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Evaluation of different *Saccharomyces cerevisiae* strains for red winemaking. Influence on the anthocyanin, pyranoanthocyanin and non-anthocyanin phenolic content and colour characteristics of wines

Maria Monagas, Carmen Gómez-Cordovés, Begoña Bartolomé *

Instituto de Fermentaciones Industriales (CSIC), Juan de la Cierva 3, 28006 Madrid, Spain

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Abstract

The possible industrial use of three previously-selected *Saccharomyces cerevisiae* strains (1EV, 2EV and 7EV) has been studied in musts derived from Tempranillo and Cabernet Sauvignon. The anthocyanin, pyranoanthocyanin and non-anthocyanin phenolic content, and colour characteristics of the resulting wines have been compared to those of a commercial strain. Anthocyanins were the compounds most influenced by the yeast strain. Independently of the grape variety, wines derived from 2EV presented significantly higher anthocyanin concentrations than those derived from 1EV and 7EV, which presented similar contents. With the exception of hydroxycinnamic acids and derivatives, no particular influence of the yeast strain was observed on the remaining non-anthocyanin phenolic compounds (i.e, hydroxybenzoic acids and flavanols). Pyranoanthocyanins and metabolites resulting from the alcoholic fermentation such as tyrosol and tryptophol, seemed to be more influenced by the must composition and pH, and thus, by the grape variety, than by the yeast strain.

Keywords: *Saccharomyces cerevisiae*; Wine; Anthocyanin; Pyranoanthocyanin; Colour

1. Introduction

Colour is one of the main quality attributes of red wines and a matter of primary importance to the winemaker. The initial colour of red wines is mainly due to monomeric anthocyanins extracted from grape skins during maceration and fermentation, principally as flavylium cations (red) and quinoidal anhydro-bases (blue), and to the phenomenon of self-association and copigmentation with other phenols present in wine (i.e., flavanols, flavonols and hydroxycinnamic acids) (Haslam, 1980). However, during wine maturation and aging, anthocyanins participate in numerous condensation reactions that result in the formation of new oligomeric and polymeric pigments that present more stable structures and modify the initial bright-red colour of young wines towards more brick-orange hues (Ribéreau-Gayon, 1982; Somers, 1971).

The implication of wine yeast in red wine colour is two-fold. On one hand, wine yeast influences the extraction of grape anthocyanins during maceration and fermentation, depending on their alcohol production capacity. They also influence the formation of more stable anthocyanin forms during maturation and ageing. On the other hand, yeast can promote anthocyanin degradation and participate in certain interactions with pigments that result in colour loss.

Saccharomyces cerevisiae wine yeast possesses pectinases (polygalacturonases) that catalyse the hydrolysis of skin pectins which favours anthocyanin extraction (Blanco, Sieiro, & Villa, 1999; Gainvors & Belardi, 1995). These enzymes are activated during the primary stage of fermentation (Takayanagi, Uchibori, & Yokotsuka, 2001).

* Corresponding author. Tel.: +34 91 562 29 00; fax: +34 91 564 48 53.
E-mail address: bartolome@ifi.csic.es (B. Bartolomé).

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Secondary metabolites produced during yeast fermentation are involved in the formation of anthocyanin-derived pigments. Acetaldehyde mediates the reaction between anthocyanins and flavanols giving rise to anthocyanin-ethyl-flavanol adducts which are more stable to pH and to SO_2 decolouration than monomeric anthocyanins (Escribano-Bailón, Alvarez-García, Rivas-Gonzalo, Heredia, & Santos-Buelga, 2001; Timberlake & Bridle, 1976). Together with other metabolites that present keto-enol tautomerism, such as pyruvic acid and acetone, acetaldehyde also participates in C4–C5 anthocyanin cycloaddition producing the so called pyranoanthocyanins (Bakker & Timberlake, 1997; Fulcrand, Benabdeljalil, Rigaud, Cheynier, & Moutounet, 1998; Hayasaka & Asenstorfer, 2002). Other pigments in which an anthocyanin is linked to a flavanol moeity through a vinyl fragment (anthocyanin-vinylflavanol adducts), could also be generated in the presence of acetaldehyde (Francia-Aricha, Guerra, Rivas-Gonzalo, & Santos-Buelga, 1997). 4-Vinylphenol and 4-vinylguaiacol produced from the decarboxylation of *p*-coumaric and ferulic acid, respectively, via *S. cerevisiae* cinnamate decarboxylase (Chatonnet, Dubourdieu, Boidron, & Lavigne, 1993), can also lead to the formation of pyranoanthocyanins (Fulcrand, Cameira Dos Santos, Sarni-Manchado, Cheynier, & FabreBonvin, 1996). These pigments exhibit a red–orange colour and due to the substitution at C-4, are more resistant to pH changes and SO_2 bleaching than monomeric anthocyanins (Bakker & Timberlake, 1997; Fulcrand et al., 1996; Vivar-Quintana, Santos-Buelga, & Rivas-Gonzalo, 2002).

The β-glucosidase activity of certain strains of *S. cerevisiae* wine yeast, which has a positive effect in wine aroma releasing the volatile aglycone of terpenol glycosides (Dubordieu, 1994), could have a negative effect on wine colour as the anthocyanidin resulting from the breakdown of the glucosidic bond of the anthocyanidin-3-glucosides is a less stable form and could easily be degraded during wine ageing. Another occurrence that results in wine colour loss is the adsorption of anthocyanins on the yeast cell wall (Morata, Gómez-Cordovés, Colomo, & Suárez, 2005; Morata, Gómez-Cordovés, Suberviola et al., 2003; Vasserot, Caillet, & Maujean, 1997). The main structural constituents of *S. cerevisiae* yeast cell wall are glucans and mannans with a minor proportion of chitin (Walker, 1998). Mannoproteins are located in the outer layer of the yeast cell wall and determine most of the surface properties of the wall, including its capacity to adsorb wine molecules such as anthocyanins (Morata, Gómez-Cordovés, Suberviola et al., 2003; Morata et al., 2005; Vasserot et al., 1997), aroma compounds (Lubber, Charpentier, Feuillat, & Voilley, 1994) and fatty acids (Larue, Geneix, Lafon-Lafourcade, Bertrand, & Ribéreau-Gayon, 1984).

Due to the demand for more sophisticated wines with special and original quality attributes, the selection of yeast strains with improved or novel properties, in addition to classical enological parameters, is becoming a very important task in Enology. In this sense, the influence of the yeast strain on the phenolic composition and colour of the resulting wines has been adopted as an additional criterion for yeast selection. In any case, selected strains must be well adapted to the characteristics of the producing area, viticultural practices and winemaking techniques. The present work is a continuation of a project that consisted in the selection of new yeast strains from the skins of *Vitis vinifera* L. red grapes belonging to the Spanish *Denomination of Origin* Navarra. The selected *Saccharomyces cerevisiae* yeast strains (1EV, 7EV and 2EV) have been tested for their production of pyruvic acid and acetaldehyde (Morata, Gómez-Cordovés, Colomo, & Suárez, 2003), and subsequent formation of pyranoanthocyanins (vitisins A and B, respectively). The adsorption of anthocyanins on the cell walls of these yeast strains has also been studied (Morata, Gómez-Cordovés, Suberviola et al., 2003; Morata et al., 2005). However, these experiments have been conducted at lab-scale. To finally evaluate the possible industrial use of these selected yeast strains, semi-industrial scaled fermentation of must derived from two very distinct *V. vinifera* L. red varieties (Spanish Tempranillo and French Cabernet Sauvignon) was performed. The anthocyanin, pyranoanthocyanin and non-anthocyanin phenolic content of the resulting wines as well as their colour characteristics, have been compared to those of a commercial yeast strain. There is still relatively little published research on the influence of yeast strain on wine phenolic compounds [see Sacchi, Bisson, and Adams (2005) for review].

2. Materials and methods

2.1. Yeast strains

The following yeast strains were used in this study: *S. cerevisiae* Na33/*S. bayanus* EC1118 (mixture, 80/20) from Lallemand Inc. (Canada) (commercial strain), and *S. cerevisiae* 1EV, 7EV and 2EV. The two former yeast strains (1EV, 7EV) were previously isolated from the grape skins of *V. vinifera* cv. Tempranillo, and the latter (2EV) from *V. vinifera* cv. Graciano grown in Olite, Navarra, Spain (Morata, Gómez-Cordovés, Suberviola, et al., 2003). Some enological properties of the tested strains are: ethanol tolerance, $\leqslant$16% v/v alcohol; volatile acidity production, $<$0.3 g/l expressed as acetic acid; glycerine production, $>$8 g/l; SO_2 tolerance, $\leqslant$200 mg/l; production of SH_2, low.

2.2. Winemaking

Monovarietal young red wines made from grapes of *V. vinifera* cv. Tempranillo and Cabernet Sauvignon were used for this study. Grapes (vintage 2001) were grown in the same geographical area (Olite, Navarra; Spain) and the wines were elaborated at the Viticulture and Enology Station of Navarra (EVENA). A batch of 100 kg of grapes of each variety was de-stemmed, crushed and the must stored in 200 l stainless-steel wine vats. The solid parts were

Table 1
Characteristics of musts from *Vitis vinifera* L. cv. Tempranillo and Cabernet Sauvignon

	Tempranillo	Cabernet Sauvignon
Potential titratable alcohol (% v/v)	13.2 ± 0.5	14.0 ± 0.4
Total acidity (g/l tartaric acid)	4.85 ± 0.73	6.45 ± 0.26
pH	3.82 ± 0.05	3.57 ± 0.05

Mean ($n = 8$) ± SD

stored between 0 and 1 °C until needed. The potential titratable alcohol, total acidity and pH of the Tempranillo and Cabernet Sauvignon musts are presented in Table 1. Semi-industrial scaled fermentations were performed with the four different yeast strains described above (commercial strain, 1EV, 7EV and 2EV) using an inoculum of 25 g/hl and a fermentation temperature ⩽25 °C. When the fermentation was active (approximately 2 days after yeast inoculation), the solid parts were added. The cap was punched down twice a day until it remained submerged during a 14 day maceration period. At the end of the alcoholic fermentation, the pomace was pressed off and sodium metabisulfite (6 g/hl) was added. The wines were then racked and stabilized for a period of 1 month at 0–1 °C. At the end of this period, the wines were filtrated through SEITZ K-250 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. Fermentations were carried out in duplicate. Wine analyses were carried out after 1 month of bottling.

2.3. HPLC-DAD analysis of anthocyanins

A Waters (Milford, MA) liquid chromatography system equipped with a 600-MS controller, a 717Plus autosampler, and a 996 photodiode-array detector (DAD) was used. Separation was performed on a reverse-phase Waters Nova-Pak C_{18} (150 mm × 3.9 mm, 4 μm) at room temperature, as described by Monagas, Gómez-Cordovés, and Bartolomé (2005). 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 0.8 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 (100 μl) of wine, previously filtered through a 0.45 μm membrane, was injected into 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 (Estrasynthèse, France) by a standard calibration curve.

2.4. Extraction of non-anthocyanin phenolics

A volume of 50 ml of wine was concentrated to 15 ml under vacuum at 30 °C and extracted three times with diethyl ether (10 + 15 + 15 ml) and 3 times with ethyl acetate (10 + 15 + 15 ml). 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 2 ml of methanol/water (1:1, v/v) and finally filtered (0.45 μm) and injected (10 μl) into the HPLC column.

2.5. HPLC-DAD analysis of non-anthocyanin phenolic compounds

The same liquid chromatography system described above was used for the analysis of non-anthocyanin phenolic compounds. Separation was performed on a reverse-phase Waters Nova-Pak C_{18} (300 mm × 3.9 mm, 4 μm) column at room temperature, as described by Monagas, Bartolomé, and Gómez-Cordovés (2005). 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. Diode-array detection (DAD) was performed from 220 to 380 nm. Quantification was carried out by external standard calibration curves. Hydroxybenzoic acids, phenolic alcohols and other related compounds, and flavanols were quantified at 280 nm; caffeic acid and its derivatives at 320 nm; and *p*-coumaric acid and its derivatives at 310 nm. Caffeic and *p*-coumaric acid derivatives were quantified by the calibration curve of their respective free form. Monomeric and dimeric flavan-3-ols were quantified using the (−)-epicatechin calibration curve.

2.6. Determination of the chromatic characteristics of wines

CIE (1986) tristimulus values (X, Y, Z), and CIELAB rectangular (L^*, a^*, b^*) and cylindrical (L^*, C^*, h) coordinates (illuminant/standard observer conditions: D65/CIE 1964 10°), were calculated by the simplified method described by Pérez-Caballero, Ayala, Echávarri, and Negueruela (2003) using the software MSCV for Windows 95/98 (http://www.unirioja.es/dptos/dq/fa/color/color.html), developed by the same authors. This method has been proposed as an OIV method for colour determination (Negueruela, Echávarri, & Ayala, 2001). Coordinate a^* is a measure of red colour if $a^* > 0$, and of green colour if $a^* < 0$; b^* is a measure of yellow colour if $b^* > 0$, and of blue colour if $b^* < 0$; L^* (lightness) is the lightness of a coloured object judged relative to the lightness of another object that appears white ($L^* = 100$, white; $L^* = 0$, black); C^* (chroma) is the chromaticness relative to the achromatic stimulus (white or grey); and h (hue angle) is the correlate of hue, the attribute of appearance. Under the MSCV software, CIELAB coordinates are referred to a 2 mm path length. The colour difference (ΔE^*) in CIELAB units between two wines (1 and 2), was calculated by the equation: $\Delta E^*_{1,2} = [(\Delta L^*_{1,2})^2 + (\Delta a^*_{1,2})^2 + (\Delta b^*_{1,2})^2]^{1/2}$.

2.7. Statistical analysis

ANOVA, principal component and discriminant analysis were performed using the PC software package SPSS (version 11.01; SPSS Inc., Chicago, IL, 2001).

3. Results and discussion

3.1. Anthocyanins and pyranoanthocyanins

Anthocyanidin-3-glucosides, -3-(6-acetyl)-glucosides, -3-(6-*p*-coumaroyl)-glucosides, including the *cis* isomer of malvidin-3-(6-*p*-coumaroylglucoside), and -3-(6-caffeoyl)-glucosides, as well as the pyranoanthocyanins resulting from the C-4/C-5 cycloaddition of anthocyanins with pyruvic acid and 4-vinylphenol were identified by HPLC/ESI-MS according to Monagas, Núñez, Bartolomé, and Gómez-Cordovés (2003). Table 2 summarizes the individual anthocyanin and pyranoanthocyanin concentration in wines from *V. vinifera* L. cv. Tempranillo and Cabernet Sauvignon resulting from fermentation with the different yeast strains. The anthocyanin profile of these two grape varieties is well established. Considering the acylated derivatives, wines from Tempranillo usually present a higher proportion of cinnamoyl- than of acetyl-glucosides (mean values: 12.7% and 8.1%, respectively) whereas the opposite is characteristic for those from Cabernet Sauvignon (mean values: 6.2% and 33.7%, respectively) (Monagas et al., 2003). As with other *V. vinifera* L. grape varieties, anthocyanidin-3-glucosides (simple glucosides) were the most abundant group of pigments in these wines (mean values: 79.1% and 60.1% for Tempranillo and Cabernet Sauvignon, respectively).

The Tempranillo wine from yeast strain 2EV showed a significantly higher anthocyanin concentration than the wines from the commercial strain, 1EV and 7EV, with very few exceptions (Table 2). The content of the three groups of anthocyanins according to their acylation pattern (i.e., Σsimple glucosides, Σacetyl-glucosides and Σcinnamoyl-glucosides) as well as the total anthocyanin content, also revealed a significantly higher anthocyanin concentration for the wines from 2EV than for the ones from 1EV, 7EV and the commercial strain (Table 2). Wines resulting from the fermentation with yeast strains 1EV and 7EV presented a similar content of most of the pigments quantified, with the exception of some cinnamoyl-3-glucosides (i.e., MCaf, PtCum and Mcum-*trans*) (Table 2). A similar trend was observed for the Cabernet Sauvignon wines. In general, it was found that the anthocyanin content of the wines resulting from the fermentation with 1EV and 7EV was similar and closer to the commercial strain while wines from 2EV presented higher levels (Table 2). However, when the anthocyanin absorption of the three selected yeast strains was tested using a Cabernet Sauvignon wine, 2EV showed the greatest absorption for most of the anthocyanin compounds (Morata, Gómez-Cordovés, Suberviola, et al., 2003). Therefore, other yeast properties influencing wine phenolic composition (such as yeast β-glucosidase activity) could be responsible for the results found.

Regarding anthocyanin–pyruvic acid adducts (MPy and MAcPy), the commercial strain and 2EV were the yeasts resulting in significantly higher levels of MPy in Tempranillo wines. In the case of Cabernet Sauvignon, wines from the commercial yeast strain presented the highest concentration of both pigments and 2EV the lowest concentration (Table 2). Pyruvic acid is a product resulting from the glycolysis of yeast metabolism during fermentation; its decarboxylation leads to the formation of acetaldehyde, which in turn is reduced to ethanol. Fermentation is the most important stage for the production of malvidin-3-glucoside-pyruvate, attaining maximum concentration in the period corresponding to 20–85% of glucose utilization, which coincides with the maximum concentration of both precursors, malvidin-3-glucoside and pyruvic acid (Asenstorfer, Markides, Iland, & Jones, 2003). Morata, Gómez-Cordovés, Colomo, et al. (2003) have reported a direct relationship between the concentration of this pigment and the production of pyruvic acid by the yeast after 96 h of fermentation. The results found in this work suggest that the must composition and pH, which differed in relation to the grape variety (Table 1), could be another variable affecting the yield of pyruvic acid resulting from yeast metabolism and the subsequent synthesis of anthocyanin-pyruvic acid adducts in wine. In fact, the pH has been described as a variable influencing the reaction between malvidin-3-glucoside and pyruvic acid in model solutions (Romero & Bakker, 1999). The difference in pH and acidity found between Tempranillo and Cabernet Sauvignon musts (Table 1) could partly explain why Cabernet Sauvignon wines presented higher levels of MPy despite presenting a similar concentration of malvidin-3-glucoside (precursor compound) (Table 2).

Considering the formation of malvidin–vinylphenol adduct (Mvinyl), wines from the commercial yeast strain and 2EV in Cabernet Sauvignon and only the former in Tempranillo, presented the highest concentration of this pigment (Table 2). In addition to the mechanism involving the decarboxylation of *p*-coumaric acid to 4-vinylphenol by yeast cinnamate decarboxylase (Chatonnet et al., 1993), another mechanism involving the free hydroxycinnamic acid and the anthocyanin without enzymatic support has been proposed for the formation of malvidin–vinylphenol adduct (Schwarz, Wabnitz, & Winterhalter, 2003). Therefore, both the yeast cinnamate decarboxylase activity and the content of free *p*-coumaric acid (higher in Tempranillo wines, see below) can affect the concentration of the malvidin–vinylphenol adduct in the wines from Tempranillo and Cabernet Sauvignon.

3.2. Non-anthocyanin phenolic compounds

Table 3 shows the individual concentration of the different non-flavonoid (hydroxybenzoic and hydroxycinnamic

Table 2
Individual anthocyanin and pyranoanthocyanin concentration (mg/l) in wines from *Vitis vinifera* L. cv. Tempranillo and Cabernet Sauvignon resulting from the fermentation with different yeast strains

	Tempranillo				Cabernet Sauvignon			
	Commercial strain	1EV	7EV	2EV	Commercial strain	1EV	7EV	2EV
Simple glucosides								
Delphinidin-3-glc (DG)	15.9 ± 0.4b	8.6 ± 0.13a	9.47 ± 0.20a	16.7 ± 1.0b	12.9 ± 0.2c	10.5 ± 0.1b	9.91 ± 0.10a	10.6 ± 0.2b
Petunidin-3-glc (PtG)	29.1 ± 1.3b	20.3 ± 0.5a	22.1 ± 0.6a	27.3 ± 2.1b	17.6 ± 0.1d	15.9 ± 0.1a	16.4 ± 0.1b	17.2 ± 0.2c
Peonidin-3-glc (PnG)	3.64 ± 0.04c	1.57 ± 0.18a	1.63 ± 0.17a	2.87 ± 0.34b	3.94 ± 0.16b	3.79 ± 0.01b	3.28 ± 0.07a	5.52 ± 0.11c
Malvidin-3-glc (MG)	167 ± 9a	195 ± 7b	194 ± 4b	195 ± 9b	157 ± 6a	157 ± 1a	165 ± 3a	184 ± 1b
ΣSimple glucosides	215 ± 8a	225 ± 7ab	227 ± 4ab	242 ± 11b	192 ± 7a	188 ± 1a	194 ± 3a	217 ± 2b
Acetyl-glucosides								
Delphinidin-3-(6-acetyl)-glc (DAc)	1.26 ± 0.02a	1.18 ± 0.10a	1.36 ± 0.07a	1.70 ± 0.11b	7.56 ± 0.14c	5.79 ± 0.02b	5.29 ± 0.09a	5.49 ± 0.23ab
Petunidin-3-(6-acetyl)-glc (PtAc)	1.70 ± 0.18a	1.60 ± 0.21a	1.71 ± 0.08a	2.00 ± 0.04a	6.57 ± 0.13a	6.31 ± 0.11a	7.32 ± 0.07b	7.24 ± 0.32b
Malvidin-3-(6-acetyl)-glc (MAc)	18.0 ± 0.5a	19.6 ± 1.5a	19.6 ± 1.4a	24.1 ± 1.0b	95.8 ± 0.7b	93.1 ± 0.5a	96.7 ± 0.5b	105 ± 1c
ΣAcetyl-glucosides	21.0 ± 0.3a	22.4 ± 1.6a	22.7 ± 1.4a	27.8 ± 0.9b	110 ± 1b	105 ± 1a	109 ± 1b	118 ± 2c
Cinnamoyl-glucosides								
Malvidin-3-(6-caffeoyl)-glc (MCaf)	2.82 ± 0.04c	2.06 ± 0.05a	2.39 ± 0.04b	3.16 ± 0.18d	2.38 ± 0.06a	2.54 ± 0.03ab	2.50 ± 0.07a	2.70 ± 0.07b
Malvidin-3-(6-*p*-coumaroyl)-glc (*cis*) (MCum-*cis*)	1.71 ± 0.04a	2.00 ± 0.07ab	1.82 ± 0.08ab	2.16 ± 0.24b	0.725 ± 0.017a	0.690 ± 0.010a	0.796 ± 0.074ab	0.873 ± 0.018b
Petunidin-3-(6-*p*-coumaroyl)-glc (PtCum)	4.24 ± 0.08b	3.71 ± 0.20a	4.25 ± 0.12b	5.00 ± 0.21c	1.36 ± 0.02a	1.29 ± 0.05a	1.29 ± 0.03a	1.47 ± 0.01b
Peonidin-3-(6-*p*-coumaroyl)-glc (PnCum)	1.71 ± 0.14ab	1.53 ± 0.15a	1.51 ± 0.01a	1.93 ± 0.06b	1.21 ± 0.04b	1.01 ± 0.06a	0.86 ± 0.01a	0.958 ± 0.07a
Malvidin-3-(6-*p*-coumaroyl)-glc (*trans*) (MCum-*trans*)	23.9 ± 0.3ab	23.2 ± 1.0a	25.5 ± 0.1b	32.5 ± 0.8c	14.0 ± 0.6a	14.2 ± 0.6a	15.0 ± 0.2ab	16.1 ± 0.7b
ΣCinnamoyl-glucosides	34.4 ± 0.4ab	32.5 ± 1.1a	35.5 ± 0.2b	44.7 ± 1.5c	19.7 ± 0.5a	19.7 ± 0.7a	20.4 ± 0.3a	22.1 ± 0.6b
Anthocyanin-derived pigments								
Malvidin-3-glc pyruvte (MPy)	0.888 ± 0.028b	0.607 ± 0.071a	0.654 ± 0.008a	0.790 ± 0.047b	3.29 ± 0.06d	2.82 ± 0.01c	2.32 ± 0.02b	1.25 ± 0.09a
Malvidin-3-(6-acetyl)-glc pyruvate (MAcPy)	tr	tr	tr	tr	3.38 ± 0.08c	1.86 ± 0.02b	1.90 ± 0.02b	1.10 ± 0.07a
Malvidin-3-glc vinylphenol (MVinyl)	0.555 ± 0.046b	0.372 ± 0.060a	0.424 ± 0.020a	0.365 ± 0.008a	0.234 ± 0.016bc	0.212 ± 0.016b	0.168 ± 0.009a	0.256 ± 0.005c
Total anthocyanins	271 ± 7a	280 ± 4a	286 ± 2a	315 ± 14b	321 ± 7ab	313 ± 3a	324 ± 3b	357 ± 1c

Mean ($n = 2$) ± SD. For each variety, different letters in the same row indicate significant difference at $p < 0.05$.
tr = Trace values.

Table 3
Individual non-anthocyanin phenolic compounds (mg/l) in wines from *Vitis vinifera* L. cv. Tempranillo and Cabernet Sauvignon resulting from the fermentation with different yeast strains

	Tempranillo				Cabernet Sauvignon			
	Commercial strain	1EV	7EV	2EV	Commercial strain	1EV	7EV	2EV
Hydroxybenzoic acids								
Gallic acid	8.88 ± 0.18b	8.52 ± 0.12b	7.31 ± 0.37a	7.82 ± 0.20a	9.60 ± 0.09a	11.0 ± 0.3b	10.2 ± 0.5ab	10.9 ± 0.3b
Methyl gallate	2.67 ± 0.06b	2.50 ± 0.06ab	2.59 ± 0.16ab	2.36 ± 0.04a	1.54 ± 0.06a	1.61 ± 0.02a	1.76 ± 0.13ab	1.99 ± 0.11b
Ethyl gallate	2.95 ± 0.19b	2.32 ± 0.13a	2.18 ± 0.14a	2.56 ± 0.12ab	3.41 ± 0.05a	3.60 ± 0.33a	3.29 ± 0.09a	3.54 ± 0.27a
Protocatechuic acid	1.89 ± 0.14b	1.55 ± 0.14a	1.36 ± 0.05a	1.38 ± 0.10a	1.29 ± 0.01a	1.19 ± 0.09a	1.09 ± 0.04a	1.10 ± 0.12a
Syringic acid	3.65 ± 0.09ab	3.90 ± 0.19bc	3.33 ± 0.20a	4.16 ± 0.18c	4.20 ± 0.06a	4.17 ± 0.08a	4.26 ± 0.08a	4.82 ± 0.05b
Vanillic acid	1.75 ± 0.07a	1.67 ± 0.11a	1.84 ± 0.10a	1.69 ± 0.09a	2.56 ± 0.06b	2.22 ± 0.08a	2.19 ± 0.20a	2.59 ± 0.08b
Hydroxycinnamic acids								
trans-Caftaric acid	0.059 ± 0.002a	0.126 ± 0.017b	tr	tr	1.90 ± 0.11c	0.332 ± 0.022b	0.247 ± 0.026ab	0.171 ± 0.009a
trans-Coutaric acid	0.090 ± 0.003b	0.168 ± 0.006c	0.069 ± 0.006a	tr	0.554 ± 0.023c	0.165 ± 0.008b	0.104 ± 0.003a	0.096 ± 0.012a
trans-Caffeic acid	4.09 ± 0.05c	1.09 ± 0.03a	1.78 ± 0.13b	3.90 ± 0.10c	0.607 ± 0.040a	0.655 ± 0.016a	0.847 ± 0.067b	0.609 ± 0.011a
trans-*p*-Coumaric acid	4.16 ± 0.04c	2.87 ± 0.20a	3.36 ± 0.16b	4.69 ± 0.18d	0.759 ± 0.005a	1.51 ± 0.05b	1.63 ± 0.10b	1.47 ± 0.15b
Phenolic alcohols and other related compounds								
Tyrosol	5.79 ± 0.03a	11.8 ± 0.1c	8.95 ± 0.40b	9.11 ± 0.22b	21.8 ± 0.8a	21.7 ± 0.2a	21.4 ± 0.9a	28.2 ± 0.7b
Tryptophol	7.58 ± 0.51a	9.15 ± 0.39b	8.15 ± 0.29ab	7.17 ± 0.38a	5.16 ± 0.25ab	4.88 ± 0.07a	5.44 ± 0.35ab	5.94 ± 0.51b
Flavanols								
(+)-Catechin	10.0 ± 0.1d	6.36 ± 0.25b	4.38 ± 0.14a	8.68 ± 0.59c	27.8 ± 1.4a	34.1 ± 0.2bc	31.9 ± 0.2b	34.4 ± 0.8c
(−)-Epicatechin	6.80 ± 0.32b	5.55 ± 0.46a	8.08 ± 0.49c	6.95 ± 0.34bc	14.5 ± 1.0a	19.1 ± 1.0c	16.4 ± 0.3ab	18.3 ± 0.9bc
Procyanidin B1	5.00 ± 0.27ab	6.22 ± 0.55b	4.23 ± 0.60a	4.84 ± 0.23a	4.16 ± 0.23a	8.71 ± 0.72b	8.31 ± 0.62b	7.40 ± 0.37b
Procyanidin B2	5.27 ± 0.19c	4.34 ± 0.36b	3.49 ± 0.24a	3.59 ± 0.17a	5.73 ± 0.08a	8.47 ± 0.11b	7.83 ± 0.44b	6.43 ± 0.44a
Total non-anthocyanins	70.7 ± 1.2b	68.2 ± 1.1b	61.1 ± 0.1a	68.9 ± 1.0b	106 ± 3a	123 ± 2bc	117 ± 1b	128 ± 3c

Mean ($n = 2$) ± SD. For each variety, different letters in the same row indicate significant difference at $p < 0.05$.
tr = Trace values.

acids and their derivatives, and phenolic alcohols and other related compounds) and flavonoid (flavanols) phenolic compounds in wines from *V. vinifera* L. cv Tempranillo and Cabernet Sauvignon. Flavanols and free hydroxycinnamic acids marked distinction among Cabernet Sauvignon and Tempranillo wines. As previously reported (Monagas, Bartolomé, et al., 2005), Cabernet Sauvignon wines usually present a higher content of monomeric flavanols than Tempranillo, whereas wines from the latter variety are characterized by a higher concentration of free hydroxycinnamic acids.

Gallic acid is the only native hydroxybenzoic acid of *V. vinifera* grapes. The Tempranillo wines from the commercial yeast strain showed the highest content of gallic acid and methyl and ethyl gallates, although it was not significantly different from all of the selected strains (i.e., from 1EV for the gallic acid content, from 1EV and 7EV for the methyl gallate content, and from 2EV for the ethyl gallate content) (Table 3). In the case of Cabernet Sauvignon wines, a contrary pattern was observed: the gallic acid and methyl gallate contents in the wines derived from the selected yeast strains were higher than in the ones from the commercial strain: significant differences were observed from 1EV and 2EV in the gallic acid content, and from 2EV in the methyl gallate content (Table 3). However, no differences were found in the concentration of ethyl gallate among the yeast strains studied. The concentration of gallic acid in wine mostly depends on its extraction from the grape seeds during the maceration and fermentation processes (Zou, Kilmartin, Inglis, & Frost, 2002), and its esterification with methanol and ethanol – respectively leading to methyl and ethyl gallate –, occurs as the result of yeast metabolism. These results suggest that the grape varietal characteristics influence the yeast phenolic metabolism in a different manner among the strains studied.

Considering the remaining hydroxybenzoic acids studied, a similar situation was found for vanillic acid since a different pattern among yeast strains was found for Tempranillo and Cabernet Sauvignon (Table 3). However, for both varieties, the highest content of protocatechuic and syringic acids corresponded, respectively, to wines from the commercial strain and from 2EV, although significant differences were only observed for Tempranillo wines concerning the content of protocatechuic acid and for Cabernet Sauvignon concerning the content of syringic acid (Table 3).

An inverse relationship was found between the content of *trans*-caftaric and *trans*-cutaric acids and their corresponding free forms, *trans*-caffeic and *trans*-*p*-coumaric acids (Table 3). For example, Tempranillo wines derived from 2EV showing trace values of *trans*-caftaric acids, presented a high concentration of *trans*-caffeic acid. A similar situation was also observed for *trans*-coutaric and *trans*-*p*-coumaric acids. Data from Cabernet Sauvignon wines also suggested that hydrolysis of both tartaric acid esters was higher in wines derived from 7EV and 2EV yeast strains. The decrease in hydroxycinnamates during vinification has been associated with enzymatic activities and with adsorptive interactions by yeast (Somers, Vérette, & Pocock, 1987), which could explain differences in the free hydroxycinnamic acid content among wines elaborated with different yeast strains.

The phenolic alcohol tyrosol and the non-phenolic alcohol tryptophol are compounds formed during yeast fermentation from tyrosine (3-(4-hydroxyphenyl)-alanine) and tryptophan (2-amino-3-(3-indolyl)-propionic acid), respectively. In the case of Tempranillo, wines derived from the 1EV yeast strain presented the highest concentration of both tyrosol and tryptophol, whereas in the case of Cabernet Sauvignon, the highest concentration for both compounds was found in wines derived from the 2EV yeast strain (Table 3).

The contents of mononeric flavanols, (+)-catechin and (−)-epicatechin, did not show any correlation with the yeast strain used, although in the case of Cabernet Sauvignon, values were closer among the selected strains and higher than the commercial strain (Table 3). Considering the dimeric procyanidins B1 and B2, no differences were found between Tempranillo wines derived from 7EV and 2EV, but in the case of Cabernet Sauvignon, this situation was found between 1EV and 7EV (Table 3). These results are probably ascribed to differences in the extraction of flavanols from the solid parts of the berry (seeds and skins) during maceration and fermentation.

The total concentration of non-anthocyanin phenolic compounds finally revealed differences between the wines derived from the commercial strain and the selected strains. Contrary to the results found in relation to the anthocyanins from which the 2EV yeast strain seemed to behave in a similar way in both Tempranillo and Cabernet Sauvignon, in the case of non-anthocyanin phenolic compounds the behavior of a particular yeast strain was not expected to be the same in both varieties. As described above, this could be explained by the fact that the concentrations of most of the non-anthocyanin phenolic compounds studied are mainly influenced by the grape variety and by their extraction during maceration and fermentation. On the other hand, the occurrence of compounds derived from yeast alcoholic fermentation such as methyl and ethyl gallates, tyrosol and tryptofol, is highly dependent on the must composition and pH of each variety (Table 1), factors that affect the growth and metabolism of yeast cells.

In order to determine the phenolic compounds that could differentiate the wines from the 4 yeast strains studied, a forward stepwise discriminant analysis was applied. Values of 4.0 and 3.9 were used for the *F*-statistic to enter and to remove variables, respectively. In order of significance, the anthocyanins DG, MCaf, and PnG, and procyanidin B2 and syringic acid, were selected as the most discriminant variables, resulting in a 100% correct classification of the samples in their original groups and in 81.3% by the cross-validation procedure (only three samples would be misclassified). Morata, Gómez-Cordovés, Suber-

viola, et al. (2003) also found that DG (i.e., trihydroxylated anthocyanin) was the anthocyanin most affected by the yeast strain. Previous studies have found differences in the phenolic content, mainly based on spectrophotometer determinations, of red wines elaborated with different selected yeast strains (Sacchi et al., 2005; for review). In this paper, it is statistically proven that the content of individual phenolic compounds can differentiate wines in relation to the yeast used in their elaboration.

3.3. Chromatic characteristics of wines

Fig. 1 illustrates the colour characteristics in function of CIELAB variables of the wines from *V. vinifera* L. Tempranillo and Cabernet Sauvignon resulting from the fermentation with different yeast strains. The colour difference in CIELAB units (ΔE^*) between the wines derived from the commercial strain and each of the selected strains is shown in Table 4. Tempranillo wines presented higher L^* and lower C^* and a^* than Cabernet Sauvingon wines (Fig. 1). As reported by Monagas, Bartolomé, et al. (2005), this is partly attributed to the characteristically high pH of Tempranillo wines (pH 4.1, mean value) which results in a lower concentration of anthocyanin species in the form of red flavylium cation in comparison with Cabernet Sauvignon wines (pH 3.7, mean value). The main differences among Tempranillo wines elaborated with different yeast strains were found for the colour variables C^* and a^* (exhibiting higher values for the commercial strain to the others) and

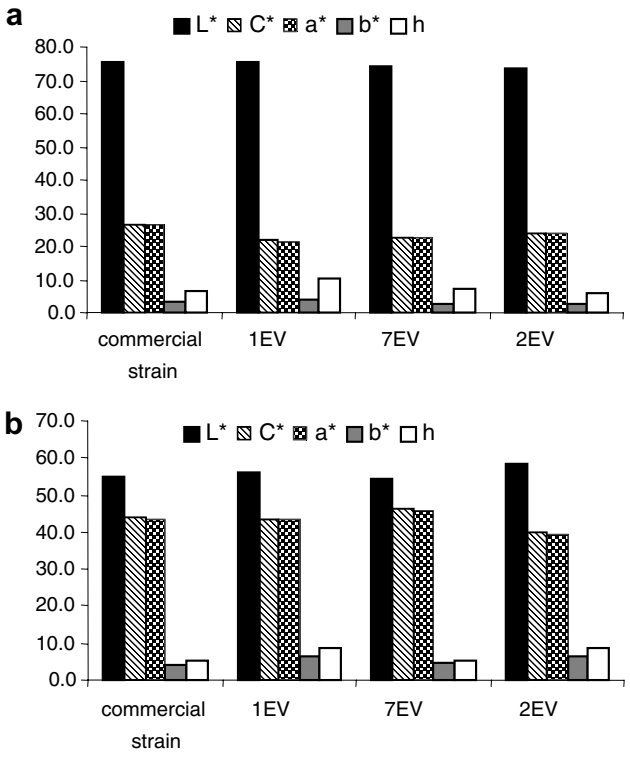

Fig. 1. Colour characteristics of wines in function of CIELAB variables. (a) Tempranillo; (b) Cabernet Sauvignon.

Table 4
Colour difference in CIELAB units (ΔE^*) between the wines derived from the commercial strain and each one of the selected strains

	Tempranillo	Cabernet Sauvignon
1EV	7.03	2.90
7EV	5.20	2.37
2EV	3.89	5.92

Mean ($n = 8$) ± SD.

h (higher value for the 1EV strain) (Fig. 1a). Consequently, the colour difference between the Tempranillo wine derived from the commercial strain and from the selected strains was greater for 1EV ($\Delta E^* = 7.03$), followed by 7EV ($\Delta E^* = 5.20$) and 2EV ($\Delta E^* = 3.89$) (Table 4). It is important to highlight that in all cases the ΔE^* values were >2.7 CIELAB units indicating that the colour differences between wines could be perceived by the human eye (Martínez, Melgosa, Pérez, Hita, & Negueruela, 2001). In the case of Cabernet Sauvignon, ΔE^* values revealed a per-

Table 5
Component matrix resulting from the PCA

Variable	PC1	PC2	PC3	PC4	PC5
MAc	0.986				
PtCum	−0.986				
PtAc	0.979				
L^*	−0.976				
C^*	0.975				
MCum-*cis*	−0.974				
(+)-Catechin	0.974				
a^*	0.972				
DAc	0.961				
(−)-Epicatechin	0.952				
Tyrosol	0.936				
Methyl gallate	−0.933				
MCum-*trans*	−0.930				
trans-*p*-Coumaric acid	−0.929		0.291		
PnCum	−0.909	0.299			
Vanillic acid	0.887			0.254	
Gallic acid	0.887		0.310		
Tryptophol	−0.884	−0.352			
MAcPy	0.878	0.282	−0.368		
Ethyl gallate	0.874	0.264	0.256		
MVynil	−0.861			−0.279	0.295
MPy	0.856	0.305	−0.297		
Procyanidin B2	0.856		0.253	−0.412	
PtG	−0.851	0.455			
b^*	0.808	−0.403	0.264		
trans-Caffeic acid	−0.769	0.535	0.324		
Protocatechuic acid	−0.710			−0.491	0.383
MG	−0.692	−0.429		0.523	
Syringic acid	0.679		0.441	0.401	0.255
PnG	0.674	0.339	0.445		0.409
Procyanidin B1	0.602	−0.451	0.494	−0.312	
DG	−0.333	0.869	0.280		
h		−0.831			0.450
trans-Coutaric acid	0.501	0.280	−0.752		0.289
MCaf		0.633	0.696		
trans-Caftaric acid	0.536	0.427	−0.670		

Variables with values >0.700 were considered strongly correlated with each component.

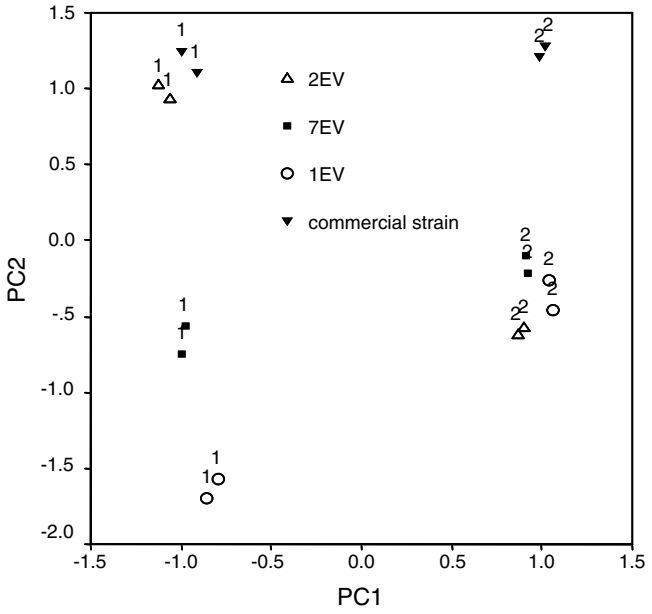

Fig. 2. Representation of the wines on the plane defined by the first two principal components, PC1 and PC2. Sample labels: 1 = Tempranillo; 2 = Cabernet Sauvignon.

ceptible colour difference between the wine from the commercial strain and 2EV, but scarce or null difference in comparison with 1EV and 7EV, respectively (Table 4). As in Tempranillo wines, the CIELAB variables that showed greater differences among Cabernet Sauvignon wines were C^* and a^* (both exhibiting lower values for the 2EV strain in comparison to the others) and h (higher values for the 1EV and 2EV strain) (Fig. 1b)

3.4. Relation between colour and phenolic compounds

A principal component analysis (PCA) was applied in order to study the interrelation between the different variables (phenolic and colour variables = 36 variables) and the samples studied. Five principal components (PC1, PC2, PC3, PC4 and PC5) were obtained. PC1 explained 69% of the total variance and was strongly correlated ($r^2 > 0.700$) with most of the CIELAB variables and phenolic compounds studied (Table 5). PC2 explained 11% of total variance and was correlated ($r^2 > 0.700$) with variables DG (0.869) and h (−0.831). Fig. 2 illustrates the bidimensional representation of the wine samples in the plane defined by these two components. Separation of wines from the two grape varieties was achieved in PC1, with Cabernet Sauvignon wines presenting higher values than the Tempranillo ones. In PC2, the commercial strain presented similar values in both varieties. However, differences were found in the values of the selected strains in function of the grape variety. In Tempranillo, wines derived from the selected strains showed very different values in PC2: 2EV presented the highest value that was similar to the commercial strain, followed by 7EV and finally by 1EV, whereas in Cabernet Sauvignon wines values were closer among the selected yeast strains and much lower than that of the commercial strain. These PCA results indicate that grape variety determines the degree of influence of a yeast strain on the phenolic composition and colour characteristics of red wines.

4. Conclusions

A detailed study on the influence of *S. cerevisiae* yeast strains on the anthocyanin, pyranoanthocyanin and non-anthocyanin phenolic compounds of red wines has been conducted. Of all the phenolic compounds studied, anthocyanins, and in particular DG, were the compounds most affected by the yeast strain independently of the grape variety, Tempranillo or Cabernet Sauvignon. However, with the exception of hydroxycinnamic acids and derivatives, the remaining non-anthocyanin phenolic compounds (i.e., hydroxybenzoic acids and flavanols) were less influenced by the yeast strain used for fermentation, which is in line with the fact that the concentration of these compounds in wine is mainly dependent on their solubility in the alcoholic medium. The content of pyranoanthocyanins and metabolites resulting from alcoholic fermentation such as methyl and ethyl gallates, tyrosol and tryptophol, seemed to be more influenced by the must composition and pH, and thus, by the grape variety, than by the yeast strain. In fact, the PCA revealed that the degree of influence of a particular yeast strain on the phenolic composition and colour characteristics of wines was marked by the grape variety. In Tempranillo, the phenolic composition and colour of wines derived from the selected strains were more variable than in Cabernet Sauvignon, indicating a greater influence of the yeast strain on this grape variety. Due to the higher anthocyanin content of the resulting wines, 2EV was the most adequate yeast strain for industrial scale fermentations of must from both grape varieties. 1EV and 7EV yeast strains presented a very similar behavior in both grape varieties but it seemed to give an overall better result in Cabernet Sauvignon.

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