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PHENOLIC COMPOSITION AND COLOUR OF *Vitis vinifera* L. cv MERLOT WINES FROM DIFFERENT VINTAGES AND AGING TIME IN BOTTLE

COMPOSIÇÃO FENÓLICA E COR DOS VINHOS DE *Vitis vinifera* L. cv MERLOT DE DIFERENTES VINDIMAS E TEMPOS DE ESTÁGIO EM GARRAFA

R. Suárez, M. Monagas, B. Bartolomé and C. Gómez-Cordovés *

Instituto de Fermentaciones Industriales, CSIC. Juan de la Cierva 3, 28006 Madrid, Spain.Tel: (34) 91 562 29 00; Fax. (34) 91 564 48 53; e-mail address: cgcordoves@ifi.csic.es

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SUMMARY

The effect of aging time in bottle on both anthocyanin and non-anthocyanin phenolic compounds, and on the wine colour characteristics, has been studied in *Vitis vinifera* cv. Merlot wines elaborated from grapes of the same vineyard in 5 consecutive vintages (1997-2001) and presenting different aging time (1-5 years) in bottle (non-oxidative conditions). A total of 22 anthocyanin and 27 non-anthocyanin compounds were quantified in the different wines in order to study the evolution trends and changes in the relative content of these compounds during aging in bottle. Grape anthocyanins and their pyruvate derivatives (vitisins A) showed a significant decrease during the aging time in bottle, whereas vinylphenol derivatives presented an increase in concentration during the same period. In relation to the non-anthocyanin compounds, aging time in bottle seemed to have a larger influence in the concentration of main non-flavonoid phenolics (hydroxybenzoic and hydroxycinnamic acids and their derivatives, stilbenes, and alcohols and other related compounds) than in that of flavonoid phenolics (flavanols and flavonols). Despite of these changes, the relative contents of the main groups of anthocyanin and non-anthocyanin phenolic compounds were slightly modified under the non-oxidative conditions present during bottle aging. Finally, the most important changes in the wine color characteristics (decrease in red, and increase in yellow and blue components) coincided with the largest changes in anthocyanin content.

RESUMO

É estudado o efeito do tempo em garrafa sobre compostos antociânicos e não antociânicos e na cor dos vinhos da casta Merlot, provenientes de uvas da mesma vinha, em 5 vindimas consecutivas (1997-2001), e com diferentes tempos (1 a 5 anos) em garrafa (condições não oxidativa). Um total de 22 antocianinas e 27 não antocianinas foram quantificados nos diferentes vinhos, para o estudo da evolução e das diferenças relativas nos teores destes compostos ao longo do estágio em garrafa. As antocianinas das uvas e os seus derivados pirúvicos (vitisina A) exibiram um decréscimo significativo durante o estágio em garrafa, enquanto os derivados vinilfenol apresentam um acréscimo de teores durante o mesmo período. Em relação aos compostos não antociânicos, o tempo em garrafa parece ter maior influência na concentração dos principais não flavonóides (ácidos hidroxibenzóicos e hidroxicinâmicos e seus derivados, estilbenos, alcoóis e outros compostos relacionados) do que nos flavonóides (flavanóis e flavonóis). Apesar destas diferenças, os teores relativos dos diferentes grupos de antocianinas e de não antocianinas sofreram apenas ligeira modificação nas condições não oxidativas dos estágios em garrafas. Finalmente, as mais importantes diferenças nas características da cor dos vinhos (decréscimo dos componentes vermelho e aumento do amarelo e azul) coincidem com as maiores diferenças nos teores em antocianinas.

Key Words: Merlot, anthocyanins, phenolics, aging in the bottle, color.
Palavras Chave: Merlot, anthocianinas, fenólicas, envelhecimento em garrafa, cor

INTRODUCTION

Phenolic compounds contribute to wine organoleptic characteristics, particularly color, astringency, and bitterness. Wine phenolics belong to two main groups: non-flavonoid (i.e., hydroxybenzoic and hydroxycinnamic acids and their derivatives, stilbenes and phenolic alcohols) and flavonoid (i.e., anthocyanins, flavanols, flavonols and dihydroflavonols) compounds. The main anthocyanins identified in *Vitis vinifera* spp. wines are the 3-*O*-glucosides, 3-*O*-acetyl glucosides and 3-*O*-*p*-coumaroyl glucosides of delphinidin, cyanidin, petunidin, peonidin and malvidin, as well as the 3-*O*-caffeoyl glucosides of malvidin and peonidin. During wine maturation and aging there is a progressive loss of anthocyanin pigments, mainly due to their participation in numerous chemical reactions that lead to the formation of more stable anthocyanin-derived pigments such as the products resulting from the direct and acetaldehyde-mediated anthocyanin-tannin condensation reactions, as well as the products originated from the C-4/C-5 cycloaddition reaction of anthocyanins with other molecules bearing a polarizable double bond (pyruvic acid, 4-vinylphenols, hydroxycinnamic acids, vinylflavanols, acetaldehyde, acetone, among others), giving rise to so called pyranoanthocyanins (Monagas *et al*., 2005a). All these oligomeric and polymeric pigments give rise to wine color changes (from bright red to brick red hues) (Ribéreau-Gayon, 1982).

Non-anthocyanin phenolic compounds, especially hydroxycinnamic acids, flavanols and flavonols, act as copigments of anthocyanins and participate in the

color stabilization of red wine (Eiro and Heinonen. 2002). Flavanols are also responsible for astringency and bitterness of red wines (Gawel, 1998). Besides, several beneficial physiological effects associated with wine consumption have been related to wine phenolic compounds (Scalbert *et al*., 2005). Stilbenes possess anti-inflammatory and anticarcenogenic properties (Jang *et al*., 1997); they inhibit oxidation of human low-density lipoproteins (Frankel *et al*., 1993) and platelet aggregation (Pace-Asciak *et al*., 1995), all of which contributes to limit the risk of cardiovascular diseases.

Young red wines bottled after the end of the winemaking process undergo changes under non-oxidative conditions (bottle aging) until the time they are consumed (Pérez-Magariño and González-San José, 2004; de Beer *et a*l., 2005; Sun and Spranger, 2005; Monagas *et al*., 2005b,c). The real market situation is that wine consumers often choose the wines according to the vintage, but each wine would have a different aging time in the bottle within the commercial life of the product. Therefore, aging time in the bottle is another quality factor that needs to be considered by wine producers. Although it has been reported that the characteristics of the vintage (i.e., weather conditions, viticulture practices, time of harvest, production yield) could affect the phenolic content of grapes (Mazza *et al*., 1999; Boselli *et al*., 2004; Pérez-Magariño and González-San José, 2005), the distribution of the different compounds can be largely maintained from year to year. Thus, it would be possible to study the changes in the relative contents of the different families of phenolic compounds during aging as well as their relationship with wine quality parameters, such as a color, in a certain moment of the wine commercial life. This experimental approach has been adopted by other authors to study the evolution trend of anthocyanin pigments in wines from *Vitis vinifera* L cv. Pinotage (Schwarz *et al*., 2004) and Tannat (Boido *et al*., 2006).

In this paper, we have studied the effect of aging time in the bottle on both anthocyanin and non-anthocyanin compounds, and wine color in a vertical row of young wines elaborated from grapes of *Vitis vinifera* L. cv Merlot (vintages 2001, 2000, 1999, 1998 and 1997) cultivated in the same vineyard and elaborated under the same winemaking conditions. Wines were simultaneously analyzed, their age finally corresponding to 1-5 years old at the time of analysis.

MATERIALS AND METHODS

Materials

Methyl gallate, ethyl gallate and malvidin-3-glucoside were purchased from Extrasynthèse (France). (+)-Catechin, (-)-epicatechin, myricetin, quercetin, tryptophol, *trans*-resveratrol and, gallic, *trans*-caffeic, *trans*-*p*-coumaric, vanillic, protocatechuic and ellagic acids were purchased from Sigma (USA). Syringic acid and tyrosol were purchased from Aldrich (Germany). *Cis*-resveratrol was obtained from the standard of *trans*-resveratrol after exposure to UV light (340 nm) for 1 h. The irradiated solution was used as *cis*-resveratrol standard.

Winemaking

Wines from grapes of *Vitis vinifera* L. cv. Merlot (clon 343) (vintages 2001, 2000, 1999, 1998 and 1997), cultivated in the same vineyard (Navarra, Spain) were elaborated at EVENA (Viticulture and Enology Station of Navarra) under the same vinification conditions. Thus, the parameter associated with a particular growing region that usually influence wine composition (i.e. soil, climatic conditions, etc.), as well as the winemaking techniques, are kept to a minimum from year to year. A lot of 220 kg of grapes of each vintage was de-stemmed, crushed and collected into 200 L stainless-steel wine vats. Semi-industrial scaled fermentations were performed with a yeast inoculum of 25 g/hL (80% EVENA *Saccharomyces cerevisiae* Na33 yeast strain; 20% Lallemand *Saccharomyces bayanus* EC1118 yeast strain) at a temperature not higher than 30ºC. The cap was punched down twice a day until it remained submerged during a 14 day maceration period. After a period of stabilization of one month at –2ºC, the wines were racked, filtrated through SEITZ K250 filters (2.5-3.0 µm) (Sert Schenk Filter System GmB, Bad Krevznach, Germany) and finally bottled after correcting the free SO_2 level to 30 mg/L. The classical wine parameters of the young wines are shown in Table I. Wine bottles were stored at 13ºC and 80-85% relative humidity. Two wine samples from each vintage (1997-2001) were simultaneously analyzed, the age of the wines finally corresponding to 1-5 years old at the time of the analysis. Analysis of phenolic compounds were carried out in duplicate.

Extraction of non-anthocyanin phenolic compounds

A volume of 50 mL of wine was concentrated to 15 mL under vacuum at 30 °C and extracted 3 times with diethyl ether (10 x 15 x 15 mL) and 3 times ethyl acetate (10 x 15 x 15 mL), as described by Fernández de Simón *et al.* (1990). The organic phases were combined and dried with anhydrous Na_2SO_4 for 30 min. The extract was then taken to dryness under vacuum, dissolved in 1 mL of methanol/water (1:1) and finally filtrated (0.45 µm) and injected into the HPLC column. The recovery of phenolic compounds in diethyl ether and ethyl acetate varies in function of their chemical structure (mean recovery value= 87%; mean SD value = 6%) (Fernández de Simón *et al.,* 1990). The extraction of phenolic compounds was performed in duplicate.

TABLE I

Enological parameters determined in the different Merlot wines.
Parâmetros enológicos determinados nos diferentes vinhos Merlot

Parameter	Vintage (aging time in bottle)				
	2001 (1 year)	2000 (2 years)	1999 (3 years)	1998 (4 years)	1997 (5 years)
pH	3.40	3.44	3.70	3.76	4.05
Alcohol (% vol)	14.75	13.45	14.00	14.05	13.55
Volatile acidity (g/L)	0.30	0.25	0.39	0.30	0.47
Total acidity (g/L)	5.4	5.9	4.4	5.0	3.8
Total dried extract (g/L)	27.2	27.5	27.1	28.0	30.0

HPLC analysis of anthocyanin phenolic compounds

A Waters (Milford, MA) HPLC chromatograph equipped with a 600-MS controller, a 7171 plus autosampler, and a 996 photodiode-array detector was used. A gradient 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 to a reverse-phase Nova-Pack C_{18} column (150 x 3.9mm) as follows: 15-80 %B linear (0.8mL/min) from 0 to 30 min, 80 %B isocratic (0.8mL/min) from 30 to 43 min and washing (100% methanol) and re-equilibration of the column from 43 to 75 min. One-hundred microliters of wine, previously filtered through a 0.45-mm membrane, was injected onto the column. Diode-array detection (DAD) 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 by a standard calibration curve. Anthocyanin analysis were performed in duplicate.

For identification purposes, a Hewlett Packard series 1100 (Palo Alto, CA) chromatograph equipped with DAD and MS detectors was used (Monagas *et al*., 2003). Chromatographic conditions were the same than reported above except the flow that was 0.7mL/min. Nitrogen was used as the nebulizing and drying gas. Operation was in positive mode and ESI conditions were as follows: nitrogen pressure, 55 psi; drying gas, 10 mL/min at 350 °C; ion spray voltage, 4000 V; and fragmentador voltage, 100 V from 0 to 24 min and 120 V from 24 to 55 min.

HPLC analysis of non-anthocyanin phenolic compounds

The same chromatographic system described above was used for the analysis of non-anthocyanin phenolic compounds. Separation (5-10 µL of wine extract) was performed on a reverse-phase Waters Nova-Pak C_{18} (300 mm x 3.9 mm, 4µm) column at room temperature. A gradient consisting of solvent A (water/acetic acid, 98:2, v/v) and solvent B (water/acetonitrile/acetic acid, 78:20:2, v/v/v) was applied at a flow rate of 1.0 mL/min as follows: 0-80 % B linear from 0 to 55 min, 80-90% B linear, from 55 to 57 min, 90% B isocratic from 57 to 70 min, 90-95% B linear from 70 to 80 min, 95-100% B from 80 to 90 min, followed by washing (methanol) and re-equilibration of the column from 90-120 min. DAD detection was performed from 220 to 380 nm. Hydroxybenzoic acids, stilbenes, phenolic alcohols, flavanols and flavonols were quantified at 280 nm, caffeic acid and its derivatives at 340 nm and, *p*-coumaric acid and its derivatives at 310 nm. Quantification was carried out by external standard calibration curves. Caffeic and *p*-coumaric acid derivatives, flavonol glycosides and stilbene glucosides were quantified by their respective free form calibration curve. Monomeric and dimeric flavanols were quantified using the (-)-epicatechin calibration curve. Non-anthocyanin phenolic analysis were performed in duplicate.

For identification purposes, a Hewlett Packard series 1100 (Palo Alto, CA) chromatograph equipped with DAD and MS detectors was used (Monagas *et al*., 2005d). Chromatographic conditions were the same than reported above except the flow that was 0.7 mL/min. The ESI parameters were: drying gas (N_2) flow and temperature, 10 L/min and 350 ºC; nebulizer pressure, 40 psi, and capillary voltage, 4000 V. The ESI was operated in negative mode scanning from *m/z* 100 to *m/z* 3000 using the following fragmentation program: from *m/z* 0-200 (100 V) and from *m/z* 200-3000 (200 V).

Condensed tannins

Wines were assayed for proanthocyanidin (condensed tannins) content as described by Ribéreau-Gayon and Stonestreet (1966). Results were expressed as mg of cyanidin/L.

Colorimetric indexes

The absorbance spectra of the wine (200-780 nm) was registered in a 1mm-width quartz cuvette using a Beckman spectrophotometer (Beckman DU-70, USA) at a speed of 2400 nm/min. From the absorbance values at 420, 520 and 620 nm, the

following colorimetric indexes were calculated as described by Glories (1984): Color Intensity (CI), Red %, Yellow %, Blue %, and %dA (pure red). The Tint was calculated according to Sudraud (1958). Color analysis was carried out in duplicate.

Statistical analysis

ANOVA was performed using the PC software package Statgraphics Plus 2.1 (Graphics Software Systems, Rockwille, MD, USA).

RESULTS AND DISCUSSION

Changes in the anthocyanin composition of Merlot wines during aging

Anthocyanidin-3-glucosides,-3-(6-acetyl)-glucosides, -3-(6-*p*-coumaroyl)-glucosides and -3-(6-caffeoyl)-glucosides, as well as several groups of more complex anthocyanin-derived pigments, including pyranoanthocyanins resulting from the C-4/C-5 cycloaddition of anthocyanins with pyruvic acid and vinylphenols/hydroxycinnamic acids, were identified in the different wines by HPLC-DAD/ESI-MS according to Monagas *et al*. (2003) (Figure 1). A total of 22 pigments were quantified in the Merlot wines from the different vintages and aging time in bottle (Table II).

As described for other *Vitis vinifera* spp., the simple glucosides were the most abundant anthocyanin group (Table II). In Merlot wines, acetyl glucosides are presented in higher concentration than cinnamoyl glucosides, as occurs in other French varieties such as Cabernet Sauvignon (Mazza *et al*., 1999; González-Neves *et al*., 2001). A decrease in the anthocyanin content was registered in the vertical row of Merlot wines as the aging time in bottle progressed due to the participation of anthocyanins in condensation and degradation reactions. A 80% loss in the total anthocyanin content was registered from the 1 year-old wine (244.60 mg/L) to the 5 year-old wine (48.37 mg/L) (Table II). These results are in accordance with previous studies carried out with Graciano, Cabernet Sauvignon and Tempranillo wines (vintage 2000) elaborated under the same vinification conditions than those reported in this study and aged during 26 months in bottle (Monagas *et al*., 2005b). The ANOVA analysis carried out between the different groups of anthocyanins (Σ of simple, acetyl and cinnamoyl glucosides) revealed that the 1 and 2 year-old wines were significantly different ($p<0.05$) from each other and from the rest of the wines, whereas the 3 year-old one was not significantly different either from the 4 year-old wine (in the case of simple and cinnamoyl glucosides) or from the 5 year-old wine (in the case of acetyl glucosides). These

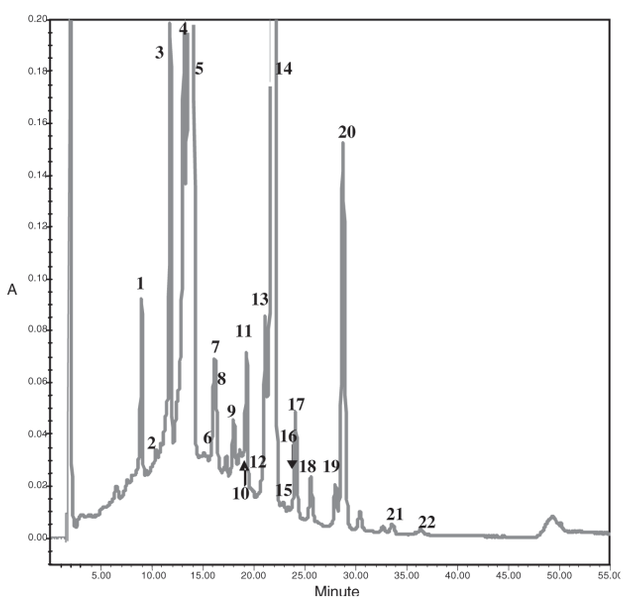

Figure 3 - HPLC-DAD chromatogram of anthocyanins identified in wines from *Vitis vinifera* L. cv Merlot.
Cromatogramas HPLC-DAD das antocianinas identificadas em vinhos de Vitis vinifera L. cv Merlot.

1. Delphinidin-3-glucoside, 2. Cyanidin-3-glucoside, 3. Petunidin-3-glucoside, 4. Peonidin-3-glucoside, 5. Malvidin-3-glucoside, 6. Peonidin-3-glucoside pyruvate, 7. Delphinidin-3-(6-acetyl)-glucoside, 8. Malvidin-3-glucoside pyruvate, 9. Malvidin-3-(6-acetyl)-glucoside pyruvate, 10. Cyanidin-3-(6-acetyl)-glucoside, 11. Petunidin-3-(6-acetyl)-glucoside, 12. Malvidin-3-(6-p-coumaroyl)-glucoside pyruvate, 13. Peonidin-3-(6-acetyl)-glucoside, 14. Malvidin-3-(6-acetyl)-glucoside, 15. Cyanidin-3-(6-p-coumaroyl)-glucoside, 16. Peonidin-3-(6-caffeoyl)-glucoside, 17. Malvidin-3-(6-caffeoyl)-glucoside, 18. Petunidin-3-(6-p-coumaroyl)-glucoside, 19. Peonidin-3-(6-p-coumaroyl)-glucoside, 20. Malvidin-3-(6-p-coumaroyl)-glucoside, 21. Malvidin-3-glucoside-4-vinylcatechol, 22. Malvidin-3-glucoside-4-vinylphenol.

TABLE II

Anthocyanin content (mg/L) of the different Merlot wines.
Teores em antocianinas (mg/L) dos diferentes vinhos Merlot.

Compound	Vintage (aging time in bottle)				
	2001 (1 year)	2000 (2 years)	1999 (3 years)	1998 (4 years)	1997 (5 years)
Grape anthocyanins					
Delphinidin-3-glucoside	18.07±0.48^{d}	4.58±0.33^{c}	2.51±0.38^{ab}	3.47±0.58^{b}	2.17±0.19^{a}
Cyanidin-3-glucoside	3.39±0.15^{c}	0.46±0.08^{b}	0.32±0.03^{ab}	0.16±0.04^{a}	0.35±0.02^{ab}
Petunidin-3-glucoside	23.44±0.44^{c}	7.00±0.60^{b}	3.51±0.39^{a}	3.84±0.33^{a}	2.89±0.14^{a}
Peonidin-3-glucoside	19.40±0.58^{c}	4.95±0.25^{b}	4.23±0.12^{b}	3.33±0.30^{a}	4.31±0.09^{b}
Malvidin-3-glucoside	102.91±1.07^{d}	62.24±0.54^{c}	28.14±0.10^{b}	25.58±2.36^{b}	20.20±0.99^{a}
Σ Simple glucosides	167.21±2.72^{d}	79.23±1.80^{c}	38.71±1.02^{b}	36.38±3.61^{b}	29.92±1.43^{a}
Delphinidin-3-(6-acetyl)-glucoside	5.16±0.28^{c}	1.22±0.26^{b}	0.69±0.05^{a}	1.10±0.11^{ab}	0.78±0.01^{ab}
Cyanidin-3-(6-acetyl)-glucoside	2.79±0.01^{e}	0.43±0.01^{d}	0.32±0.01^{b}	0.37±0.02^{c}	0.23±0.01^{a}
Petunidin-3-(6-acetyl)-glucoside	6.19±0.08^{e}	2.07±0.01^{d}	0.87±0.06^{b}	1.21±0.13^{c}	0.08±0.01^{a}
Peonidin-3-(6-acetyl)-glucoside	5.54±0.01^{c}	1.59±0.06^{b}	1.09±0.11^{a}	1.79±0.15^{b}	1.72±0.10^{b}
Malvidin-3-(6-acetyl)-glucoside	31.67±0.10^{d}	19.08±0.10^{c}	7.43±0.10^{ab}	8.30±0.64^{b}	6.20±0.43^{a}
Σ Acetyl glucosides	51.35±0.48^{d}	24.39±0.44^{c}	10.40±0.33^{a}	12.77±1.05^{b}	9.01±0.56^{a}
Peonidin-3-(6-caffeoyl)-glucoside	0.52±0.07^{b}	0.15±0.07^{a}	0.08±0.01^{a}	0.08±0.01^{a}	0.10±0.01^{a}
Malvidin-3-(6-caffeoyl)-glucoside	1.52±0.08^{c}	0.88±0.10^{b}	0.22±0.01^{a}	0.16±0.01^{a}	0.09±0.01^{a}
Cyanidin-3-(6-*p*-coumaroyl)-glucoside	1.11±0.01^{d}	0.42±0.03^{c}	0.22±0.01^{b}	0.10±0.01^{a}	0.13±0.01^{a}
Petunidin-3-(6-*p*-coumaroyl)-glucoside	0.80±0.02^{d}	0.65±0.02^{c}	0.16±0.03^{a}	0.20±0.01^{a}	0.29±0.01^{b}
Peonidin-3-(6-*p*-coumaroyl)-glucoside	10.31±0.06^{d}	9.24±0.03^{c}	4.47±0.01^{a}	4.64±0.01^{b}	4.79±0.12^{b}
Malvidin-3-(6-*p*-coumaroyl)-glucoside	6.29±0.01^{d}	5.60±0.01^{c}	1.21±0.04^{a}	1.26±0.09^{a}	1.65±0.19^{b}
Σ Cinnamoyl glucosides	20.55±0.25^{d}	16.94±0.26^{c}	6.36±0.11^{a}	6.44±0.14^{a}	7.05±0.35^{b}
Anthocyanidin derived pigments					
Peonidin-3-glucoside pyruvate	0.16±0.01^{a}	0.24±0.02^{b}	0.17±0.03^{a}	0.22±0.02^{b}	0.23±0.01^{b}
Malvidin-3-glucoside pyruvate	3.15±0.01^{d}	3.38±0.17^{d}	1.01±0.01^{b}	1.47±0.32^{c}	0.41±0.01^{a}
Malvidin-3-(6-acetyl)-glucoside pyruvate	0.41±0.01^{b}	0.41±0.01^{b}	0.23±0.22^{a}	0.38±0.01^{a}	0.22±0.03^{a}
Malvidin-3-(6-*p*-coumaroyl)-glucoside pyruvate	1.67±0.01^{d}	0.64±0.06^{c}	0.34±0.01^{b}	0.19±0.03^{a}	0.41±0.01^{b}
Σ Pyruvate derivatives	5.39±0.04^{b}	4.67±0.26^{b}	1.75±0.27^{a}	2.26±0.38^{a}	1.27±0.06^{a}
Malvidin-3-glucoside vinylcatechol	0.10±0.01^{a}	0.76±0.06^{b}	0.19±0.02^{a}	0.17±0.02^{a}	0.84±0.07^{b}
Malvidin-3-glucoside vinylphenol	nd	nd	0.07±0.01^{b}	0.18±0.01^{b}	0.28±0.02^{c}
Σ Vinylphenol derivatives	0.10±0.01^{a}	0.76±0.06^{c}	0.26±0.03^{ab}	0.35±0.03^{b}	1.12±0.09^{d}
TOTAL Anthocyanin Content	244.60±3.5^{c}	125.99±3.08^{b}	57.48±1.76^{a}	58.20±5.21^{a}	48.37±2.49^{a}

Different letters in the same row indicate significant differences at $p<0.05$.
nd – not detected

results indicate that the largest changes in the anthocyanin content occurred during the first 3 years of aging in bottle and that some stabilization occurred after that time (Table II).

Significant differences between wines were also found for the pyruvate derivatives (vitisins A), which also showed a decline in concentration (76% loss) after the second year of aging (Table II). Other authors (Mateus and De Freitas, 2001; Schwarz *et al*. 2003a; Monagas *et al*., 2005b) have also described a decline in the levels of vitisins A during aging. In the case of vinylphenol derivatives, the highest concentration of malvidin-3-glucoside-vinylcatechol was registered for the 2-year and 5-year wines, whereas the pigment malvidin-3-glucoside-vinylphenol was only detected after three years of aging. This is in agreement with the results of Schwarz *et al*. (2003b, 2004), who reported that the major synthesis of the pigment malvidin-3-glucoside-vinylcatechol in Pinotage wines occurred after a long aging time in bottle (≥ 2.5 years).

Figure 2 illustrates the distribution of anthocyanins in the vertical row of Merlot wines. Despite the changes registered during bottle aging, little differences were found in the distribution of grape anthocyanin from the 1 to the 5 year-old wines in the case of simple glucosides (68.4-61.9%) and acetyl-

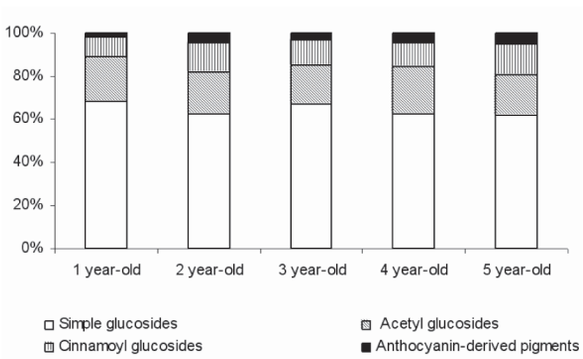

Figure 2 - Distribution of anthocyanin compounds in the different Merlot wines.
Distribuição dos compostos antociânicos nos diferentes vinhos Merlot

glucosides (21.0-18.6%), although it varied for minor groups such as of cinnamoyl-glucosides (8.4-14.6%) and anthocyanin-derived pigments (pyruvate + vinylphenol derivatives; 2.2-4.9%). These results indicate that during a 5 year- aging period in bottle (non-oxidative conditions), the initial anthocyanin profile of Merlot wines is largely maintained, taking into account the variables analyzed in this study. However, this was not the case of wines aged under other conditions, since Boido et al. (2006) found that

anthocyanins in a vertical row of Tannat wines, with different aging time in oak and in bottle, underwent faster disappearance rates and larger changes in relative contents. In addition, it is important to point out that the contribution of polymeric pigments, which is very difficult to estimate using currently analytical techniques, have not been taking into account in this kind of study.

Changes in the non-anthocyanin phenolic composition of Merlot wines during aging

The HPLC-DAD chromatogram of the non-anthocyanin phenolic compounds identified in the Merlot wines studied is illustrated in Figure 3. A total

In comparison to other studies, the non-anthocyanin phenolic compounds identified in the Spanish Merlot wines analyzed in this work was similar to those recently reported for Merlot wines from Italy (La Torre *et al*., 2006) and South African (de Villiers *et al*., 2004), although some compounds such as tryptophol and quercetin-3-glucuronide were not reported in these studies. On the other hand, ferulic acid was reported to occur in these latter wines, but it was not detected in our study.

In contrast to anthocyanins, no significant differences (p>0.05) were found in the total content of non-anthocyanin phenolic compounds as the ageing time in bottle progressed (Table III). However, some

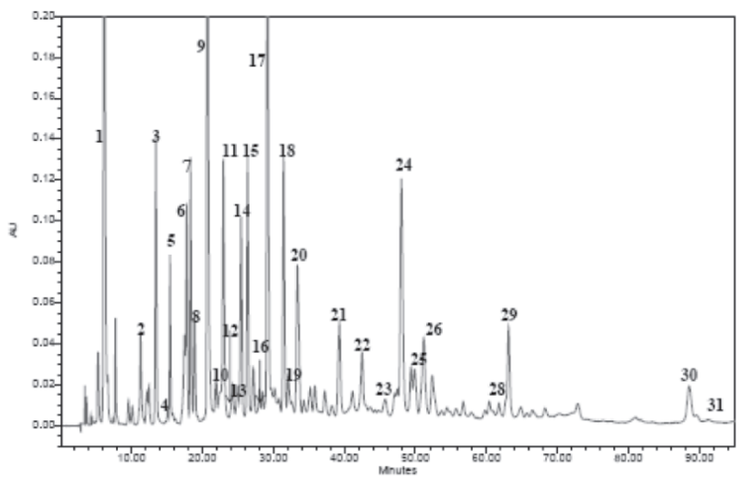

Figure 3. HPLC-DAD chromatogram of non-anthocyanin phenolic compounds identified in wines from *Vitis vinifera* L. cv Merlot.
Cromatogramas HPLC-DAD dos compostos não antocianicos identificadas em vinhos de Vitis vinifera L. cv Merlot.

1. Gallic acid, **2.** Protocatechuic acid, **3.** *trans*-Caftaric acid, **4.** 2,3-dihydroxy-1-(4-hydroxy-3-methoxyphenyl)-propan-1-one, **5.** Methyl gallate, **6.** Procyanidin B1, **7.** Tyrosol, **8.** *trans*-Cutaric acid, **9.** (+)-Catechin, **10.** Procyanidin T2, **11.** Ácido vainillínico, **12.** *trans*-Caffeic acid, **13.** Hexose ester of trans-p-coumaric acid (1), **14.** Procyanidin B2, **15.** Syringic acid, **16.** Hexose ester of trans-p-coumaric acid (2), **17.** (-)-Epicatechin, **18.** Ethyl gallate, **19.** Procyanidin C1, **20.** *trans-p*-Coumaric acid, **21.** Myricetin-3-*O*-glucoside, **22.** *trans*-Resveratrol-3-*O*-glucoside, **23.** Ellagic acid, **24.** Quercetin-3-*O*-glucuronide, **25.** Quercetin-3-*O*-glucoside, **26.** Tryptophol, **27.** Myricetin, **28.** *cis*-Resveratrol-3-*O*-glucoside, **29.** *trans*-Resveratrol, **30.** Quercetin, **31.** *cis*-Resveratrol.

of 27 of non-flavonoid and flavonoid phenolic compounds were quantified in the different wines (Table III). Non-flavonoid compounds included: hydroxybenzoic acids and their derivatives (gallic, protocatechuic, vanillic and syringic acids, methyl gallate and ethyl gallate), hydroxycinnamic acids and their derivatives (trans-caftaric, trans-cutaric, trans-caffeic and trans-p-coumaric acids, and two hexose esters of trans-p-coumaric acid), stilbenes (trans and cis resveratrol, and trans and cis resveratrol-3-O-glucoside) and phenolic alcohols and other related compounds (tyrosol and tryptophol). Flavonoid compounds quantified comprised: flavanols [(+)-catechin, (-)-epicatechin and dimeric procyanidins B1 and B2] and flavonols (myricetin-3-O-glucoside, quercetin-3-O-glucuronide, quercetin-3-O-glucoside, myricetin and quercetin). Ellagic acid and 2,3-dihydroxy-1-(4-hydroxy-3-methoxyphenyl)-propan-1-one, as well as the trimeric procyanidins T2 and C1, were not quantified due to their low contents.

changes were presented in the content of the different structural groups during this period.

Hydroxybenzoic acids and their derivatives.

The oldest wine (5 year-old) presented the lowest content of total hydroxybenzoic acids (10.75 mg/L), whereas the rest of wines (1-4 year-old) presented closer values (18.46-22.19 mg/L) (Table III). This difference was mainly attributed to the concentration of gallic acid, which was more than 50% lower in the 5 year-old wine in comparison to the remaining ones. In accordance to this, the latter wine also presented the lowest concentration of methyl and ethyl gallates, compounds that are generated by the esterification of gallic acid with methanol and ethanol, respectively during the winemaking process.

Concerning the stability of hydroxybenzoic acids during wine aging in bottle, Revilla and González-San José (2003) did not find significant changes in the concentration of gallic, protocatechuic and vanillic

TABLE III

Non-anthocyanin content (mg/L) of the different Merlot wines.
Teores em compostos não antociânicos (mg/L) dos diferentes vinhos Merlot.

Compound	Vintage (aging time in bottle) 2001 (1 year)	2000 (2 years)	1999 (3 years)	1998 (4 years)	1997 (5 years)
• **NON-FLAVONOID PHENOLIC COMPOUNDS**					
Hydroxybenzoic acids and derivatives					
Gallic acid	11.16±0.77^{b}	15.29±0.03^{d}	15.78±0.33^{d}	13.05±0.51^{c}	5.18±0.55^{a}
Protocatechuic acid	1.74±0.02^{d}	0.81±0.01^{b}	0.78±0.01^{b}	1.15±0.10^{c}	0.47±0.01^{a}
Vanillic acid	3.87±031^{c}	2.35±0.06^{a}	3.32±0.07^{b}	2.67±0.16^{a}	3.40±0.26^{bc}
Syringic acid	2.26±0.13^{b}	2.60±0.01^{b}	2.31±0.14^{b}	1.59±0.02^{a}	1.70±0.28^{a}
Methyl gallate	1.20±0.07^{cd}	1.07±0.01^{ab}	1.32±0.04^{d}	1.08±0.06^{ab}	0.96±0.06^{a}
Ethyl gallate	3.19±0.26^{b}	5.04±0.05^{cd}	4.23±0.05^{c}	5.74±0.62^{d}	2.08±0.02^{a}
Σ Hydroxybenzoic acids	19.03±1.56^{bc}	21.05±0.11^{cd}	22.19±0.55^{d}	18.46±0.79^{b}	10.75±1.10^{a}
Σ Hydroxybenzoic acid derivatives	4.39±0.33^{b}	6.11±0.06^{cd}	5.55±0.09^{c}	6.82±0.68^{d}	3.04±0.08^{a}
Hydroxycinnamic acids and derivatives					
Trans-Caftaric acid	2.20±0.14^{c}	2.15±0.01^{c}	0.70±0.01^{b}	0.76±0.09^{b}	0.45±0.05^{a}
Trans-Coutaric acid	0.55±0.04^{b}	0.80±0.01^{c}	0.37±0.01^{a}	0.39±0.01^{a}	0.39±0.01^{a}
Trans-Caffeic acid	0.36±0.03^{a}	0.41±0.01^{a}	1.13±0.07^{b}	0.53±0.09^{a}	3.55±0.45^{c}
Trans-p-Coumaric acid	1.33±0.01^{a}	0.99±0.01^{a}	2.68±0.27^{b}	0.97±0.01^{a}	4.39±0.56^{c}
Hexose ester of *trans-p*-coumaric acid (1)	0.19±0.01^{c}	0.20±0.02^{cd}	0.08±0.01^{a}	0.22±0.01^{d}	0.13±0.02^{b}
Hexose ester of *trans-p*-coumaric acid (2)	0.15±0.01^{b}	0.26±0.01^{e}	0.23±0.01^{d}	0.20±0.01^{c}	0.09±0.01^{a}
Σ Hydroxycinnamic acids	1.69±0.04^{a}	1.40±0.02^{a}	3.81±0.34^{b}	1.50±0.10^{a}	7.94±1.01^{c}
Σ Hydroxycinnamic acid derivatives	3.09±0.20^{c}	3.41±0.05^{d}	1.38±0.04^{b}	1.57±0.12^{b}	1.06±0.09^{a}
Stilbenes					
Trans-Resveratrol-3-*O*-glucoside	0.76±0.04^{b}	1.22±0.03^{c}	0.43±0.05^{a}	1.25±0.04^{c}	0.68±0.07^{b}
cis- Resveratrol-3-*O*-glucoside	0.25±0.02^{a}	0.36±0.01^{b}	0.26±0.02^{a}	0.55±0.05^{c}	0.23±0.02^{a}
Trans-Resveratrol	0.13±0.01^{a}	0.21±0.01^{a}	0.46±0.01^{a}	1.49±0.03^{b}	4.09±0.55^{c}
cis-Resveratrol	0.01±0.01^{a}	0.05±0.01^{b}	0.25±0.01^{d}	0.09±0.01^{c}	0.50±0.03^{e}
Σ Stilbenes	0.14±0.02^{a}	0.26±0.02^{a}	0.71±0.02^{a}	1.58±0.04^{b}	4.59±0.58^{c}
Σ Stilbene glucosides	1.01±0.06^{b}	1.58±0.04^{c}	0.69±0.07^{a}	1.80±0.09^{d}	0.91±0.09^{b}
Phenolic alcohols and other related compounds					
Tyrosol	9.46±0.66^{a}	12.64±0.04^{b}	13.87±0.01^{bc}	15.10±0.72^{c}	14.38±1.07^{c}
Tryptophol	0.58±0.08^{a}	1.07±0.03^{b}	1.49±0.21^{b}	2.48±0.31^{c}	1.23±0.15^{b}
Σ Phenolic alcohols and other related compounds	10.04±0.74^{a}	13.71±0.07^{b}	15.36±0.22^{b}	17.58±1.03^{c}	15.61±1.22^{bc}
• **FLAVONOID PHENOLIC COMPOUNDS**					
Flavanols					
(+)-Catechin	33.48±2.44^{d}	27.09±0.07^{ab}	26.18±0.28^{ab}	30.14±2.45^{cd}	24.70±2.96^{a}
(-)-Epicatechin	27.29±2.03^{c}	19.33±0.08^{a}	20.69±0.18^{a}	24.04±1.15^{b}	19.16±1.40^{a}
Procyanidin B1	5.54±0.52^{a}	4.96±0.07^{a}	8.20±0.01^{b}	11.44±1.14^{c}	7.28±0.34^{b}
Procyanidin B2	8.01±0.79^{a}	6.97±0.13^{a}	7.96±0.63^{a}	10.02±1.00^{b}	6.14±0.84^{a}
Σ Monomeric Flavanols	60.77±2.47^{d}	46.42±0.15^{ab}	46.87±0.46^{ab}	54.18±3.60^{cd}	43.86±4.36^{a}
Σ Dimeric Flavanols	13.55±1.31^{ab}	11.93±0.20^{a}	16.16±0.64^{b}	21.46±2.14^{c}	13.42±1.18^{ab}
Flavonols					
Myricetin-3-*O*-glucoside	4.20±0.28^{c}	4.69±0.05^{c}	0.77±0.08^{a}	1.86±0.01^{b}	5.78±0.74^{d}
Quercetin-3-*O*-glucoside	1.34±0.20^{c}	1.07±0.04^{b}	1.29±0.01^{bc}	1.50±0.03^{c}	0.73±0.04^{a}
Quercetin-3-*O*-glucuronide	11.42±0.94^{d}	8.50±0.10^{c}	4.34±0.01^{a}	6.61±0.01^{b}	6.81±0.71^{b}
Myricetin	0.64±0.01^{a}	1.33±0.04^{b}	0.54±0.03^{a}	2.10±0.03^{c}	2.50±0.02^{d}
Quercetin	2.63±0.13^{b}	4.67±0.02^{c}	1.26±0.01^{a}	6.25±0.67^{d}	2.15±0.02^{b}
Σ Flavonol aglycones	3.27±0.14^{b}	6.00±0.06^{d}	1.80±0.04^{a}	8.35±0.70^{e}	4.65±0.01^{c}
Σ Flavonol glycosides	16.96±1.42^{d}	14.26±0.19^{c}	6.40±0.10^{a}	9.97±0.05^{b}	13.32±1.49^{c}
TOTAL Non-Anthocyanin Content	133.94±8.29^{a}	126.23±0.97^{a}	120.92±2.57^{a}	143.27±9.34^{a}	119.15±11.21^{a}

Different letters in the same row indicate significant differences at $p<0.05$.

acids in Tempranillo wines after 24 months of bottle aging. Our results also suggest that these compounds seem to be quite stable in wine.

Hydroxycinnamic acids and their derivatives.

With the exception of the 3 year-old wine, the 1, 2, and 4 year-old wines did not present significant differences ($p<0.05$) in relation to the total concentration of hydroxycinnamic acids (*trans*-caffeic and *trans-p*-coumaric acids) (Table III). However, the oldest wine marked distinction, presenting the highest levels of these compounds (470% of the 1 year-old wine hydroxycinnamic acid content). The opposite situation was observed for the hydroxycinnamic acid derivatives (tartaric esters of *trans-p*-coumaric and caffeic acids, and the hexose esters of *trans-p*-coumaric acid), since the youngest wines (1 and 2 year-old wines) presented the highest concentration and the oldest wine (5 year-old), the lowest one (34% of the 1 year-old wine hydroxycinnamic derivative content) (Table III). Therefore, some conversion of the combined forms into the free forms likely occurred during wine aging.

These results are consistent with the typical evolution profile of hydroxycinnamates during wine aging. Somers *et al.* (1987) first reported that the accumulation of free hydroxycinnamic acids during wine aging arised from the hydrolysis of the corresponding combined forms. Zafrilla *et al.* (2003) and Monagas *et al.* (2005c) later confirmed the occurrence of these compositional changes during wine aging in bottle.

Stilbenes and their glucosides.

The highest levels of stilbene glucosides were found in the 2 and 4-year old wines, whereas the 3 year-old wine showed the lowest concentration (Table III). A different situation was observed for the aglycones. In this case, the concentration was higher for wines with a prolonged aging time in bottle (4 and 5 year-old wines) (Table III). Hydrolysis of glucosilated stilbenes leading to free forms has been reported to occur during

vinification (Jeandet and Bessis, 1995; Mattivi *et al*., 1995; Pezet and Cuenat, 1996). However, there is no much information in the literature concerning the evolution of stilbenes during post-fermentation and aging (Sun *et al*., 2006). The results found in this work suggest that certain degree of hydrolysis must occur during wine aging.

Phenolic alcohols and other related compounds.

Concentrations of both tyrosol and tryptophol, secondary products of yeast alcoholic fermentation, increased as the wine aged from 1 to 4 years, but then slightly decreased in the 5 year-old wine (Table III). The content of these compounds as a total in the 4 year-old wine represented 175% of the content of the youngest one. This results are not in accordance with those of Sapis and Ribéreau-Gayon (1969), who found that tryptophol was practically absent in aged wines, whereas tyrosol concentration kept relative constant during aging.

Monomeric and dimeric flavanols.

The highest concentration of total monomeric flavan-3-ols was registered for the 1 and 4 year-old wines, whereas no significant differences (p>0.05) in concentration were found between the rest of wines (Table III). In the case of dimeric flavan-3-ols, the 4 year-old wine also presented the highest level. Again, no significant differences in terms of concentration were observed among the remaining wines (Table III).

Although the concentration of flavanols has been reported to decline during wine aging possibly due to their participation in condensation reactions (Revilla and González-San José, 2003; Gómez-Plaza *et al*., 2000), a clear decrease in flavanols was not observed in our study. The acid-catalyzed C-C bond-breaking of polymeric forms, known to occur during wine aging, could have compensated the possible loss in concentration of monomers and dimers (Haslam, 1980).

Flavonol glycosides and aglycones.

The highest level of total flavonol glycosides was registered for the youngest wines (1 year-old) whereas the 3 year-old wine presented the lowest concentration (Table III). For the aglycones, the concentration was largely variable between the different wines, significant differences (p<0.05) being found between the five wines analyzed (Table III). A certain degree of hydrolysis of the glycosilated forms to the corresponding aglycones has been reported to occur during wine aging (Zafrilla *et al*., 2003). However, this conclusion could not be drawn from our results probably due to climatic conditions of each vintage such as the degree of sun exposure that the berries have received during ripening, which is known to affect the flavonol content (Price *et al*., 1995; Haselgrove *et al*., 2000; McDonald *et al*., 1998).

Figure 4 shows the distribution of non-anthocyanin phenolic compounds. In general, most of the groups

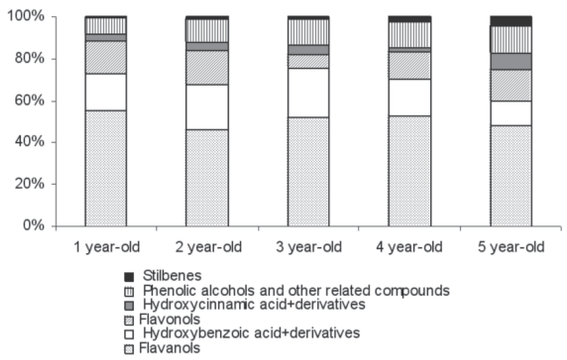

Figure 4 - Distribution of non-anthocyanin phenolic compounds in the different Merlot wines.
Distribuição das fenólicas não antociânicas nos diferentes vinhos de Merlot.

of non-flavonoid and flavonoid compounds only presented small changes in proportion during aging in bottle (with the exception of the 3 year-old wine for flavonols). From the 1 year-old to the 5 year-old wine, major groups such as hydroxybenzoic acids and their derivatives (from 17.5 to 11.6%), and flavanols (from 55.5 to 48.15%) presented a slightly decrease. However, an increase was registered in the relative contents of minor groups including hydroxycinnamic acids and their derivatives (from 3.6 to 7.6%), stilbenes (from 0.9 to 4.6%) and phenolic alcohols and other related compounds (from 7.5 to 13.1%).

Changes in the content of condensed tannins of Merlot wines during aging

The content of oligo/polymeric flavan-3-ols (condensed tannins) was measured by the Bate-Smith method, based on the acid-catalyzed oxidative cleavage of the C-C interflavanic bond of proanthocyanidins in alcohol-water mixtures. The oldest wine (1997) showed a significantly higher content of condensed tannins (1196 mg of cyaniding/ L) in comparison to the rest of the wines: 1998 (1040 mg/L), 1999 (975 mg/L), 2000 (1060 mg/L) and 2001 (965 mg/L).

Changes in the colour characteristics of Merlot wines during aging

A decrease in the red component (Red %) and an increase in the yellow component (Yellow %) was registered as the aging time in bottle progressed (Figure 5). As expected, the tint (Abs 420 nm/ Abs 520 nm) was also found to increase with the aging time in bottle. In relation to the blue component (Blue %), older wines (3-4 year-old wines) presented higher values than the younger ones (1 and 2 year-old wines) (Figure 5). These changes are consistent with the typical evolution trend of the wine color from purple-red to brick-red hues (Ribéreau-Gayon, 1982). The slight difference observed between the trend of the tint and the yellow component indicated that the loss in red component was also compensated, besides from an increase in the yellow component, by an increase

in the blue component, which is the other modifier of the base red (Figure 5). In fact, a better

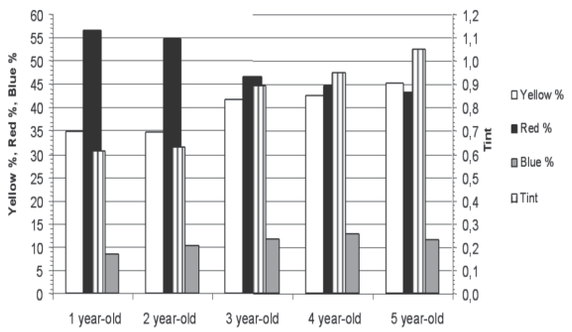

Figure 5 - Colour characteristics of the different Merlot wines. *Características da cor dos diferentes vinhos Merlot.*

correspondence was found between red component and the relationship (Abs 420 nm + Abs 620nm)/Abs 520nm, which included both the yellow and the blue components in the tint (data not shown). Finally, the largest modification in the wine color characteristics seemed to occur during the first three years of bottle aging, following the same trend as the anthocyanin content.

CONCLUSIONS

This paper reports data about the evolution trend and relative proportion of both anthocyanin and non-anthocyanin phenolic compounds in Merlot wines elaborated from grapes of the same vineyard in 5 consecutive vintages (1997-2001) with different aging time in bottle (1-5 years old). This experimental approach allowed confirming in a quick and convenient way the most important changes that occur in the phenolic composition and color during aging in bottle when a wine from a single vintage is monitored during a particular time period. Grape anthocyanins and pyruvate derivatives (vitisins A) decreased during aging time in bottle, whereas vinylphenol derivatives presented an increase in concentration during the same period. The largest changes in anthocyanin content occurred during the first three years of aging, period which also coincided with the largest modification of the wine color characteristics. In contrast, no significant differences were found in the total content of non-anthocyanin phenolic compounds of the different wines although changes were found particularly in the content of non-flavonoid compounds (hydroxybenzoic and hydroxycinnamic acids and their derivatives, stilbenes, and phenolic alcohols and other related compounds). Despite of all the changes described above, the distribution of the main groups of anthocyanin and non-anthocyanin phenolic compounds were only slightly modified under the non-oxidative conditions present during bottle aging.

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