JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Occurrence of Pyranoanthocyanins in Sparkling Wines Manufactured with Red Grape Varieties

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The anthocyanin pigments in rosé (*Vitis vinifera* cv. Garnacha) and blanc de noir (*V. vinifera* cv. Monastrell) base and sparkling wines were studied by LC-DAD/ESI-MS. Anthocyanins of grape origin and pyranoanthocyanins resulting from C-4/C-5 cycloaddition of the former ones with pyruvic acid, acetaldehyde, 4-vinylphenol, 4-vinylguaiacol, and 4-vinylcatechol were identified in the different wines. Rosé wines presented a higher total pigment content than blanc de noir wines. Pyranoanthocyanins represented 68.9−76.0% of total pigment content in rosé wines and 49.4−60.7% in blanc de noir wines. Malvidin 3-glucoside-pyruvate was the most abundant pigment in both rosé and blanc de noir base wines. Important qualitative and quantitative changes were observed in terms of the anthocyanin and pyranoanthocyanin pigments after the second (bottle) fermentation and 9 months of aging on yeast lees, but not after a further time (3−9 additional months) of aging on lees. Evaluation of the wine color characteristics was consistent with a greater color stability for the rosé sparkling wines that could be associated with the high content, structural diversity, and spectroscopic features of the pyranoanthocyanins present in these wines.

KEYWORDS: Sparkling wines; rosé; blanc de noir; color; pyranoanthocyanins; yeast

INTRODUCTION

Although sparkling wines are usually produced with white grape varieties, red varieties are often used in sparkling winemaking for producing rosé (partially fermented with skins) and blanc de noir (fermented without skins) sparkling wines. The anthocyanin content of these wines depends on the grape variety, press intensity, maceration time, and fermentation temperature employed. Color is therefore an important parameter for evaluating the quality of sparkling wines produced with red grape varieties.

During winemaking and aging, grape anthocyanins are progressively converted into more stable pigments which are associated with important changes in the color and flavor characteristics of wines. The products resulting from the direct (*1*−*3*) and acetaldehyde-mediated (*2*−*4*) condensation reactions between anthocyanins and flavanols, first postulated as the main mechanisms of formation of these new pigments (*5*, *6*), have now been confirmed in red wines. More recently, a new class of stable anthocyanin-derived pigments (pyranoanthocyanins) resulting from the cycloaddition of C-4 and the hydroxyl group at C-5 of the anthocyanin with two double-bonded carbons of another molecule have been detected in wine (*7*). Many of the molecules capable of giving rise to pyranoanthocyanins comprise secondary metabolism products arising from yeast glycolysis during alcoholic fementation such as pyruvic acid (*8*−*10*), acetaldehyde (*11*), and acetone (*11*, *12*). Other secondary products exhibiting keto−enolic tautomerism, including α-ketoglutaric acid, 3-hydroxybutan-2-one (acetoine) (*11*), and 2,3-butanedione (diacetyl) (*13*), are also susceptible to undergoing anthocyanin C-4/C-5 cycloaddition reactions. Adducts of anthocyanins with 4-vinylphenol and 4-vinylguaiacol, volatile phenols resulting, respectively, from the enzymatic decarboxylation of *p*-coumaric and ferulic acids by yeast cinnamate decarboxylase (*14*), have also been identified in red wines. Moreover, the occurrence of the pigments malvidin 3-glucoside-4-vinylcatechol and malvidin 3-glucoside-4-vinylsyringol in red wines, tentatively proposed by Hayasaka and Asenstorfer (*12*), has recently been confirmed (*15*, *16*). Vinylcatechin, vinylepicatechin, or vinyldicatechin (procyanidin B2) adducts of malvidin 3-glucoside, first reported to occur in model solutions containing malvidin 3-glucoside, acetaldehyde, and the respective flavan-3-ol (*17*), have also been established in wines (*4*, *18*−*20*). Pyranoanthocyanins exhibit orange-red hues and because of their structure properties, they are very stable, present greater resistance to color bleaching by sulfur dioxide, and express more color at higher pH values than their precursor anthocyanins (*3*, *21*, *22*). Recently, it has been demonstrated that some pyranoantochyanins (anthocyanin−pyruvic acid adducts) can react with vinylflavanol derivatives, resulting in novel pigments that exhibit a blue color (*23*).

The occurrence of anthocyanin-derived pigments in wine may be influenced by the concentration of the anthocyanin and non-

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10.1021/jf030639x CCC: $27.50
Published on Web 02/07/2004

anthocyanin precursors, which in turn depends on the grape variety used and the winemaking technology (yeast strain, fermentation, and aging conditions) (*4*, *24*−*26*). Recently, the maximum rate of malvidin−pyruvic acid synthesis has been reported to occur in the period corresponding to 20−85% of glucose utilization, coinciding with the maximum concentration of pyruvic acid (*27*). These findings agree with the fact that this adduct is the main pigment in aged Port wine (*24*), the alcoholic fermentation of which is stopped when ∼50% of the sugars have been utilized by yeast. However, to our knowledge there is no information concerning the occurrence and evolution of anthocyanin-derived pigments in wines that undergo a second fermentation and a postfermentation aging on yeast lees under reducing conditions (bottle), as occur during the production of sparkling wines made with red grape varieties. The aim of the present work was to the determine the occurrence of anthocyanin-derived pigments in sparkling wines (rosé and blanc de noir) at different stages of the traditional (champenoise) production. The influence of these compounds on the color characteristics of these wines is discussed.

MATERIALS AND METHODS

Samples. Two varietal base wines were industrially manufactured from Garnacha and Monastrell red grape varieties. The Garnacha wine was a rosé (partially fermented with skins), and the Monastrell wine was a blanc de noir (fermented without skins). From both base wines, a batch of sparkling wines was industrially manufactured by using the "méthode champenoise". Disgorging was performed after 9 and 12 months of aging on yeast lees for the Garnacha wine and after 9 and 18 months of aging on yeast lees for the Monastrell wine. Malolactic fermentation took place in both base wines before the second fermentation. For each wine, two bottles were mixed and homogenized before sampling. Homogenized wines were centrifuged at 5000*g* for 15 min at 15 °C. The Garnacha base wine had a pH of 3.06 and an alcoholic degree of 10.7% (v/v), which increased 1 degree during the second alcoholic fermentation. The Monastrell base wine had a pH of 3.29 and an alcoholic degree of 10.9% (v/v), which increased 1.5 degrees during the second alcoholic fermentation.

Sample Preparation. A volume of 15 mL of wine was lyophilized and reconstituted in 1 mL of water/ethanol (9:1, v/v) and then centrifuged at 2200*g* for 10 min. The supernatant was collected and analyzed by liquid chromatography−electrospray mass spectrometry (LC-DAD/ESI-MS).

LC-DAD/ESI-MS. A Hewlett-Packard series 1100 (Palo Alto, CA) chromatography system equipped with a diode array detector (DAD) and a quadrupole mass spectrometer (Hewlett-Packard series 1100 MSD) with a electrospay interface was used. Separation was performed on a 150 mm × 3.9 mm i.d., 4 μm, reverse-phase Waters Nova-Pak C_{18} column (Waters, Milford, MA) at room temperature, according to the method described by Monagas et al. (*25*). A gradient consisting of solvent A (water/formic acid, 90:10, v/v) and solvent B (water/methanol/formic acid, 45:45:10, v/v/v) was applied at a flow rate of 0.7 mL/min as follows: 15−80% B linear from 0 to 30 min, 80% B isocratic from 30 to 43 min, followed by washing (methanol) and re-equilibration of the column from 43 to 75 min. One hundred microliters of wine, previously filtered through a 0.45 μm membrane, was injected onto the column. Diode array detection was performed from 260 to 600 nm. Quantification was carried out by area measurements at 530 nm, and the anthocyanin content was expressed as malvidin 3-glucoside (Estrasynthese, Genay, France) by a standard calibration curve; coefficients of variation were ≤2.0%. The ESI parameters were as follows: drying gas (N_2) flow and temperature, 10 L/min and 350 °C; nebulizer pressure, 380 Pa (55 psi); and capillary voltage, 4000 V. The ESI was operated in positive mode scanning from *m*/*z* 100 to 1500 using the following fragmentator voltage gradient: 100 V from 0 to 17 min and 120 V from 17 to 55 min. Analysis were conducted in duplicate.

Measurement of Wine Color Variables. A direct measurement of wine absorbance at 420, 520, and 620 nm was carried out in a DU 70 Beckman spectrophotometer (Beckman, Los Angeles, CA), using 1 mm (for rosé wines) and 5 mm (for blanc de noir) path length quartz cuvettes. The following color variables were calculated as described by Glories (*28*): color intensity, percent of red, yellow, and blue components, and % dA (proportion of red component produced by the flavilium cations of the free and bound anthocyanins). The tint was calculated according to the method of Saudraud (*29*). Color determinations were conducted in duplicate.

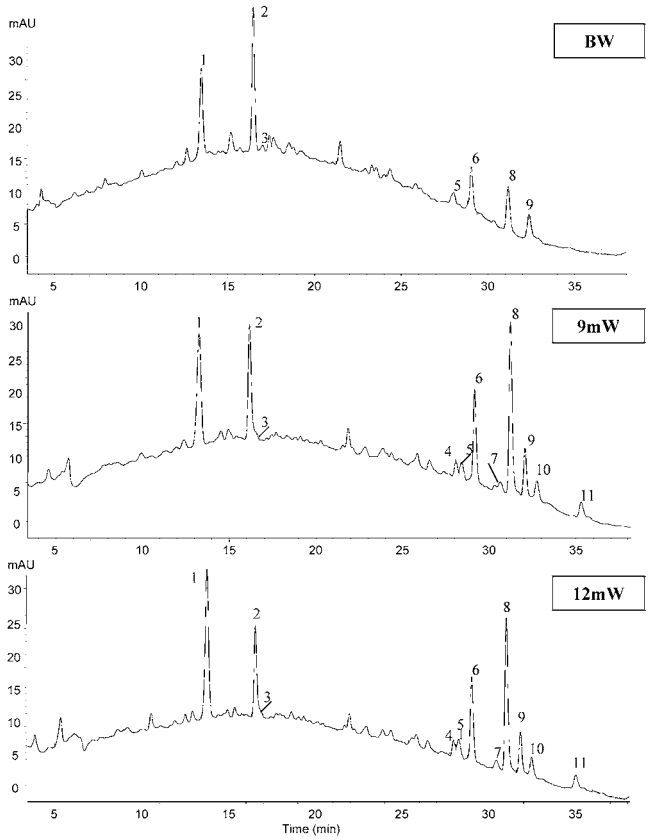

Figure 1. LC chromatograms at 530 nm of rosé (cv. Garnacha) wines: base wine **(**BW**)** and sparkling wines after 9 **(**9mW**)** and 12 **(**12mW**)** months of aging on yeast lees. Peak numbers refer to **Table 1**.

RESULTS AND DISCUSSION

Identification of Anthocyanins and Anthocyanin-Derived Pigments by LC-DAD/ESI-MS. As representative examples, the anthocyanin LC chromatograms corresponding to the rosé base wine and its corresponding sparkling wines after 9 and 12 months of aging on yeast lees are illustrated in **Figure 1**. Among the anthocyanins of grape origin, malvidin 3-glucoside (peak 1) and malvidin 3-(6″-*p*-coumaroylglucoside) (peak 5) were the only pigments identified **(Table 1)**. The molecular ions $[M]^+$ as well as the fragments corresponding to the anthocyanidin after cleavage of the (esterified) glucose moiety were observed **(Table 1)**.

The rest of the peaks identified in these wines by LC-DAD/ESI-MS corresponded to pyranoanthocyanins formed during the two different alcoholic fermentations and during the postfermentation aging on yeast lees. Peak 2, presenting signals at *m*/*z* 561 and 399 (aglycon cation), corresponded to the product resulting from the C-4/C-5 cycloaddition of pyruvic acid and malvidin 3-glucoside (*8*, *9*) **(Table 1**; **Figure 2A)**. Peak 3, showing mass signals at *m*/*z* 517 and 355, corresponded to the adduct resulting from the reaction of malvidin 3-glucoside with acetaldehyde (*11*), also known as vitisin B (*21*) **(Table 1)**.

Table 1. Anthocyanin and Pyranoanthocyanin Pigments Identified in the Rosé (cv. Garnacha) and Blanc de Noir (cv. Monastrell) Wines by LC-DAD/ESI-MS

peak	rel t_R[a]	λ_{max} (nm)	$[M]^+$ (*m*/*z*)	fragment (*m*/*z*)	compound	rosé	blanc de noir
1	1.00	526	493	331	malvidin 3-glucoside	*[b]	*
2	1.24	512	561	399	malvidin 3-glucoside pyruvate	*	*
3	1.25	512	517	355	maldivin 3-glucoside acetaldehyde	*	–
4	2.12	503	595	433	petunidin 3-glucoside-4-vinylphenol	*	–
5	2.15	535	639	331	malvidin 3-(6″-*p*-coumaroylglucoside)	*	–
6	2.20	510	625	463	malvidin 3-glucoside-4-vinylcatechol	*	*
7	2.31	504	579	417	peonidin 3-glucoside-4-vinylphenol	*	–
8	2.36	504	609	447	malvidin 3-glucoside-4-vinylphenol	*	*
9	2.42	508	639	477	malvidin 3-glucoside-4-vinylguaiacol	*	–
10	2.47	508	651	447	malvidin 3-(6″-acetylglucoside)-4-vinylphenol	*	–
11	2.67	505	755	447	malvidin 3-(6″- *p*-coumaroylglucoside)-4-vinylphenol	*	–

[a] Retention time relative to that of malvidin 3-glucoside. [b] *, detected; –, not detected.

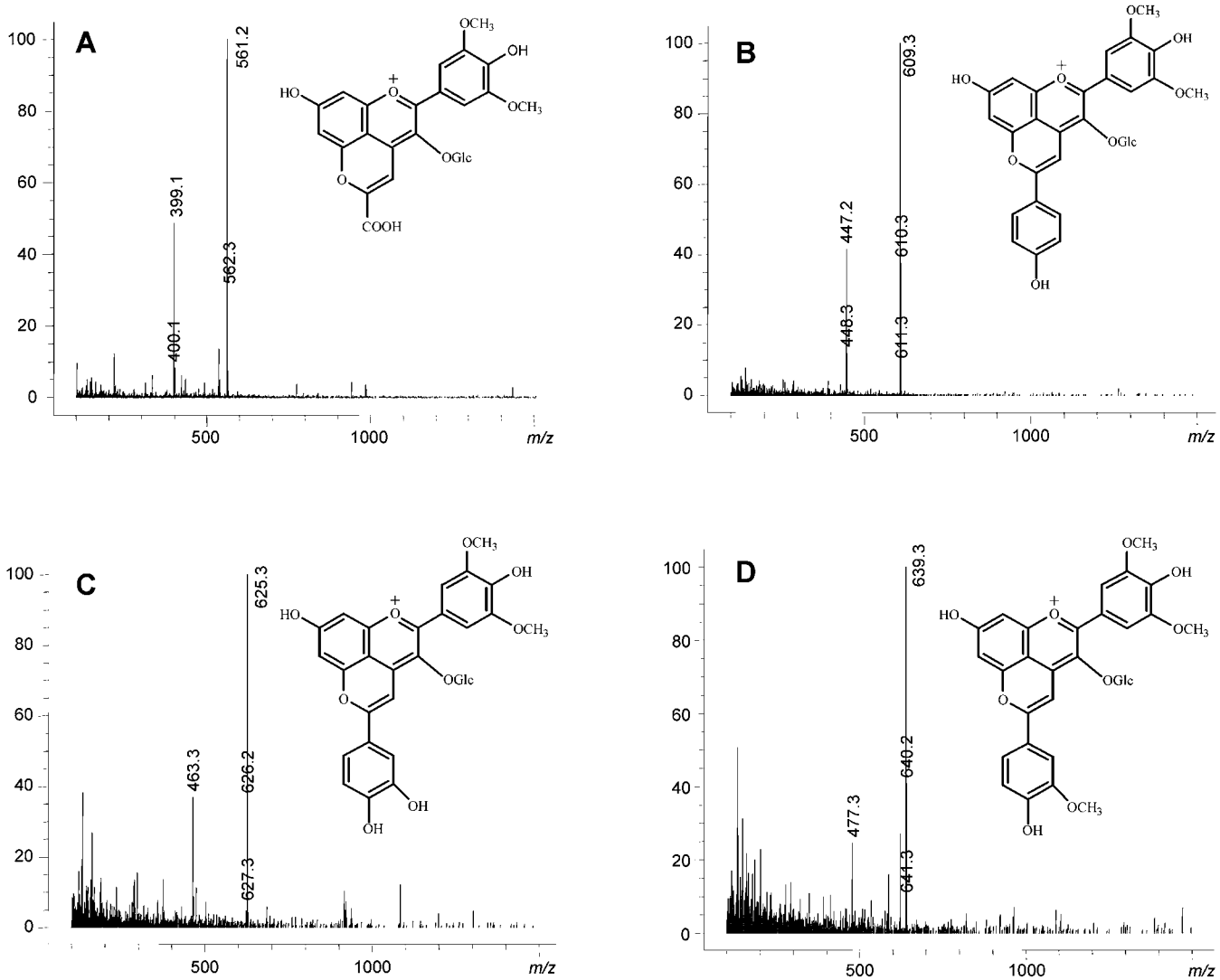

Figure 2. Mass spectra and structures of main pyranoanthocyanins identified in the wines studied: (**A**) malvidin 3-glucoside-pyruvate; (**B**) malvidin 3-glucoside-4-vinylphenol; (**C**) malvidin 3-glucoside-4-vinylcatechol; (**D**) malvidin 3-glucoside-4-vinylguaiacol.

Another series of pyranoanthocyanin pigments exhibiting lower polarity (peaks 6, 8, and 9) was also detected in the different wines **(Figure 1**; **Table 1)**. Peak 8 (*m*/*z* 609, 447) corresponded to the adduct resulting from the C-4/C-5 cycloaddtion of malvidin 3-glucoside and 4-vinylphenol **(Figure 2B)** (*7*, *30*). Peak 6 (*m*/*z* 625, 463) showed a molecular mass consistent with the pigment malvidin 3-glucoside-4-vinylcatechol **(Figure 2C)**, whereas peak 9 (*m*/*z* 639, 477) exhibited mass signals equivalent to the pigment malvidin 3-glucoside-4-vinylguaiacol **(Figure 2D)** (*12*, *15*, *16*). Anthocyanin−vinylphenol adducts are considered to be formed by the reaction of anthocyanins and vinylphenols, the latter being generated during yeast alcoholic fermentation (*7*). Although *Saccharomyces cerevisiae* possesses a type-(*E*) enzymatic activity, substituted cinnamate carboxy-lyase (SCD), which transforms *trans-p*-coumaric and ferulic acids into 4-vinylphenol and 4-vinylguaiacol, respectively, this enzyme displays no activity on caffeic acid (*14*), in contrast to the cinnamate descarboxylase

Table 2. Changes in Anthocyanin Concentration (Micrograms per Liter) during Sparkling Winemaking

		rosé, cv. Garnacha			blanc de noir, cv. Monastrell		
peak	compound	BW[a]	9mW	12mW	BW	9mW	18mW
1	malvidin 3-glucoside	40.7	68.0	79.9	24.3	10.7	10.3
2	malvidin 3-glucoside pyruvate	51.9	59.6	42.9	37.3	16.5	10.1
3	maldivin 3-glucoside acetaldehyde	tr[b]	tr	tr	–	–	–
4	petunidin 3-glucoside-4-vinylphenol	–	12.5	12.1	–	–	–
5	malvidin 3-(6″-*p*-coumaroylglucoside)	11.6	14.2	16.0	–	–	–
6	malvidin 3-glucoside-4-vinylcatechol	22.4	44.5	41.9	–	tr	tr
7	peonidin 3-glucoside-4-vinylphenol	–	10.5	10.0	–	–	–
8	malvidin 3-glucoside-4-vinylphenol	24.9	77.6	72.8	tr	tr	tr
9	malvidin 3-glucoside-4-vinylguaiacol	16.7	25.2	24.2	–	–	–
10	malvidin 3-(6″-acetylglucoside)-4-vinylphenol	–	15.2	15.5	–	–	–
11	malvidin 3-(6″-*p*-coumaroylglucoside)-4-vinylphenol	–	15.5	13.7	–	–	–
	total	168.2	342.8	329.0	61.6	27.2	20.4
	% grape anthocyanin	31.1	24.0	29.1	39.5	39.3	50.6
	% pyranoanthocyanins	68.9	76.0	70.9	60.5	60.7	49.4
	pyranoanthocyanins/grape anthocyanins	2.2	3.2	2.4	1.5	1.5	1.0

[a] BW, base wine; 9mW, 12mW, and 18mW: sparkling wine after 9, 12, and 18 months of aging on yeast lees. [b] –, not detected; tr, trace, <5 μg/L.

presented by some strains of lactic acid bacteria, which is capable of decarboxylating caffeic acid into 4-vinylcatechol (*31*, *32*). However, there is no evidence in the literature related to the formation of 4-vinylcatechol in wines. Another mechanism, recently proposed by Schwarz et al. (*16*), involving the direct reaction between the intact hydroxycinnamic acid and the anthocyanin, is most likely to be responsible for the formation of malvidin 3-glucoside-4-vinylcatechol as well as for most of the other malvidin−vinylphenol adducts identified.

Additional pyranoanthocyanins (peaks 4, 7, 10, and 11), corresponding to the 4-vinylphenol adducts of the −3-glucosides of petunidin (peak 4) and peonidin (peak 7), and malvidin 3-(6″-acetylglucoside) (peak 10) and (6″-*p*-coumaroylglucoside) (peak 11) were also identified **(Figure 1**; **Table 1)**. It is important to note that in contrast to other types of wines (*2*, *4*, *12*, *19*, *20*), the sparkling wines analyzed in this study did not contain other classes of pigments of oligomeric nature, such as C-4/C-5-substituted vinylflavanol pigments or the products resulting from the direct or acetaldehyde-mediated condensation reaction between anthocyanins and flavanol. The lack or low content of (+)-catechin, (−)-epicatechin, and dimeric procyanidins found in these wines (*33*) could explain these observations.

Evolution of Anthocyanins and Pyranoanthocyanin Pigments during Sparkling Winemaking. Due to the short skin maceration time, the rosé base wine **(**BW**)** contained low levels of anthocyanins of grape origin, mainly comprising the pigment malvidin 3-glucoside and its acylated derivative malvidin 3-(6″-*p*-coumaroylglucoside), both together representing only 31.1% of the total anthocyanin content **(Table 2**). Pyranoanthocyanins contributed to the rest of the anthocyanin content (68.9%), malvidin 3-glucoside pyruvate being the most abundant pigment, representing 30.8% of total pigments quantified. The occurrence of anthocyanin-derived pigments has been related to the concentration of the corresponding anthocyanin precursors in wines (*24*, *25*); however, to our knowledge, this is the first time that malvidin 3-glucoside pyruvate is presented in higher concentration than its precursor anthocyanin in a young wine. The same situation was observed for the blanc de noir base wine despite its lower content of malvidin 3-glucoside. However, in contrast to the rosé base wine, the blanc de noir wine did not show the *p*-coumaroyl derivative of malvidin 3-glucoside **(Table 2)**.

Rosé sparkling wines analyzed after a second fermentation in bottle and a further 9 months of aging on lees (9mW) presented changes in their anthocyanin content and distribution. Besides the qualitative changes related to the generation of new pigments (peaks 4, 7, 10, and 11), both anthocyanins of grape origin and pyranoanthocyanins already present in the base wine underwent quantitative changes, which resulted in a total pigment content increase from 168.2 to 342.8 μg/L, accompanied by a pyranoanthocyanin/grape anthocyanin ratio increase from 2.2 to 3.2 **(Table 2)**. The largest increase registered corresponded to the pigment malvidin 4-vinylphenol, which multiplied 3-fold in concentration, being the most abundant compound among individual pigments evaluated. The following hypothesis could be postulated to explain these changes. First, the increase in malvidin 3-glucoside and its *p*-coumaroyl derivative coincides with a decrease in the baseline hump from the BW to 9mW wines **(Figure 1)**, indicating a partial depolymerization of anthocyanin−tannin and/or other condensation products originated due to the short skin maceration time, considering that skin tannins are easily extracted during fermentation (*34*, *35*). In fact, acid hydrolysis of the rosé wines in butanol/HCl (50:50, v/v) (Bate-Smith reaction) revealed the presence of tannins in a concentration equivalent to a mean value of 107.4 mg/L (expressed in terms of cyanidin chloride). The acidic conditions of the wine and the presence of microbial hydrolytic enzymes, derived from the yeast autolysis that takes place during aging on yeast lees (*36*), could have facilitated the breakdown of anthocyanin-condensed products, liberating anthocyanins. However, no increase in monomeric flavan-3-ol or generation of dimeric or trimeric procyanidins was observed from rosé base wines to 9mW sparkling wines (*33*), which is in accordance with the low level of terminal units of skin tannins (high degree of polymerization) as reported by Vidal et al. (*37*). Finally, in the presence of additional anthocyanin precursors and metabolites generated through the fermentations, the cycloaddition reactions with pyruvic acid, 4-vinylphenols, and/or hydroxycinnamic acids, leading to the respective pyranoanthocyanins, could be favored.

This hypothesis could be also supported by the fact that the total anthocyanin content in the blanc de noir base wine elaborated without skin maceration did not increase, but decreased from 61.7 to 27.2 μg/L after the second fermentation in bottle and the same period of aging on lees **(**9mW**)**. The pyranoanthocyanin/grape anthocyanin ratio remained constant at value of 1.5, malvidin 4-vinylcatechol (peak 6) being the only new pyranoanthocyanin identified in these wines, although it

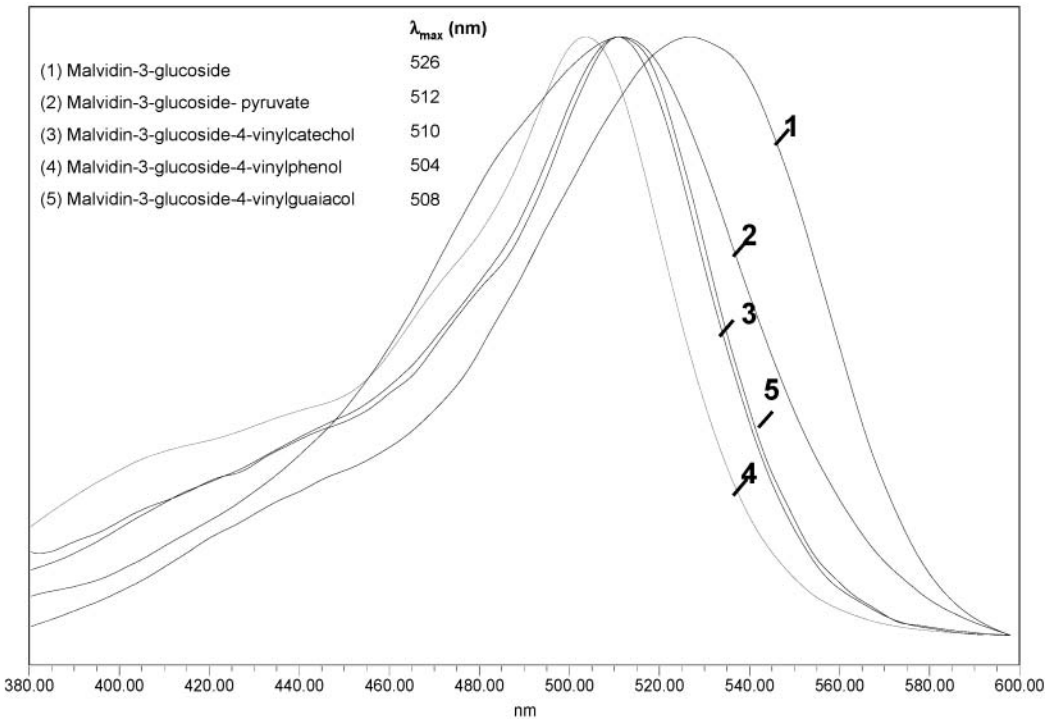

Figure 3. Visible spectra of malvidin 3-glucoside and main pyranoanthocyanins identified in the wines studied.

was detected in trace amounts **(Table 2)**. It is also important to point out that besides not containing a possible extra source of anthocyanins of grape origin, this base wine is also poorer in hydroxycinnamic acids (*33*).

An important distinction has to be made between the two different fermentations taking place during sparkling winemaking. The first alcoholic fermentation occurs under ideal conditions for yeast growth and reproduction, whereas, in the second, yeast fermentation occurs in the bottle under more reducing conditions and accompanied by other stress factors (high acidity, ethanol content, and CO_2 pressure) and by a rapid lack of nutrients that finally ends in the yeast autolysis. These differences in yeast growth and postfermentation conditions could explain the synthesis of different types of pyranoanthocyanins in the rosé sparkling wines. The first fermentation **(**BW**)** was characterized by a high synthesis of malvidin 3-glucoside-pyruvate, whereas in the second fermentation **(**9mW**)**, malvidin 4-vinylphenol was the most abundant pigment produced. The first situation is in accordance with the fact that formation of malvidin 3-glucoside-pyruvate is known to occur mainly after oxygen has been consumed by yeast in the early stages of an aerobic fermentation, coinciding with a 20−85% glucose utilization and maximum pyruvic acid concentration (*27*). In addition, the penultimate step of malvidin 3-glucoside-pyruvate synthesis requires the presence of oxidants that are believed to be the result of the yeast actively converting available oxygen into reactive oxygen species during fermentation (*27*). It seems then that the conditions present in the second fermentation were not ideal for the yeast to synthesize high levels of pyruvic acid, therefore limiting the formation of malvidin 3-glucoside-pyruvate. In contrast, the formation of malvidin 3-glucoside-4-vinylphenol, as postulated by Schwarz et al. (*16*), requires only the presence of malvidin and *p*-coumaric acid, the latter known to be present in both free and bound forms in the rosé base wine studied (*33*). Moreover, the rate of formation of anthocyanin−vinylphenol adducts does not depend on the presence of oxygen (*16*), and therefore the reaction could occur during aging on yeast lees, supporting the observed results.

A prolonged time of aging on lees did not result in the generation of new pigments. The rosé sparkling wines **(**12mW**)** experienced a further increase in the content of anthocyanins of grape origin **(Table 2)**, whereas pyranoanthocyanins remained stable or experienced a slight decrease as in the case of malvidin 3-glucoside-pyruvate, resulting in a pyranoanthocyanin/grape anthocyanin ratio decrease from 3.2 to 2.4. This latter observation was also noted in the blanc de noir sparkling wines after 18 months of aging on lees **(**18mW**)**, in which malvidin 3-glucoside-pyruvate was the pigment that experienced the largest decrease, resulting in a pyranoanthocyanin/grape anthocyanin ratio decrease from 1.5 to 1.0. Despite the described changes, the total pigment content of both wines did not exhibited significant variation during this period.

Changes in Wine Color Characteristics during Sparkling Winemaking. Figure 3 illustrates the visible spectra of the main pyranoanthocyanins identified in the wines studied in comparison with their precursor anthocyanin, malvidin 3-glucoside. Considering their abundance (68.9−76.0% in rosé and 49.4−60.7% in blanc de noir) **(Table 2)**, spectroscopic characteristics, and high structural stability in wine (*3*, *21*, *22*), these pigments may have a major impact on the color of the base and sparkling wines studied.

The rosé base wine presented a higher color intensity than the blanc de noir base wine **(Table 3**). The yellow component represented the highest percentage (50.9%) of total color in the rosé base wine, followed by the red component (42.2%), whereas, in the blanc de noir base wine the red component presented the highest contribution (48.3%) to color, followed very closely by the yellow component (45.2%). This difference in red component proportion was also reflected in the variable % dA, the highest values corresponding to the blanc de noir base wine, which indicates a major proportion of nonmodified anthocyanins of grape origin in this wine, as supported by a lower pyranoanthocyanin/grape anthocyanin ratio in comparison to the rosé base wine **(Table 2)**. In consequence, the high proportion, structural diversity, and maximum absorption wavelength (512−504 nm) **(Figure 3)** of pyranoanthocyanins in the

Table 3. Changes in Wine Color Characteristics during Sparkling Winemaking

	rosé, cv. Garnacha			blanc de noir, cv. Monastrell		
color variable	BW[a]	9mW	12mW	BW	9mW	18mW
color intensity[b]	0.07	0.05	0.05	0.02	0.01	0.01
tint	1.21	1.11	1.48	0.94	1.32	1.47
% yellow	50.9	49.8	55.8	45.2	54.0	56.5
% red	42.2	45.0	37.7	48.3	40.8	38.5
% blue	6.9	5.2	6.5	6.5	5.2	5.0
% da	31.5	38.8	17.4	46.5	27.6	20.2
% yellow/% blue	7.4	9.6	8.6	6.9	10.4	11.3

[a] BW, base wine; 9mW, 12mW, and 18mW, sparkling wine after 9, 12, and 18 months of aging on yeast lees. [b] Expressed in 1 mm path length cuvette.

rosé base wine may be partly responsible for the observed distribution between the red and yellow components, which results in the characteristic red-orange hues typical of this rosé wine. Tannins as well as pigmented polymers in the rosé base wine may also be responsible for the observed color characteristics.

Despite the formation of new pyranoanthocyanins and the increase in total pigment content observed in the rosé sparkling wines after the second fermentation in bottle and 9 months of aging on lees **(**9mW**) (Table 2)**, the yellow component showed approximately the same contribution (49.8%) to the color, but was accompanied by a slight increase in the red component contribution **(Table 3)**. This was not the case of the blanc de noir sparkling wines of the same age **(**9mW**)**, in which the decrease in total anthocyanin content and scarce formation of new pigments observed during the same period of time **(Table 2)** resulted in an 8.0% reduction in red component proportion accompanied by the same increase in yellow proportion. The relationship between the red color modifiers, yellow and blue (% yellow/% blue), also exhibited a higher increase in the blanc de noir sparkling wines. These observations are consistent with a higher color stability for the rosé sparkling wines in comparison with the blanc de noir ones. Considering the type and content of anthocyanin pigments presented in both wines **(Table 2)**, it is most likely that the compounds responsible for the yellow color component in the 9mW blanc de noir sparkling wines are of a different nature from those present in the rosé sparkling wines of the same age.

In the case of the rosé sparkling wines, a prolonged time of aging on lees **(**12mW**)** resulted in a 7.3% decrease in the red component, accompanied by a 6.0% gain in yellow proportion. This situation was also observed in the blanc de noir sparkling wines, although the variations were much less pronounced despite the longer period of aging on lees that these wines experienced **(**18mW**)**. The overall color evaluation of both aged sparkling wines was characterized by small changes in the % yellow/% blue ratio and by a significant reduction in the % dA and increase in tint.

The evidence presented in this work suggests that the red-orange hues typical of rosé sparkling wines are due not only to anthocyanin of grape origin but also to pyranoanthocyanins formed during sparkling winemaking. Differences were observed in the type of pyranoanthocyanins synthesized through the first fermentation and through the second fermentation followed by aging on yeast lees, probably due to differences in yeast performance under very distinctive fermentation conditions and to the process of yeast autolysis that occurs in the bottle after the second fermentation. The scarce presence of pyranoanthocyanins in the blanc de noir wines in comparison to the rosé ones could be associated with their lower level of anthocyanins of grape origin and of other necessary precursor compounds. To our knowledge, this is the first time that pyranoanthocyanins have been identified in rosé and blanc de noir sparkling wines, bringing a new approach to evaluate the importance of color in the quality and characterization of these wines.

ACKNOWLEDGMENT

We are grateful to Castellblanch, S.A. (San Sadurní d'Anoia, Spain) and Dr. Pilar Hidalgo (IMIA, Madrid, Spain) for elaborating the wines for the purpose of this research.

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Received for review September 2, 2003. Revised manuscript received December 8, 2003. Accepted December 8, 2003. We thank the Agencia Española de Cooperación International (AECI) for a MUTIS predoctoral scholarship to M.M. and the Spanish Comisión Interministerial de Ciencia y Tecnología (CICYT) (Project AGL2000-1569) for funding.

JF030639X