	*	*	*	*
27	41.2	693				A-type vitisin of Pt-3-(*p*-coumaroyl)glc	nd	*	*	tr	*	tr	tr
32	42.3	1093	931	585, 641, 395		Mv-3-glc-4-vinyl procyanidin dimer	nd	tr	tr	tr	tr	tr	tr
34	42.7	559	355	355, 339, 294	298, 361, 494	B-type vitisin of Mv-3-acetylglc	*	*	tr	tr	tr	tr	tr
38	44.1	1135	931, 845, 641, 503	641, 595		Mv-3-acetylglc-4-vinyl procyanidin dimer	nd	tr	tr	tr	nd	tr	tr
39	44.4	707	399	399, 383, 338, 367, 310, 355		A-type vitisin of Mv-3-(*p*-coumaroyl)glc	nd	*	*	*	*	*	*
45	46.2	581	419, 400, 383	419, 400	504	Dp-3-glc-4-vinylphenol	nd	*	*	*	*	*	*
48	46.7	611	449, 531	434, 449, 417, 406		Pt-3-glc-4-vinylcatechol	nd	tr	tr	*	tr	*	*
50	47.0	805				Mv-3-glc-4-vinylcatechin	nd	tr	tr	tr	tr	tr	tr
56	48.1	663			497	B-type vitisin of Mv-3-(*p*-coumaroyl)glc	*	*	tr	tr	tr	tr	tr
58	48.6	595	433	418, 433, 402	504	Pt-3-glc-4-vinylphenol	nd	*	*	*	*	*	*
59	48.9	805				Mv-3-glc-4-vinylepicatechin	nd	tr	tr	tr	nd	tr	tr
60	49.2	625	463	447, 463, 402, 430	300, 352, 380, 512	Mv-3-glc-4-vinylcatechol	nd	*	*	**	*	**	*
63	50.8	579	417	417, 402	275, 403, 499	Pn-3-glc-4-vinylphenol	nd	*	tr	*	tr	*	tr
64	51.1	609	447	447, 431, 386, 414, 358	294, 336, 410, 504	Mv-3-glc-4-vinylphenol	nd	*	**	**	*	**	**
65	51.3	609				Pn-3-glc-4-vinylguaiacol	nd	nd	tr	nd	tr	nd	nd
66	51.3	667	463	447, 463, 375	513	Mv-3-acetylglc-4-vinylcatechol	nd	*	*	*	tr	*	tr
67	51.6	639	477	477, 461, 445, 416, 309	512	Mv-3-glc-4-vinylguaiacol	nd	*	*	*	*	*	*
68	53.4	651	447	447, 431, 386, 358, 414	507	Mv-3-acetylglc-4-vinylphenol	nd	*	*	*	*	*	*
69	53.8	771				Mv-3-(*p*-coumaroyl)glc-4-vinylcatechol	nd	tr	tr	*	tr	*	tr
70	53.9	681			513	Mv-3-acetylglc-4-vinylguaiacol	nd	tr	tr	tr	tr	tr	tr
71	55.9	755			283, 313, 410, 508	Mv-3-(*p*-coumaroyl)glc-4-vinylphenol	nd	*	*	*	*	tr	*
						Direct Condensation Products							
1	10.7	797	635, 467, 373, 617	467, 617, 373, 509, 331		Mv-3-glc-GC	tr	*	*	*	*	*	*
2	11.0	753				Dp-3-glc-C	nd	*	*	*	*	*	tr
3	16.2	767	605, 453, 359, 587	359, 587, 453, 329, 252		Pt-3-glc-C	tr	*	*	*	*	*	*
5	20.4	751				Pn-3-glc-C	tr	*	tr	*	*	*	tr
6	21.0	781	619, 601, 373	373, 467, 601, 331	533	Mv-3-glc-C	tr	*	**	**	**	**	*
20	37.3	823	619, 601, 467, 373	467, 373, 285, 331		Mv-3-acetylglc-C	nd	*	*	*	*	*	tr
37	43.7	927	619, 467, 373	373, 467, 493, 451, 331, 257		Mv-3-(*p*-coumaroyl)glc-C	tr	*	*	*	tr	*	tr
						Acetaldehyde-Mediated Condensation Products							
25	39.9	795	343, 505, 633, 481, 435	328, 343, 205		Pt-3-glc-8-ethyl-C	nd	*	tr	tr	tr	tr	tr
26	41.0	825				Mv-3-glc-8-ethyl-GC	nd	tr	nd	nd	nd	nd	nd
29	41.4	809	357, 519, 647, 331, 657	357, 341		Mv-3-glc-8-ethyl-C	nd	*	*	tr	tr	tr	nd
30	41.5	825				Mv-3-glc-8-ethyl-GC	nd	*	tr	tr	tr	tr	tr
33	42.5	809	357, 519, 647, 331, 657	357, 341		Mv-3-glc-8-ethyl-C	nd	*	*	tr	tr	tr	tr
35	43.4	809	357, 519, 647, 331, 657	357, 341		Mv-3-glc-8-ethyl-EC	nd	*	*	tr	tr	tr	nd
46	46.2	851				Mv-3-acetylglc-8-ethyl-(epi)C	nd	*	tr	tr	tr	tr	tr
52	48.0	851				Mv-3-acetylglc-8-ethyl-(epi)C	nd	tr	tr	nd	nd	nd	nd
54	48.1	955	357, 665, 647, 803	357, 341, 325, 313		Mv-3-(*p*-coumaroyl)glc-8-ethyl-(epi)C	nd	*	*	*	*	tr	tr
57	48.6	955	357, 665, 647, 803	357, 341, 325, 313		Mv-3-(*p*-coumaroyl)glc-8-ethyl-(epi)C	nd	*	tr	tr	tr	tr	nd

[a] t_R, retention time; GRA, grape sample; W03, W02, W01, W00, W99, W98, Tannat wine samples of the different vintages (2003, 2002, 2001, 2000, 1999, and 1998, respectively); Dp, delphinidin; Cy, cyanidin; Pt, petunidin; Pn, peonidin; Mv, malvidin; Glc, glucoside; acetylglc, (6″-acetylglucoside); *p*-coumaroylglc, (6″-(*p*-coumaroyl)glucoside); C, catechin; EC, epicatechin; GC, gallocatechin; nd, not detected; tr, detected, but not quantified; *, **, ***, semiquantification, corresponding, respectively, to area values ranging from 10^6 to 10^7, from 10^7 to 10^8, and >10^8.


A
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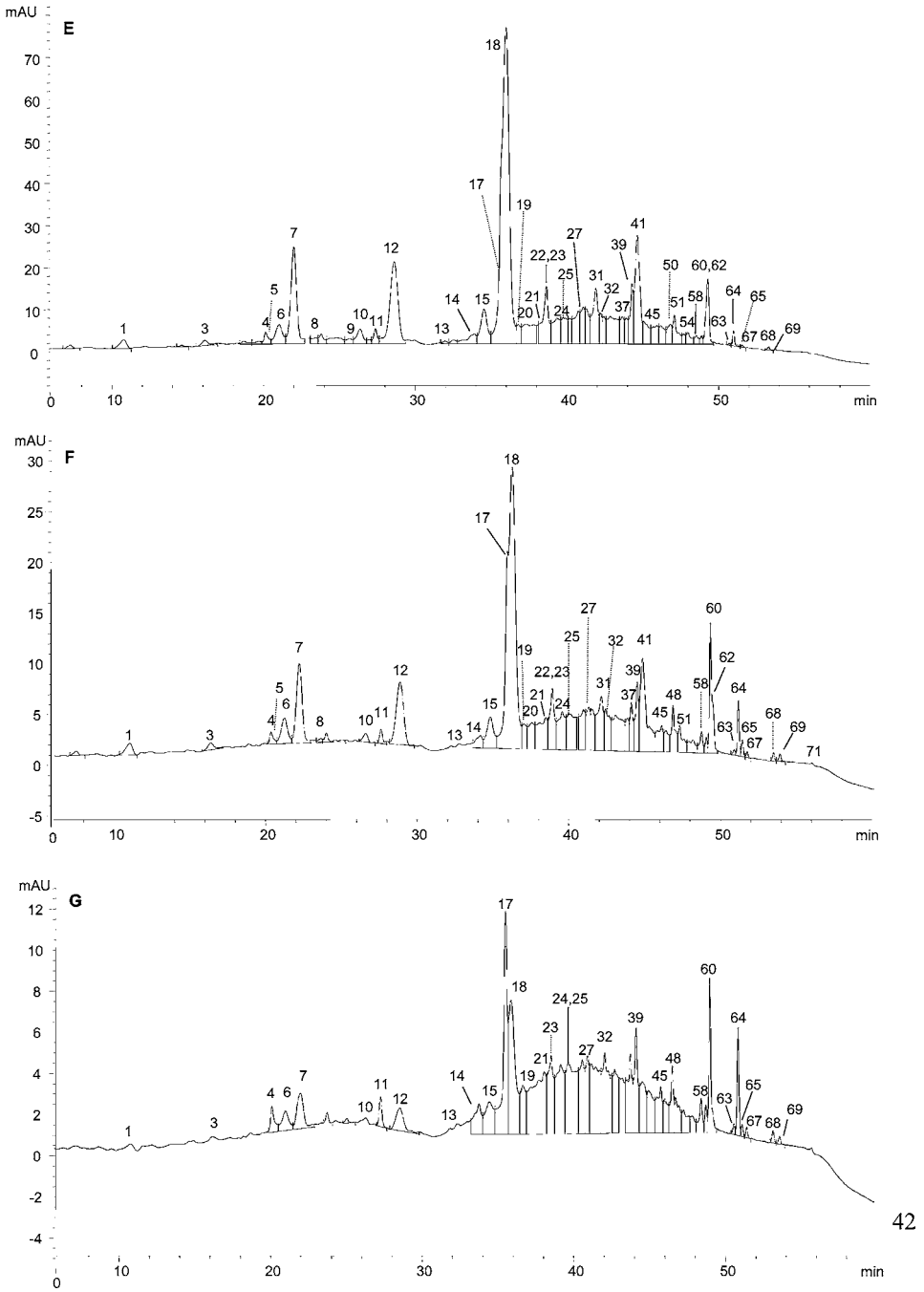

Figure 1. Chromatograms recorded at 520 nm corresponding to the grape extract (**A**) and to the wine samples of different ages (**B**, 4 months; **C**, 16 months; **D**, 28 months; **E**, 40 months; **F**, 52 months; **G**, 64 months). The identities of the peaks are shown in **Table 2**. In order to make possible the localization of these compounds in the chromatograms, they are shown each in full scale.

compounds were detected in small percentages in all of the wines analyzed.

The fragmentation in the MS^2 analysis of the molecular ion of compounds **32** and **38** (*m*/*z* 1093 and 1135, respectively) yielded the same major fragment ion at *m*/*z* 913 by losses of 162 and 204 amu (loss of a glucose and an acetylglucose moiety, respectively). In the MS^3 analysis, both compounds lost 290 amu, corresponding to the loss of one procyanidin extension unit. Thus, these compounds were identified as those originated in the cycloaddition of vinylprocyanidin dimer with malvidin-3-glucoside and malvidin-3-acetylglucoside. These compounds have been previously identified both in wines (*23*, *26*, *32*, *33*) and in model solutions (*32*).

Direct Condensation Products. Compounds **2**, **3**, **5**, and **6** showed fragmentation patterns corresponding to compounds originated by direct condensation between catechin and delphinidin-, petunidin-, peonidin-, and malvidin-3-glucosides, respectively, in agreement with previously reported data in Tannat wine (*2*).

Compounds **20** and **37** eluted later than those compounds. Their molecular ions yielded signals at *m*/*z* values of 823 and 927, respectively, which were 42 and 146 amu higher than that of compound **6** (direct condensation product between catechin and malvidin-3-glucoside). The fragment ions obtained in the MS^2 and MS^3 analyses of both compounds were the same and were identical to those obtained in the fragmentation of compound **6**. These data along with the losses of 204 and 308 amu observed in their respective fragmentations allowed their identification as the direct condensation products between catechin and malvidin-3-acetylglucoside and malvidin-3-(*p*-

coumaroyl) glucoside, respectively. These compounds have been previously reported in wines (*2*, *27*).

The UV−vis spectrum of compound **1** was similar to those of the direct condensation products analyzed, but its earlier elution indicated substitution in the molecule with more polar substituents. The fragmentation pattern allowed the identification of this compound as the product originated by the direct condensation between malvidin-3-glucoside and (epi)gallocatechin. It was proposed to be the non-epi isomer, taking into account the results of previous studies carried out in our laboratory (*2*, *11*), in which two peaks of the same features were detected, the isomer eluting first being proposed to be the direct condensation product between gallocatechin and malvidin-3-glucoside and the second one, the epigallocatechin derivative. Compound **1** was first reported in wines by Alcalde-Eon et al. (*2*).

All of these flavanol−anthocyanin direct condensation products were detected in all of the wine samples analyzed. The direct condensation products between catechin and petunidin-3-glucoside, peonidin-3-glucoside, malvidin-3-glucoside, and malvidin-3-(*p*-coumaroyl)glucoside and that between gallocatechin and malvidin-3-glucoside (compounds **3**, **5**, **6**, **37**, and **1**, respectively) were also detected in the grape sample, although in lower quantity. The presence of flavanol−anthocyanin direct condensation products in grapes has already been reported by González-Paramás et al. (*34*), who identified the dimers between (epi)catechin and peonidin- and malvidin-3-glucosides.

Acetaldehyde-Mediated Condensation Products. Compounds **29**, **33**, and **35** showed the same molecular ion (*m*/*z* 809) and fragmentation patterns. The assignment of the identity of each compound was carried out by considering previous studies performed in our laboratory on young Tannat wines (*2*) and in wines elaborated from other grape varieties (*11*, *27*). Thus, compounds **29** and **33** were assigned to the two possible diasteroisomers of malvidin-3-glucoside-8-ethylcatechin, and compound **35** was assigned as the most abundant of the two possible isomers of malvidin-3-glucoside-8-ethylepicatechin.

Compounds **26** and **30** had the same molecular ion (*m*/*z* 825), which possessed 16 amu more in their *m*/*z* ratios than compounds **29**, **33**, and **35**, which suggested the presence of an additional hydroxyl group in the molecule. These two peaks were identified as the two possible isomers of malvidin-3-glucoside-8-ethylgallocatechin by taking into account previous studies in red wines (*27*).

Compounds **54** and **57** were identified as the products resulting from the acetaldehyde-mediated condensation reaction between malvidin-3-(*p*-coumaroyl)glucoside and (epi)catechin, and compounds **46** and **52** were identified as two of the four possible dimers of malvidin-3-acetylglucoside-8-ethyl(epi)-catechin. In the case of these acylated compounds, only two isomers were detected, although in both cases the second isomer in elution order was in lower amount or could not be quantified in the oldest samples. Thus, the first isomer of each pair of compounds might be identified as the dimer containing catechin, and the second isomer might be identified as the one derived from epicatechin, in accordance with the elution order and proportions observed for the corresponding nonacylated dimers.

Quantitative Analysis. In this study, a vertical row of wine samples produced from Tannat grapes was analyzed. However, climatic conditions, time of harvesting, and course of fermentation may slightly differ from year to year and thus could influence the wine composition. Nonetheless, the influence of vintages was kept to a minimum by analyzing a wine derived from the same vineyard, thus eliminating effects of different growing regions, soils, etc., and minimizing climatic and technological factors. As a consequence, the following discussion will focus the relevance of the results on the trend of the evolution with time of the different pigments studied.

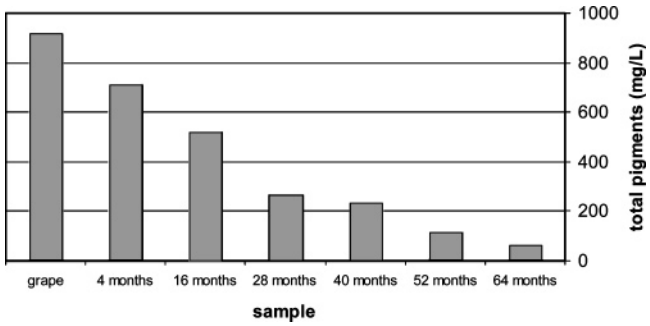

Figure 2. Tannat grape and wine total pigment contents expressed as malvidin-3-glucoside (mg/kg in grape sample and mg/L in wine samples).

Total Pigment Contents. Total pigment contents for grape (mg/kg) and wine (mg/L) samples are shown in **Figure 2**. The total pigment content was calculated from the total area of the peaks obtained in the chromatograms of each wine recorded at 520 nm (**Figure 1**), referred to a malvidin-3-glucoside calibration curve and expressed as this anthocyanin. Remarkable quantitative differences in total pigment contents were noted among the different wine samples, this content being only 9% of the initial value in the oldest samples.

Individual Quantification. The quantitative evolution of the individual compounds, belonging to the different families of anthocyanin-related compounds, was monitored through the areas obtained in the HPLC-MS analyses for their molecular ions in each sample and through their percentages over the total area, which was obtained as the sum of the areas of all the detected compounds. The higher selectivity of the MS technique allowed the monitoring of the changes produced on any individual compound during aging, without the difficulties sometimes associated with the HPLC-DAD analysis (enough peak definition and/or peak overlapping). Furthermore, in the tested concentration ranges the correlation between the area values obtained by HPLC-MS and HPLC-DAD for the anthocyanin monoglucosides was calculated and the correlation coefficient was near 0.98, which indicated a good correlation between both areas. Thus, the area value obtained by HPLC-MS was chosen to be employed in the quantitative analysis.

(a) Tannat Grapes. The contents, expressed as percentages of the area, of the identified compounds in the analyzed grape sample are given in **Table 3**. It can be seen that malvidin- and petunidin-monoglucosides were the major pigments within this family, in the analyzed samples (27.8 and 7.8%, respectively), followed by those of peonidin, delphinidin, and cyanidin. The same hierarchy, in quantitative terms, was observed for the acetyl- and coumaroylglucosides. They do not agree with those reported by other authors, who described the ring-B trisubstituted anthocyanins as the major pigments in Tannat grapes from the southern region of Uruguay (*5*).

The nonacylated monoglucosides of the anthocyanin accounted for 47.2% of the total pigment content, whereas acetylglucosides and coumaroylglucosides represented 22.2 and 28.5%, respectively. These results did not agree with those previously reported for grapes of the Tannat variety (*5*), where the acetylated forms predominated over the coumaroylated ones.

As first reported by Gonzalez-Paramás et al. (*34*), the direct condensation products between (epi)catechin and peonidin-3-glucoside and between (epi)catechin and malvidin-3-glucoside

Table 3. Percentual Grape Composition

compound	percentage
delphinidin-3-glucoside	3.3
cyanidin-3-glucoside	1.6
petunidin-3-glucoside	7.8
peonidin-3-glucoside	6.7
malvidin-3-glucoside	27.8
glucosides	47.2
delphinidin-3-(6″-acetylglucoside)	1.0
cyanidin-3-(6″-acetylglucoside)	0.4
petunidin-3-(6″-acetylglucoside)	3.7
peonidin-3-(6″-acetylglucoside)	2.4
malvidin-3-(6″-acetylglucoside)	14.6
acetylglucosides	22.2
delphinidin-3-(6″-(*p*-coumaroyl)glucoside) (*cis*)	0.2
delphinidin-3-(6″-(*p*-coumaroyl)glucoside)	1.9
cyanidin-3-(6″-(*p*-coumaroyl)glucoside) (*cis*)	0.1
petunidin-3-(6″-(*p*-coumaroyl)glucoside) (*cis*)	0.5
cyanidin-3-(6″-(*p*-coumaroyl)glucoside)	0.9
petunidin-3-(6″-(*p*-coumaroyl)glucoside)	4.0
peonidin-3-(6″-(*p*-coumaroyl)glucoside) (*cis*)	0.3
malvidin-3-(6″-(*p*-coumaroyl)glucoside) (*cis*)	2.5
peonidin-3-(6″-(*p*-coumaroyl)glucoside)	1.7
malvidin-3-(6″-(*p*-coumaroyl)glucoside)	16.7
coumaroylglucosides	28.5
malvidin-3-caffeoylglucoside	0.4
caffeoylglucosides	0.4
vitisin B type pigments	0.3
direct condensation products	0.1
other compounds	1.4

were also found in the Tannat grape sample. In addition, in this work, the dimers of petunidin-3-glucoside and malvidin-3-(*p*-coumaroyl)glucoside with catechin and that of malvidin-3-glucoside with gallocatechin were also detected and quantified. Nevertheless, the direct condensation products accounted for only 0.1% of the total area in the grape sample.

The B-type vitisins of malvidin-3-glucoside, malvidin-3-acetylglucoside, and malvidin-3-(*p*-coumaroyl)glucoside were also detected in the grape sample, and they represented 0.3% of the total pigment content, which is similar to that determined for young Tannat wines. This is a curious result considering that for the formation of B-type vitisins the presence of acetaldehyde is necessary. Nevertheless, as previously stated, the presence of vitisin B in grape juices has already been reported (*22*).

(b) Tannat Wines from Different Vintages. **Figure 3** shows the evolution of the studied families of compounds. Data are expressed as the sum of areas of all the pigments considered for each family and as the percentage of each family of compounds over the total area.

Anthocyanins. During Tannat wine aging, the anthocyanin contents (considering glucosides, acetylglucosides, and *p*-coumaroylglucosides) notably decreased (**Figure 3A**) and only 2% of the initial area (that corresponding to the 4-month sample) was determined in the oldest sample. The relationship between anthocyanins and total pigments also decreased with storage time: 87% of the total area was due to the anthocyanins in the 4-month wine and 32% in the 64-month one. It is worth pointing out that in the oldest sample, the acetylglucosides and coumaroylglucosides represented only 0.33 and 0.55%, respectively, of their initial areas, whereas, in the oldest sample, the monoglucosides still accounted for 33% of their initial area. This might indicate that the loss in the anthocyanin content and in the total pigment area could be closely related to the decreases of the acetates and coumarates. This fact has also been observed in the study of the evolutions of these compounds during the aging of Tempranillo wines, but to a lesser extent, because this study comprised only 2 years of wine life (*11*). It has been postulated (*15*) that the relative higher stability of the nonacylated anthocyanins in relation to the acylated forms might be due to the possibility of the acylated anthocyanins to be hydrolyzed and converted to glucosides, thus increasing the nonacylated compound content and, consequently, decreasing the apparent disappearance rate.

Unlike the Tannat grapes, which showed higher coumaroylglucoside contents than acetylglucoside ones, the Tannat wines, obtained from grapes of the same vineyard, showed the opposite relationship, which is in agreement with that reported for Tannat young wines (*3*). Studies carried out in six different *V. vinifera* grape varieties and in the experimental wines made from them (*10*) have demonstrated that anthocyanin fingerprints of red wines are significantly different from those of grapes used for making them. These authors also stated that these changes in the fingerprints might take place during the alcoholic fermentation. They also reported that the decrease observed in the coumaroylglucoside contents in young wines in relation to grapes was much bigger than that observed for the acetylglucosides in the six wines. In fact, it has been demonstrated that, during the fermentation of Cabernet Sauvignon and Graciano *V. vinifera* grapes by different *Saccharomyces* strains, the cinnamoyl derivatives of the anthocyanins (coumaroyl- and caffeoylglucosides) are the pigments most adsorbed in the lees (*35*, *36*).

Pyranoanthocyanins. The A-type vitisin contents (**Figure 3B**) also diminished with storage time but to a lesser extent than anthocyanins: 27% of the initial area was found in the oldest sample. Previous studies carried out in Cabernet Sauvignon (*37*) and Syrah wines (*13*) have shown that after the formation of the A-type vitisins during alcoholic fermentation, a decay in their levels is observed from malolactic fermentation and during aging. The lesser degradation of the A-type vitisins when compared to anthocyanins can be explained by their higher stability, as a result of the newly formed pyran ring protecting C-4 from nucleophilic attack (*38*). Nevertheless, their contribution to the total pigment content clearly increased with time (17.4% of the total area in the 64-month sample vs 3.5% in the youngest wine sample). The contribution to the total pigment content increased similarly in the cases of the monoglucosides and coumarates of A-type vitisins, whereas the contribution of the acetates remained almost constant with time (data not shown).

The contents of the B-type vitisins (vinyl adducts) decreased with storage time, as occurred for the vinylformic adducts, but in the case of the vinyl adducts the decrease was faster and was followed by a steady state. In fact, 66% of the initial area was lost in the 16-month sample, whereas all of the remaining samples showed levels close to 25% of the initial area. Similar behavior has been observed in Tempranillo wines (*11*). The relationship between the B-type vitisins and the total pigment content slightly increased with storage time (**Figure 3C**).

Figure 3D shows the area values found for the 4-vinylphenol derivatives, which remained almost constant during wine aging. Nevertheless, the percentage over the total pigment content increased (1% when it was 4 months old vs 21% in the 1998 wine). The evolutions of the levels with storage time of the 4-vinylcatechol and 4-vinylguaiacol derivatives were very

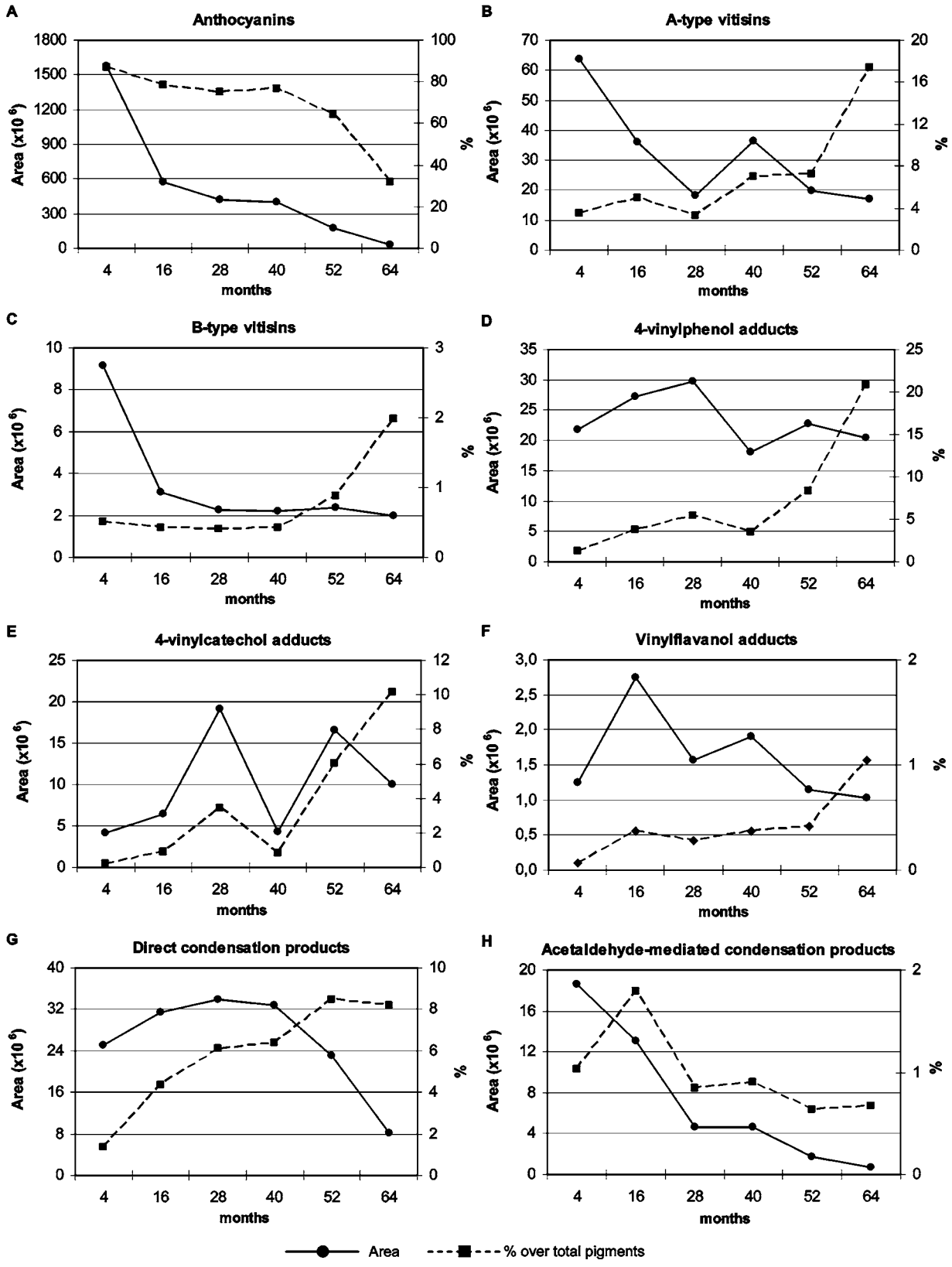

Figure 3. Evolution of the different pigment families in the Tannat wine samples.

similar. Thus, only that corresponding to the 4-vinylcatechol is shown in **Figure 3E**. The percentages over the total area increased in both types of vitisins (0.23% in the 2003 sample vs 10% in the 1998 wine for vinylcatechol adducts and 0.11 vs 2.5% for vinylguaiacol adducts). The maximum area values for the 4-vinylphenol, 4-vinylcatechol, and 4-vinylguaiacol derivatives were found between 16 and 28 months, and then their levels did not undergo an important decrease. This fact might indicate that these compounds were synthesized during all of the study period, which was in agreement with the results found by Schwartz et al. (*30*), which showed that, in addition to the rapid reaction between the anthocyanins and the products of

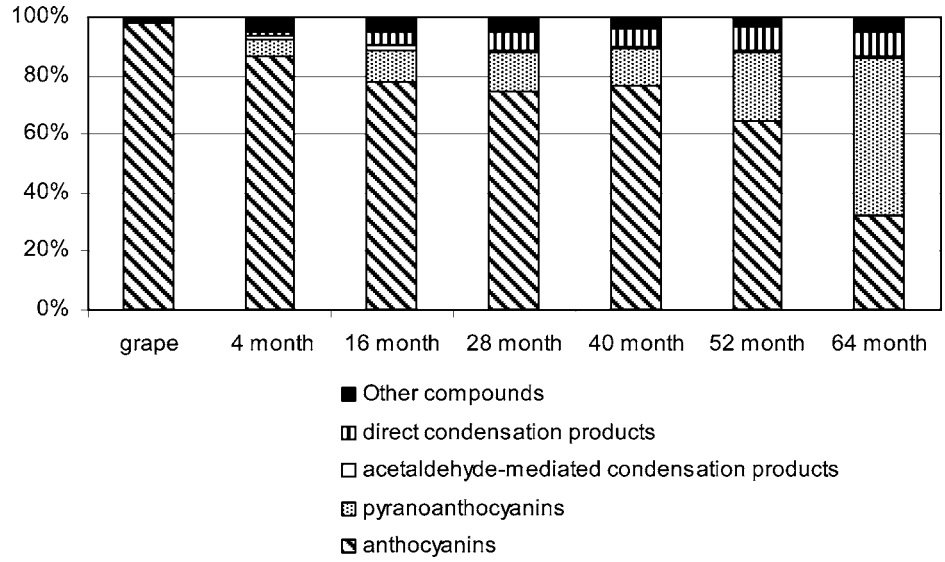

Figure 4. Relative contents of the main pigment families in the different samples analyzed.

the enzymatic decarboxylation of the hydroxycinnamic acid by yeasts (*28*), these pyranoanthocyanins could also be formed directly during aging by the reaction between the corresponding acid and the anthocyanins. Recently, the evolution with time of this kind of pyranoanthocyanins has also been explained, in Tempranillo wines, by these two mechanisms, the formation from the decarboxylation product being important during the earlier stages and the formation from the acid being important during the late maturation and aging (*11*).

The contents of the vinylflavanol (vinylcatechin, vinylepicatechin, and vinylprocyanidin dimer) adducts of the anthocyanins showed an increment in the wine samples at 16 months, but they decreased with storage time (**Figure 3F**). Moreover, as occurred in the other pyroanthocyanins, the vinylflavanol content in the Tannat samples increased with relation to total pigment content during wine aging. Nevertheless, the vinylflavanol content was significantly lower than those of the other pigments, which was in agreement with the results reported by Wang et al. (*26*) in aged Cabernet Sauvignon wines.

Direct Condensation Products. The area values obtained for these derivatives increased during the first 28−40 months and, then, they diminished with storage time (**Figure 3G**). The increase in the levels was observed in the samples for which maturation mostly took place in barrels. On the contrary, the decrease in the levels was observed in the samples that were aged mostly in the bottle. As reported by Alcalde-Eon et al. (*11*) the synthesis of the direct condensation products seems to be more favored during oxidative aging (in barrels) than during aging in the bottle, because, in the barrels, the ellagitannins extracted to the wine along with the oxygen available in them create favorable conditions for their synthesis. In spite of this decrease in the levels observed in the oldest samples, the percentage of this pigment family over the total area clearly increased with time (1% of the total pigments in the 4-month wine vs 9% in the 64-month one).

Acetaldehyde-Mediated Condensation Products. The area values found for these derivatives decreased significantly with time (96.5% of the content was lost in the 1998 sample, taking the 2003 wine as reference) (**Figure 3H**). In relation to the percentage over the total area, these compounds showed an increase to the second year of wine aging (samples still in barrels); from there onward the percentage decreased until values close to those found in the youngest sample and, then, they stayed almost stable. This would indicate that the contribution of these compounds to the total pigment content remains almost constant after wine bottling.

The first step of the formation reaction of these compounds is considered to be the reaction of the acetaldehyde with the flavanols giving rise to an unstable ethanol adduct (*39*), which could, in turn, either be protonated, originating an ethylflavanol cation, or lose a water molecule, giving rise to a vinylflavanol. Both intermediates might react with anthocyanins, originating flavanol−anthocyanin−acetaldehyde-mediated condensation products or pyranoanthocyanins. Because in the transformation of ethanol of the wine into acetaldehyde oxygen is needed (*40*), these reactions would occur only in presence of oxygen and, thus, when the wine is in barrels and not in the bottle. This fact could explain why the higher contents of these compounds were found in the youngest wine samples, because their maturation and aging occur only in barrels.

Figure 4 shows the percentage of the total area corresponding to each family of compounds in Tannat grapes and wines. The anthocyanin family, which accounted for 98% of the total area in grapes, represented only 32% of the total quantified compounds in the 1998 wine (64 months old), although the higher decrease was observed from the 40-month sample. In contrast, the percentage of pyranoanthocyanins over the total area, increased with age, representing 54% in the oldest sample. In this family, the vinyl adducts showed a relevant increase (33% of the total area in the 64-month-old sample). It is worth pointing out that the percentage of the pyranoanthocyanins increased not only by their higher stability compared with that of the anthocyanins but also because some of the members of this family were synthesized during all of the study period, thus maintaining their areas with time in relation to that of the anthocyanins and to the total pigment content. The direct condensation products between flavanols and anthocyanins showed an increasing relevance as Tannat wine aged, representing >8% of the total area in the oldest sample versus 0.1% in grapes and 1.4% in the youngest wine sample. In contrast, acetaldehyde-mediated condensation products decreased significantly after barrel aging, remaining, then, almost constant at low levels (almost 0.8% of the total pigment content).

Thus, in this work, it has been shown that during wine aging the pyranoanthocyanins and, to a lesser extent, the flavanol−anthocyanin direct condensation products acquire an increasing

Table 4. Color Parameter Values for the Wine Samples Analyzed

time of aging (months)	L^*	a^*	b^*	C^*_{ab}	h_{ab}
4	43	56	8	57	8
16	29	54	17	57	17
28	51	46	15	48	18
40	38	53	17	55	18
52	50	45	18	49	21
64	47	46	21	51	24

importance in quantitative terms, the pyranoanthocyanins being the most abundant pigments in the oldest analyzed sample (64 months old). Furthermore, at wine pH, most of these anthocyanin derivatives are present in colored forms, whereas only 15% of the anthocyanins are in flavylium colored form (*41*), which would increase the importance of the anthocyanin derivatives in the color of the wine.

Color Parameters and Correlation with the Different Pigment Families. The CIELAB parameters calculated for the six wine samples are presented in **Table 4**.

A decrease in the a^* (red) parameter along with an increase in the b^* (yellow) parameter was observed as the samples grew older. This change was also verified through the increment in the hue (h_{ab}) values, indicating a color deviation of the wine from purple-red hues to more red-orange hues. However, it was observed that the L^* values (lightness) did not show significant variations related to the aging of the samples, even when an important decrease in the total pigment content was detected.

Table 5 shows the correlation coefficients between the evolution of the different pigment families and CIELAB parameters and the evolution of the percentages over the total pigment content of each pigment family and those of the CIELAB parameters. As wine became older, the area values corresponding to anthocyanins, A-type vitisins, B-type vitisins, and acetaldehyde-mediated condensation products decreased as well as that corresponding to the total pigments, each family to a different extent (**Figure 3**). This could explain the positive and high correlations found for the evolutions of these compounds with the a^* and C^*_{ab} parameters and the negative correlations with the b^* and h_{ab} parameters, even when A- and B-type vitisins are known to possess orange hues. The levels of the vinylphenol adducts and those of the direct condensation products did not correlate either with the a^* parameter or with the b^* parameter and, consequently, either with C^*_{ab} or h_{ab}.

The correlations between the percentages over the total content and the CIELAB parameters supplied more information than the former correlations. The slight variations observed in the L^* value correlated only with the flavanol−anthocyanin acetaldehyde-mediated condensation products and negatively.

The increase observed in the h_{ab} values (see **Table 4**) as wine aged might be attributed, above all, to the increase in the percentage of the direct condensation products and to the decrease of the percentage of the anthocyanins because in both cases the correlation coefficients with h_{ab} are rather high and positive and negative, respectively. This increase of the hue from red-purple to orange-red might also be due to, but to a lesser extent, the increase in the percentage of pyranoanthocyanins and, particularly, to that of the 4-vinylphenol adducts. Considering only the visible absorbance maxima of the direct condensation products, it might be expected that the colors transmitted by these compounds were more bluish than those transmitted by the corresponding anthocyanins, because their visible maxima show a bathochromic shift in relation to those of the anthocyanins, which can be interpreted as a displacement to the blue zone in the visible transmission spectrum. Nevertheless, the higher absorption of the direct condensation products in the range of 400−480 nm (corresponding to the blue zone of the visible absorption spectrum), when compared with the corresponding anthocyanin (**Figure 5**), causes lower transmission or expression of the blue, which might explain why the increase of the direct condensation products with time correlates with

Table 5. Correlation Coefficients between Pigments and Color CIELAB Parameters in the Wine Samples with Different Aging; Coefficients Obtained in the Correlation of the CIELAB Parameters with the Direct Condensation Products and Pyranoanthocyanins in the Three Youngest and Three Oldest Samples[a]

	L^*	a^*	b^*	C^*_{ab}	h_{ab}
anthocyanins	–0.246	0.790*	–0.951**	0.647	–0.984***
pyranoanthocyanins	–0.236	0.725	–0.949**	0.564	–0.968**
A-type vitisins	–0.459	0.924**	–0.819*	0.837*	–0.919**
B-type vitisins	–0.116	0.701	–0.911*	0.570	–0.916**
vinylphenol adducts[b]	0.463	–0.643	0.136	–0.720	0.286
acetaldehyde-mediated condensation products	–0.531	0.881*	–0.803*	0.785	–0.899*
direct condensation products	–0.362	0.353	–0.360	0.251	–0.425
total pigments	–0.523	0.871*	–0.818*	0.765	–0.911*
anthocyanins (%)	–0.389	0.660	–0.738	0.514	–0.802
pyranoanthocyanins	0.390	–0.625	0.699	–0.483	0.763*
A-type vitisins (%)	0.208	–0.440	0.694	–0.272	0.704
B-type vitisins (%)	0.372	–0.506	0.565	–0.376	0.627
vinylphenol adducts (%)	0.470	–0.701	0.687	–0.575	0.775
acetaldehyde-mediated condensation products (%)	–0.885*	0.660	–0.128	0.697	–0.315
direct condensation products (%)	0.458	–0.867*	0.857*	–0.753	0.940**
Tannat 2003, 2002, 2001					
pyranoanthocyanins	0.025	0.754	–0.925	0.612	–0.994
direct condensation products	0.119	–0.841	0.861	0.719	0.968
pyranoanthocyanins (%)	0.187	–0.876	0.824	–0.765	0.948
direct condensation products (%)	0.228	–0.896	0.800	–0.792	0.934
Tannat 2000, 1999, 1998					
pyranoanthocyanins	–0.260	0.406	–0.994	0.224	–0.860
direct condensation products	–0.621	0.735	–0.957	0.592	–0.992
pyranoanthocyanins (%)	0.509	–0.636	0.988	–0.477	0.965
direct condensation products (%)	0.993	–0.999	0.474	–0.988	0.793

[a] *, $p < 005$; **, $p < 001$; ***, $p < 0001$. [b] Vinylphenol adducts include 4-vinylphenol adducts, 4-vinylcatechol adducts, 4-vinylguaiacol adducts, and vinylflavanol derivatives.

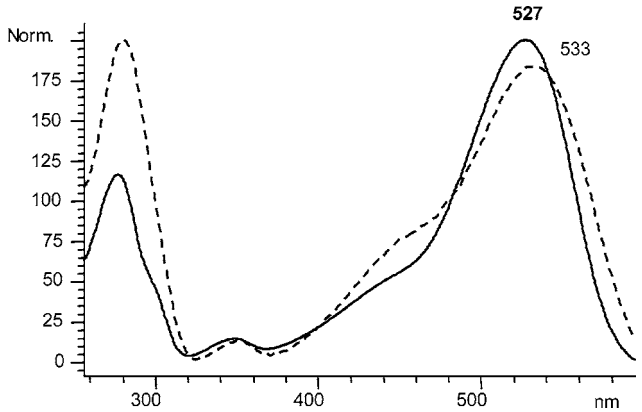

Figure 5. UV–vis spectra of the direct condensation product between malvidin-3-glucoside and catechin (compound **6**, - - -) compared to that of malvidin-3-glucoside (compound **18**, —).

the increase in the hue. Thus, taking into account the absorbances at all of the visible wavelengths, which is the correct way to draw conclusions about color from the data supplied by spectra (**Figure 5** shows only the visible absorption spectra from 270 to 600 nm), it can be expected that direct condensation products are redder and less bluish than their corresponding anthocyanins.

The decrease observed in the C^*_{ab} parameter as the wine samples grew older can be interpreted as a loss of color purity or a displacement toward duller colors in the oldest samples in relation to the youngest ones. Among all of the pigment families, the direct condensation products had a better correlation with this parameter, although the correlation coefficient was negative and not very high (−0.753).

It is worth pointing out that the highest correlations were observed for the h_{ab} parameter, which would mean that the changes observed in the color of the wines as they grew older are qualitative changes (modification of the hue from bluish-red to red-orange) rather than quantitative ones (changes in lightness or in color saturation).

The same conclusions can be drawn from the a^* and b^* parameters, because h_{ab} and C^*_{ab} are calculated from the former ones. Thus, the decrease observed in the red component is mainly due to the increase of the percentage of the direct condensation products and, to a lesser extent, the decrease of the percentage of anthocyanins. The increase in the blue-yellow component is also related to the increase of the percentage of the direct condensation products and to the increase of the percentages of the different types of vitisins. When the behavior of the h_{ab} parameter (**Table 4**) is compared with those of the different families (**Figures 3** and **4**), it can be observed that, in the last samples, the increase in the h_{ab} values did not correspond with an increase in the direct condensation product levels, which, in turn, tend to stabilize, but seemed to be more related to the increase observed in the levels of the pyranonanthocyanins. In fact, the percentage of the direct condensation products tends to stabilization, because, as mentioned previously, once the wine is in the bottle (nonoxidative aging) the synthesis of this kind of anthocyanin derivative is not favored. From this moment onward, these compounds will undergo degradation simultaneously to the decrease of the total pigment content, which could explain the stabilization of their levels. On the contrary, taking into account the results obtained in previous studies (*11*) and after observing in **Figure 3** the evolution of the absolute values of the areas of the pyranoanthocyanins, it seems that they might be synthesized even in the bottle, except in the cases of the A-type vitisins and vinylflavanol derivatives. Furthermore, these compounds are more resistant to degradation than anthocyanins, which in the oldest samples showed an important decrease in their levels (**Figures 3** and **4**). Thus, their lower degree of degradation and the possibility of being synthesized in the bottle might be the causes of the remarkable increase of the percentage of the pyranoanthocyanins in the last samples (**Figure 4**), which could correlate with the increase of the hue observed in the aged samples.

Thus, it seems that the color changes observed in the samples for which evolution mostly took place in barrels might be attributed to the increase of the percentage of the direct condensation products and pyranoanthocyanins and to the decrease of that of the anthocyanins, whereas the color changes observed in the last samples might be attributed above all to the increase of the percentage of the pyranoanthocyanins and to the decrease of that of the anthocyanins. In order to verify this hypothesis, the correlations between the CIELAB parameters and the levels and percentages of the pyranoanthocyanins and the direct condensation products have also been calculated for the three youngest samples and for the three oldest ones separately. As can be seen in **Table 5**, in the youngest samples, similar correlation coefficients have been obtained for the percentages of the direct condensation products and the pyranoanthocyanins (quite good for the h_{ab} values), whereas, in the oldest samples, the correlation coefficients for h_{ab} and these compounds were higher for the pyranoanthocyanins than for the direct condensation products.

Summarizing, the decrease in the anthocyanin levels would allow the expression of the color of the anthocyanin derivatives: pyranoanthocyanins and direct condensation products during the three first years of aging, and in the last 3 years that of the pyranoanthocyanins above all. Nevertheless, the contribution to the wine color of the direct condensation products is probably lower than that deduced from the correlation coefficients because, recently, it has been reported that the flavanol−anthocyanin adducts possessed a resistance against hydration similar to that of monomeric anthocyanins (*42*), thus increasing the contribution of the pyranoanthocyanins to the wine color.

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