MODULATION OF CHEMICAL AND SENSORY CHARACTERISTICS OF RED WINE FROM MENCÍA BY USING INDIGENOUS *SACCHAROMYCES CEREVISIAE* YEAST STRAINS

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Abstract

Aim: To evaluate the influence of native *Saccharomyces cerevisiae* strains in red wines from *Vitis vinifera* cv. Mencía: fermentative ability, inoculation success, and sensory and chemical characteristics of wines.

Methods and results: Indigenous yeast strains (Sc5, Sc11, Sc21 and Sc24) were inoculated in grape musts and their inoculation success was followed by mtDNA-RFLP (mitochondrial DNA-restriction fragment length polymorphism) at different stages of fermentation. The results showed that the added yeast strains fermented in co-dominance with a resident strain, which also controlled the spontaneous processes. Chemical analysis of basic wine parameters using official methodologies showed significant differences among wines for alcohol degree and volatile acidity. Fermentative aroma compounds were determined by gas chromatography. Wines made with different yeast strains varied in higher alcohols, ethyl ester, 2-phenylethanol, ethyl lactate and acetoin content. Sensory analysis indicated that wine from strain Sc24 had the best overall score, whereas that from strain Sc11 achieved the highest scores for colour intensity, structure and fruity character.

Conclusions: The application of selected *S. cerevisiae* strains allowed us to obtain differentiated wines from both the chemical and sensory points of view.

Significance and impact of the study: The results confirmed that indigenous yeasts can be used to elaborate singular wines and may constitute a useful tool to diversify Mencía wines.

Key words: *Saccharomyces cerevisiae*, indigenous yeasts, Galicia, Mencía, red wine

Résumé

Objectif : Évaluer l'influence de levures autochtones de *Saccharomyces cerevisiae* sur l'élaboration de vin rouge de *Vitis vinifera* cv. Mencía, en étudiant leur capacité fermentaire, leur capacité d'implantation et les caractéristiques chimiques et sensorielles des vins.

Méthodes et résultats : Des levures autochtones (Sc5, Sc11, Sc21 et Sc24) ont été inoculées dans le moût et leur capacité à s'implanter a été étudiée à différentes étapes de la fermentation par mtDNA-RFLP (polymorphisme de longueur des fragments de restriction de l'ADN mitochondrial). Les résultats indiquent que ces levures ont fermenté en codominance avec une souche déjà implantée dans la cave et également dominante dans les processus spontanés. L'analyse chimique des paramètres basiques du vin montre des différences significatives entre le titre alcoométrique volumique et l'acidité volatile. Les composés aromatiques fermentaires ont été déterminés par chromatographie en phase gazeuse. Les vins élaborés avec les différentes souches de levures varient pour leur teneur en alcools supérieurs, esters éthyliques, phényl-2 éthanol, lactate d'éthyle et acétoïne. L'analyse sensorielle indique que le vin de la souche Sc24 est le mieux évalué globalement, tandis que le vin de Sc11 a obtenu le meilleur classement pour l'intensité de la couleur, la structure en bouche et le caractère fruité.

Conclusion : L'application des souches de *S. cerevisiae* sélectionnées a permis d'obtenir des vins de qualité différente d'un point de vue chimique et sensoriel.

Signification et impact de l'étude : Les résultats ont confirmé que les levures autochtones peuvent être utilisées pour élaborer des vins de qualité, tout en étant un outil essentiel pour valoriser les vins de Mencía.

Mots clés: *Saccharomyces cerevisiae*, levures autochtones, Galicia, Mencía, vin rouge

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INTRODUCTION

The wine industry constitutes an important economic sector in Galicia (NW Spain). Five Designations of Origin (DO) are recognized in this area: Rías Baixas, Ribeiro, Ribeira Sacra, Valdeorras and Monterrei. Galician white wines are appreciated worldwide and they account for more than 80% of Galician total wine production. Despite being lower, red wine production is also relevant in Galicia, especially in DO Ribeira Sacra where it represents about 90% of total production. Besides, since the early 1990s there has been a growing interest in the protection and characterization of autochthonous or noble red grapevine varieties from Galicia in order to obtain singular wines. Galicia has a wide range of red grapevine varieties shared with proximal areas such as Portugal and Bierzo (Freijanes and Alonso, 1997; Díaz Losada *et al.*, 2011). Among them, Mencía is the main red cultivar grown in this region and it is used to elaborate monovarietal wines or in combination with other red varieties. Several studies about the volatile composition and/or sensory properties (Rebolo *et al.*, 2000; Calleja and Falqué, 2005; Vilanova and Soto, 2005; Noguerol-Pato *et al.*, 2009; Noguerol-Pato *et al.*, 2011; Vilanova *et al.*, 2012) and the phenolic composition of Mencía wines (Soto Vázquez *et al.*, 2010; Ortega-Heras *et al.*, 2012) have shown the excellent potential of this variety.

Wine chemical composition, which determines its quality, is the result of complex interactions among compounds derived from grapes (primary or varietal compounds) and secondary products originated during wine fermentation (fermentative compounds) and aging (post-fermentative compounds). Alcoholic fermentation is carried out by yeasts that transform sugar not only into ethanol and carbon dioxide, but also into a range of minor secondary metabolites including higher alcohols, fatty acids and esters (Lambrechts and Pretorius, 2000; Swiegers *et al.*, 2005; Styger *et al.*, 2011). The ability to produce these secondary metabolites depends on the yeast strain and species; therefore, it is important to determine the dynamics of yeast populations during fermentation because their metabolism has an important impact on the chemical and sensory properties of the wine (Fleet and Heard, 1993; Lema *et al.*, 1996; Egli *et al.*, 1998; Torrens *et al.*, 2008; Callejón *et al.*, 2010; Cortés and Blanco, 2011). Consequently, a wide range of selected yeast strains is available that guarantees fermentation control and quality wines. However, some wineries are interested in selecting their own yeast starters because this type of strain is better adapted to the environmental conditions. In addition, their use promotes yeast biodiversity and the elaboration of wines that preserve the typicality of a given variety and region (Callejón *et al.*, 2010; Blanco *et al.*, 2013).

Yeast strains can influence wine colour mainly by absorption of anthocyanins by yeast cell walls or by production of metabolites that react with polyphenols (Morata *et al.*, 2003; Caridi *et al.*, 2004). However, despite the importance of yeast in wine properties, to the best of our knowledge, no information is available about their role in Mencía wine composition. In this study, carried out in the Estación de Viticultura e Enoloxía de Galicia (EVEGA), four indigenous strains

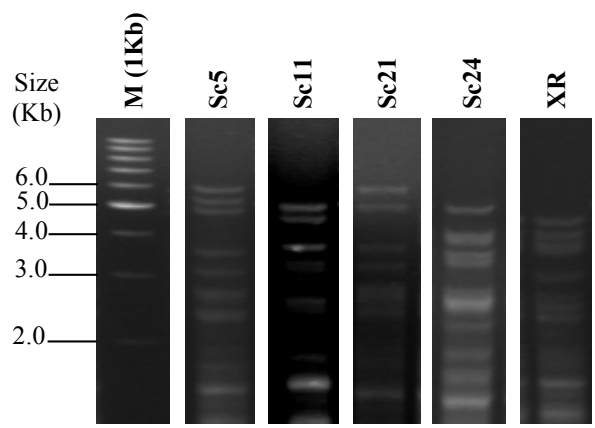


Figure 1. mtDNA restriction profiles of the *S. cerevisiae* strains used in this study.

Table 1. *Saccharomyces cerevisiae* strains used in this study

Strain	*mtDNA-RFLP**	*Origin*	*Killer phenotype*
Sc5	V	EVEGA	Sensitive
Sc11	XI	EVEGA	Neutral
Sc21	XXI	EVEGA	Neutral
Sc24	XXIV	EVEGA	Killer
Excellence XR	XXII	Lamothe-Abiet	Killer

*According to the mtDNA-RFLPs described in the yeast collection of EVEGA (Estación de Viticultura e Enoloxía de Galicia).

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of *Saccharomyces cerevisiae* were used to elaborate red wine from Mencía. The fermentative ability of these yeasts and their influence on the chemical composition, colour parameters and sensory properties of wine were evaluated.

MATERIALS AND METHODS

1. Yeast strains, media and preparation of inoculum

Five strains of *S. cerevisiae* were used in this study: four (Sc5, Sc11, Sc21 and Sc24) are indigenous strains that had been previously isolated in the experimental winery of EVEGA; the last one, Excellence XR, is a commercial yeast strain (ADY) from Lamothe-Abiet. The characteristics of these strains are summarized in Table 1 and Figure 1. Yeast strains were grown and stored in YEPD (1 % (w/v) yeast extract, 2 % (w/v) peptone and 2% (w/v) glucose). This medium was also employed for inoculum preparation. Thus, yeasts were grown in YEPD at 28 ºC for 24 h. Then, the cells were recovered by centrifugation, washed with sterile water and resuspended in physiological serum (9 ‰ NaCl) until use.

For detection of killer activity, 2 % agar plates were prepared with YEPD buffered at pH 4.2 with citrate-phosphate buffer and supplemented with 0.02 % (w/v) methylene blue. The sensitive strain *S. cerevisiae* CECT 1980 was spread on the agar plates. Then, the isolates were spotted from solid cultures and the plates were incubated at 24 ºC for 4 days. Strains were regarded as killer when they were surrounded by a clear zone of growth inhibition. To test the sensitive and neutral phenotypes, the non-killer isolates were spread on the plates, and then the reference killer strain *S. cerevisiae* EX73 (Ramírez *et al.*, 2004) was spotted on these plates. The presence or absence of an inhibition halo around the killer strain indicated that the tested strain was sensitive or neutral, respectively.

2. Fermentation

Grapes of *Vitis vinifera* cv. Mencía were manually harvested at the experimental vineyard of EVEGA in 2009, transported to the winery of EVEGA and processed following standard protocols for red wine elaboration. Thus, grapes were crushed and destemmed and homogenously distributed into twelve 35-L stainless steel tanks equipped with a system (a fixed internal steel grille) that maintains the cap immersed in the must at all times. During grape processing, 50 mg/L of SO_2 and 3 g/100 Kg of grapes of Vinozym Vintage FCE (Lamothe-Abiet, France) were added to the mass. The characteristics of the must were: Brix degree: 23.8; potential alcoholic degree: 13.5 (v/v); reducing sugars: 235.8 g/L; pH: 4.02; total acidity: 3.78 g/L of tartaric acid; tartaric acid: 4.6 g/L; and malic acid: 2.2 g/L. Fermentations were carried out in duplicate at room temperature (22-24 ºC). The indigenous yeast strains (Sc5, Sc11, Sc21 and Sc24) were inoculated in two tanks each, at a final concentration of 10^6 cells/mL, after a previous step of adaptation to must conditions. Excellence XR was added to another two fermentation tanks following the instructions of the manufacturer; thus, yeast cells were rehydrated in sugared water at 37 ºC for 20 min and, after the adaptation step to must conditions, added to the tanks at a concentration of 10^6 cells/mL. Finally, the remaining two tanks were allowed to ferment spontaneously (Esp). Fermentation evolution was followed by daily monitoring of density and temperature. After 8 days of fermentation (residual sugars <2 g/L), wines were pressed, racked into new tanks, and kept at room temperature (22-24 ºC) to allow the development of spontaneous malolactic fermentation. Samples were taken weekly to follow the evolution of malic acid and lactic acid contents. When malic acid content was not detected (after 30 days), the wines were racked again and sulphited (25 mg/L of free SO_2). Finally, after a period of 3 months of cold clarification and stabilization, the wines were bottled and stored until further chemical and sensory analysis.

3. Microbiological control

Samples for yeast isolation were taken at the beginning, middle and end of alcoholic fermentation to control the inoculation success of the strains. All samples were collected in sterile tubes, adequately diluted in sterile water, and spread on WL nutrient agar (Scharlau Microbiology, Barcelona, Spain). The plates were incubated at 28 ºC for 48 h. Thereafter, 20 colonies from each sample were randomly selected and isolated on YEPD for further characterization. Lysine agar medium (Scharlau Microbiology, Barcelona, Spain) was used to distinguish between *Saccharomyces* and non-*Saccharomyces* yeast isolates, since the former are not able to grow on this medium. The strains identified as *Saccharomyces* were characterized according to their killer phenotype as described above and at the strain level by analysis of mitochondrial DNA restriction profiles (mtDNA-RFLPs). Total yeast DNA was obtained as described by Querol *et al.* (1992) and digested with the restriction endonuclease *Hinf* I (Promega). The restriction fragments were separated by gel electrophoresis on a 0.8% (w/v) agarose gel in 1X TAE buffer. After staining with ethidium bromide (0.5 µg/mL), the DNA pattern bands were visualized

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under UV light and documented using a GelPrinter Plus system (TDI).

4. Chemical analysis

Basic parameters of wines (alcohol content, reducing sugars, pH, titratable and volatile acidity, tartaric, malic and lactic acids, and glycerol) were determined by Fourier transform infrared spectrometry (FTIR) using a WineScan FT120 analyzer (FOSS Electric) calibrated according to the official methods for wine analysis (OIV, 2009). In addition, free and total sulphur dioxides were determined using official methods (OIV, 2009*)*.

Wine colour parameters including intensity, tonality, total polyphenol index (TPI) and total anthocyanins were determined following the methods described by Zamora (2003), using a Perkin Elmer UV/VIS spectrophotometer.

Volatile compounds were determined by gas chromatography (GC). The concentration of major volatile compounds was determined by GC following the method of direct injection proposed and validated by Peinado *et al.* (2004) with some modifications. Thus, 50 µL of an internal standard solution (5 g/L of 4-methyl-2-pentanol in 50% ethanol) was added to 5 mL of wine. An aliquot of 2 µL of this mixture was injected (split 1:30) into a CP-WAX 57CB fused-silica capillary column of 50 m x 0.25 mm and 0.2-µm film thickness (Chrompack, Middelburg, The Netherlands). The analyses were carried out using an Agilent 7890A gas chromatograph (Agilent Technologies Deutschland GmbH, Waldbronn, Germany) equipped with a split/splitless injector, EFC and a flame ionization detector (FID). Instrumental conditions were as follows: injector temperature of 275 ºC, detector temperature of 300 ºC, hydrogen as carrier gas at 3.3 mL/min, and nitrogen as make-up gas at 30 mL/min. The flow rates of detector gas hydrogen and air were 40 mL/min and 400 mL/min, respectively. The temperature program was as follows: 50 ºC for 5 min, raised to 200 ºC at 4 ºC/min and held at 200 ºC for 15 min. Total running time was 57.5 min.

Volatile fatty acids, ethyl esters and higher alcohols acetates were extracted (liquid-liquid extraction) according to the methods described by Bertrand (1981) and Lema *et al.* (1996) with some modifications. A total of 2 mL of 3-octanol (50 mg/L) and 2 mL of heptanoic acid (70 mg/L) as internal standards and 1 mL of sulphuric acid (1/3) were added to 50 mL of wine. Then, this sample was extracted three times with 4, 2 and 2 mL of diethyl ether-hexane (1:1, v/v), respectively, for 5 min with shaking. Once the organic phases were totally recovered, an aliquot of 2 µL was injected in splitless mode (0.5 min; split ratio: 1:20). The analyses were carried out in an HP 5890 series II gas chromatograph equipped with a FID and connected to a Hewlett-Packard Integrator 3393A. The compounds were separated on a HP crosslinked FFAP fused-silica capillary column (polyethylene glycol stationary phase; 50 m x 0.2 mm internal diameter with 0.33-µm film thickness; Agilent). Instrumental conditions were: injector temperature of 220 ºC, detector temperature of 250 ºC, hydrogen at 1.4 mL/min as carrier gas, and nitrogen at 30 mL/min as make-up gas. The detector gas flow rates were hydrogen at 40 mL/min and air at 400 mL/min. The temperature program was as follows: initial temperature at 50 ºC (0 min), raised to 200 ºC at 3ºC/min and held at 200 ºC for 25 min. Total running time was 75 min.

Volatile compounds were identified by comparing their retention times with those of commercial pure standards. All determinations were performed in triplicate.

To estimate the sensory contribution to the overall wine flavour, the odour activity value (OAV) of certain compounds was calculated by dividing their concentration by their odour threshold value (Guth, 1997).

5. Statistical analysis

One-way ANOVA was used to check the differences in basic and colour wine parameters and volatile compounds taking into account the yeast strain as a factor. The Tukey HSD test was used to separate means. These analyses were carried out using SPSS 15.0 for Windows. Principal component analysis (PCA) was used to separate the wines produced with different yeasts according to their volatile composition. PCA was done using XLSTAT 2010 (Addinsoft).

6. Sensory analysis

Sensory evaluation was done by a panel of seven wine experts (three females and four males, 30-60 years old) with experience in tasting Galician red wines in order to differentiate wines made with different yeast strains. A descriptive scorecard including colour intensity and aroma and taste attributes was used. Aroma analysis comprised descriptors like floral, fruity, red fruits, spicy and lactic. Taste evaluation included alcohol, acidity, astringency, structure, persistence and balance. The descriptors considered were scaled from 0 (not present) to 9 (most intense). Wine samples (30 mL) coded with random numbers

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were served in clear tulip-shaped glasses. Data were processed using Big Sensory Soft 1.02 (Centro Studi Assagiatori).

RESULTS AND DISCUSSION

1. Fermentation and microbiological control

All yeasts showed a similar fermentation curve, including both inoculated and spontaneous processes (data not shown). Alcoholic fermentation started within the first 24 h after yeast addition, even in the spontaneous tanks where no yeasts were added, and it took 8 days to consume sugars. These findings contrast with those described for spontaneous vinification of white varieties, which delayed the beginning of fermentation by a few days compared to inoculated processes (Blanco *et al.*, 2006; Cortés and Blanco, 2011). During fermentation, samples for assessing yeast inoculation success were taken. Isolates were characterized according to their killer phenotype and mtDNA-RFLPs. The results indicated that the commercial strain XR clearly dominated the fermentation; however, the native yeasts fermented in co-dominance (approximately 50%) with a resident yeast, whose mtDNA-RFLP was similar to that of XR (Table 2) (Blanco *et al.*, 2011). The latter was also the leader strain in the spontaneous fermentation. In addition, it was shown that when a non-killer strain was inoculated, the genetic analysis confirmed the results from the killer test. Therefore, in those specific cases, this trait (killer phenotype) could be a rapid and useful tool for monitoring yeast implantation.

2. Chemical composition of wines: general and colour parameters

The results of the basic composition of wines showed significant differences, depending on the yeast strain, in alcohol, volatile acidity, and tartaric and lactic acids (Table 3). Sc5 wines presented the highest alcohol content. Sc24 produced the wine with the highest value of volatile acidity and XR the lowest one, although their alcohol content was similar. The influence of yeast strains on certain basic parameters of wine such as alcohol content, total and volatile acidity, and glycerol have been reported for white (Torrens *et al.*, 2008; Cortés and Blanco, 2011; Blanco *et al.*, 2013) and red wines (Caridi *et al.*, 2004; Callejón *et al.*, 2010; Blazquez Rojas *et al.*, 2012). Compared to previous data for wines from Mencía, the alcohol content was higher than that reported for Mencía wines from O Bierzo (Ortega-Heras *et al.*, 2012) and DO Rías Baixas (Vilanova *et al.*, 2012). As expected, the wines from the current study also had a lower total acidity and higher pH values than those aforementioned. Therefore, our findings confirmed that the strains of *S. cerevisiae* leading the fermentation influenced wine properties in the wines of this study. However, vintage and area are also important factors that determine the wine's final characteristics and their effect on wine attributes is sometimes greater than that exerted by the yeasts.

Regarding colour parameters, no differences were found for intensity, tonality, TPI or total anthocyanins (Table 3). Nevertheless, certain tendencies were observed: Sc5 and Esp wines presented higher values for intensity, tonality and TPI, whereas XR, Sc5 and Sc24 wines had a higher concentration of anthocyanins. Other authors found that yeast strain affected the content of polyphenols and anthocyanins in wine (Morata *et al.*, 2003; Caridi *et al.*, 2004), but further research is needed to elucidate if the strains used in this work could influence the chromatic characteristics of wine. The values of TPI and anthocyanins were similar to those reported previously for Mencía wine under different winemaking techniques (Soto Vázquez *et al.*, 2010).

Table 2. Frequency (in percentage) of killer phenotype and mtDNA-RFLPs of isolates from the different fermentations performed in this study.

	Fermentation					
	Esp	Sc5	Sc11	Sc21	Sc24	XR*
Killer phenotype						
Killer	93.8	54.5	40.0	51.7	97.5	90.5
Non-Killer	6.3	45.5	60.0	48.3	2.5	9.5
mtDNA-RFLP						
Inoculated strain	--	49	48	49	55	97
XXII	94	48	50	50	42	97
Other profiles	6	3	2	1	3	3

*mtDNA-RFLP of the inoculated strain XR is XXII. Esp (spontaneous fermentation), Sc5, Sc11, Sc21 and Sc24 (indigenous *S. cerevisiae* strains), and XR (commercial *S. cerevisiae* strain).

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Table 3. General composition and colour characteristics of Mencía wines made with different *S. cerevisiae* strains.

	Strain of *Saccharomyces cerevisiae*					
	Esp	**Sc5**	**Sc11**	**Sc21**	**Sc24**	**XR**
Alcohol content at 20ºC (% vol)	14.30a±0.00	14.50b±0.00	14.35ab±0.07	14.30a±0.00	14.25a±0.07	14.25a±0.07
Reducing sugars (g/L)	0.25a±0.07	0.20a±0.14	0.15a±0.07	0.35a±0.21	0.20a±0.14	0.05a±0.07
Total acidity (g/L tartaric acid)	3.90a±0.00	3.95a±0.07	4.05a±0.07	3.85a±0.07	3.95a±0.07	3.90a±0.00
Volatile acidity (g/L acetic acid)	0.39ab±0.02	0.40ab±0.01	0.47b±0.01	0.46b±0.04	0.51b±0.07	0.36a±0.01
pH	4.00a±0.03	4.02a±0.01	3.95a±0.03	4.00a±0.01	3.98a±0.01	3.98a±0.01
Tartaric acid (g/L)	2.00c±0.00	2.05c±0.01	1.65a±0.07	1.90bc±0.00	1.75ab±0.01	2.05c±0.07
Malic acid (g/L)	nd	nd	nd	nd	nd	nd
Lactic acid (g/L)	1.65ab±0.07	1.75b±0.07	1.50a±0.00	1.65ab±0.07	1.70b±0.00	1.70b±0.00
Free sulphur dioxide (mg/L)	49.0a±2.82	52.0a±1.14	56.0a±0.00	51.5a±10.61	54.0a±1.41	49.0a±1.41
Total sulphur dioxide (mg/L)	92.0a±2.83	111.5a±11.10	112.0a±0.00	96.0a±12.73	110.0a±0.00	96.5a±2.12
Glycerol (g/L)	9.00a±0.00	9.05a±0.35	8.80a±0.00	8.75a±0.21	8.45a±0.07	8.90a±0.00
Intensity	7.67a±0.21	7.59a±0.16	7.01a±1.09	7.67a±0.41	6.87a±0.67	7.33a±0.14
Tonality	0.84a±0.30	0.80a±2.80	0.81a±0.20	0.84a±0.21	0.75a±6.05	0.77a±6.01
Total polyphenol index (TPI)	52.6a±0.57	51.6a±5.49	48.9a±4.73	53.5a±1.46	46.6a±0.00	47.3a±5.00
Total anthocyanins (mg/L)	545a±2.47	579a±51.14	544a±10.10	541a±23.92	585a±130.13	625a±143.13

Data are mean values of two repetitions ± Standard Deviation. Different letters in the same row indicate significant differences according to the Tukey test ($p<0.05$). nd - not detected.

3. Volatile fermentation-derived compounds

Volatile fermentative compounds derived from yeast metabolism, including higher alcohols, acetates, ethyl esters and organic acids, were determined by GC. The results, summarized in Table 4, indicated that 14 from a total of 34 compounds presented significant differences among wines made with different yeasts.

Higher alcohols are important for wine quality due to their strict relation to yeast metabolism and because they are quantitatively the largest group of volatile compounds in wine. Higher alcohols impart a strong, pungent smell and taste; however, at moderate concentrations they contribute to wine aroma complexity (Giudici *et al.*, 1990; Lambrechts and Pretorius, 2000; Swiegers *et al.*, 2005). Wines from this study varied in their total contents of alcohols. Thus, wines made with strains Sc5, Sc11 and spontaneous fermentations presented higher content of alcohols, whereas wines Sc21 and Sc24 had the lowest values of these compounds, mainly due to the concentration of isoamyl alcohols (2-methyl-1-butanol and 3-methyl-1-butanol) (Figure 2 and Table 4). It was also noticeable that the highest concentration of 2-phenylethanol was in wines that fermented spontaneously (Table 4), because this aromatic alcohol is characterized by a pleasant floral odour (Aznar *et al.*, 2001).

Although they appeared in wine at a lower concentration than higher alcohols, acetates and ethyl esters are a qualitatively relevant group of compounds because they contribute desirable fruity and floral notes to wine and their concentration in wine is generally above their sensory threshold levels (Lambrechts and Pretorius, 2000). In this study, a trend towards higher concentrations of total acetates in wines from indigenous strains was observed (Figure 2). For instance, wines made with Sc11 presented the highest content of isoamyl acetate and ethyl acetate (Table 4); however, the differences found were not always significant. Similar results were obtained for ethyl esters: wines from indigenous strains had a significantly higher concentration of these compounds (Figure 2), mainly due to the content of ethyl hexanoate and ethyl octanoate (Table 4).

Volatile fatty acids are related to negative properties like rancid, fatty or cheese notes, but they are important for the aromatic equilibrium and complexity of wine (Callejón *et al.*, 2010). Figure 2 shows a trend

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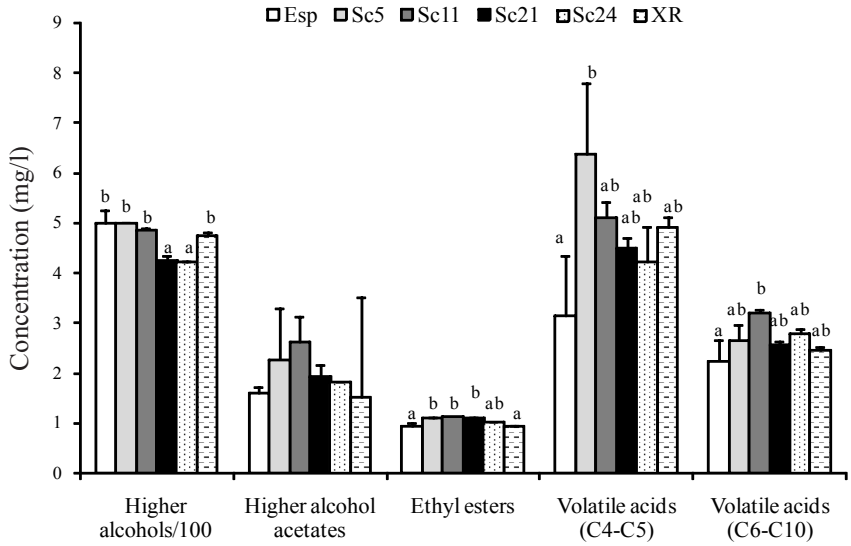


Figure 2. Total concentration (mg/L) of several groups of fermentative compounds (higher alcohols, acetates, ethyl esters and volatile acids) in wines made with different yeast strains.
Bars with different letters indicate significant differences according to the Tukey test at $p<0.05$.

Table 4. Volatile composition (mg/L) of Mencía wines made with different *S. cerevisiae* strains.

		***S. cerevisiae* strain**					
	Compound	**Esp**	**Sc5**	**Sc11**	**Sc21**	**Sc24**	**XR**
Alcohols							
	Methanol	190.7a±22.7	199.5a±5.4	201.6a±5.7	203.2a±0.9	178.3a±1.3	194.4a±5.3
	1-propanol	26.24a±0.17	28.90b±0.83	27.43ab±0.33	26.30a±0.55	27.83ab±0.40	28.82b±0.25
	2-methyl-1-propanol	58.21ab±4.30	63.19b±0.39	59.55ab±0.53	55.06a±1.24	56.55ab±0.37	60.84ab±1.16
	2-methyl-1-butanol	103.7b±5.7	101.7b±0.5	97.0b±0.8	84.7a±1.9	82.5a±1.6	96.6b±2.1
	3-methyl-1-butanol	311.2b±18.2	306.6b±0.9	302.9b±4.4	260.4a±5.6	254.9a±3.4	288.3a±5.6
	1-hexanol	2.51a±0.46	2.50a±0.10	2.94a±0.17	2.64a±0.08	2.52a±0.14	2.51a±0.27
	Trans 3-hexen-1-ol	0.07a±0.03	0.08a±0.01	0.09a±0.01	0.07a±0.02	0.08a±0.01	0.09a±0.01
	Cis 3-hexen-1-ol	0.03a±0.01	0.03a±0.00	0.03a±0.00	0.03a±0.01	0.04a±0.01	0.04a±0.01
	Benzylic alcohol	0.18a±0.10	0.52a±0.37	0.70a±0.03	0.81a±0.58	0.81a±0.64	0.29a±0.01
	2-phenylethanol	78.23b±7.6	57.97a±3.6	56.71a±3.0	49.82a±0.0	46.98a±0.4	59.99a±3.5
Acetates							
	Isoamyl acetate	1.31a±0.13	1.29a±0.10	1.66b±0.05	1.44ab±0.05	1.39ab±0.10	1.18a±0.01
	Hexyl acetate	0.015a±0.003	0.015a±0.003	0.020a±0.002	0.019a±0.003	0.020a±0.002	0.015a±0.001
	Phenylethyl acetate	0.27a±0.02	0.97a±0.93	0.94a±0.56	0.48a±0.28	0.41a±0.10	0.32a±0.01
	Ethyl acetate	46.20a±0.6	48.04ab±1.4	55.11c±0.6	52.09bc±0.8	51.19ab±2.8	46.71a±0.9
Ethyl Esters							
	Ethyl butyrate	0.44a±0.21	0.73a±0.05	0.75a±0.04	0.74a±0.00	0.73a±0.04	0.73a±0.00
	Ethyl hexanoate	0.42a±0.002	0.49c±0.003	0.49c±0.001	0.45b±0.002	0.42a±0.008	0.41a±0.012
	Ethyl octanoate	0.40a±0.05	0.47ab±0.02	0.49b±0.00	0.47ab±0.01	0.44ab±0.02	0.41ab±0.01
	Ethyl decanoate	0.11a±0.02	0.14a±0.00	0.14a±0.01	0.16a±0.03	0.15a±0.02	0.11a±0.00
	Ethyl dodecanoate	0.02a±0.00	0.03a±0.03	0.06a±0.01	0.06a±0.05	0.05a±0.04	0.02a±0.00
	Ethyl lactate	39.25a±2.1	46.69ab±2.3	43.00a±0.6	44.08ab±0.9	51.90b±3.1	46.14ab±2.8
Volatile organic acids							
	Iso-butyric acid	1.75a±0.59	2.66a±0.09	2.58a±0.15	2.37a±0.08	2.27a±0.46	2.56a±0.18
	Butyric acid	0.38a±0.21	2.22a±1.32	0.87a±0.11	0.83a±0.09	0.76a±0.09	0.77a±0.06
	Iso-valeric acid	1.01a±0.41	1.50a±0.02	1.68a±0.05	1.30a±0.04	1.17a±0.17	1.57a±0.00
	Caproic acid	1.26a±0.05	1.55ab±0.11	1.82b±0.04	1.55ab±0.10	1.60ab±0.18	1.40a±0.03
	Caprylic acid	0.91a±0.32	1.02a±0.22	1.26a±0.01	0.91a±0.12	1.08a±0.04	0.98a±0.05
	Capric acid	0.07a±0.05	0.08a±0.01	0.12a±0.01	0.10a±0.06	0.10a±0.05	0.07a±0.02
Other compounds							
	Acetaldehyde	11.13ab±0.81	11.95b±0.42	12.59b±0.50	9.74a±0.33	14.76c±0.26	10.50a±0.65
	Benzaldhyde	0.04a±0.00	0.03a±0.00	0.03a±0.00	0.03a±0.01	0.04a±0.00	0.04a±0.00
	Diethyl succinate	3.17a±0.43	3.16a±0.15	4.98a±1.28	3.47a±0.08	3.91a±0.05	3.45a±0.15
	Acetoin	20.59ab±5.31	20.26ab±1.47	20.94b±0.96	9.75a±1.19	26.20b±3.45	15.38a±2.48
	Acetol	23.13a±6.13	27.14a±1.24	29.19a±2.11	25.29a±1.22	27.03a±1.58	26.98a±2.07
	2,3-butanediol-levo	462.7a±55.6	455.9a±17.1	446.8a±9.6	466.5a±10.3	485.2a±2.0	452.9a±3.7
	2,3-butanediol-meso	158.7a±6.8	155.5a±4.7	154.3a±2.0	182.2b±3.7	176.2b±4.7	167.9ab±5.9
	γ-β Lactone	47.30a±5.4	47.72a±7.5	49.45a±5.6	51.46a±3.3	52.52a±2.8	63.24a±4.5

Data are mean values of two repetitions ± Standard Deviation. Different letters in the same row indicate significant differences according to the Tukey test ($p<0.05$).

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Table 5. Odour activity values (OAV) of compounds that reached a concentration above their odour threshold (OAV >1) for Mencía wines made with different *S. cerevisiae* strains.

Compound*	Aroma threshold (mg/L)	Aroma descriptor	Esp	Sc5	Sc11	Sc21	Sc24	XR
2-methyl-1-propanol*	40[a]	fusel, green	1.46	1.58	1.49	1.38	1.41	1.52
2-methyl-1-butanol *	40[a]	fusel, alcoholic	2.59	2.54	2.43	2.12	2.06	2.42
3-methyl-1-butanol*	30[a]	harsh, sweet, alcoholic, fusel	10.37	10.22	10.10	8.68	8.50	9.61
Acetaldehyde*	0.5[a]	bruised apple	22.26	23.88	25.16	19.48	29.52	21.00
Ethyl acetate*	7.5[a]	nail polish, fruity	6.16	6.41	7.35	6.94	6.83	6.23
Isoamyl acetate*	0.03[a]	banana	43.63	42.90	55.40	47.93	46.37	39.30
Phenylethyl acetate	0.25[a]	flowery, rose, fruity, honey	1.08	3.88	3.75	1.90	1.63	1.29
Ethyl butyrate	0.02[a]	floral, fruity, apple	22.05	37.15	37.35	36.75	36.65	36.65
Ethyl hexanoate*	0.014[b]	green apple, fruit	29.79	34.93	35.29	30.36	29.86	29.29
Ethyl octanoate*	0.005[b]	sweet, fruity, fresh	80.00	94.20	97.20	91.60	88.00	82.20
2-phenylethanol*	14[b]	floral, rose	5.59	4.14	4.05	3.56	3.36	4.28
Iso-butyric acid	2.3[b]	rancid, butter, cheese	0.76	1.15	1.12	1.03	0.99	1.11
Butyric acid	0.173[b]	rancid, cheese, sweat	2.17	12.82	5.03	4.79	4.40	4.37
Iso-valeric acid	0.033[b]	cheese, fatty, rancid, sweat	30.61	45.36	50.3	39.24	35.30	47.61
Caproic acid*	0.42[b]	fatty, cheese, sweat	3.00	3.70	4.34	3.68	3.81	3.33
Caprylic acid	0.5[b]	fatty, rancid, sweat, cheese	1.83	2.05	2.47	1.83	2.16	1.95

*Significant differences among wines (see Table 4). [a]Guth (1997); [b]Ferreira *et al.* (2000).

of indigenous yeasts to produce wines with higher contents of these compounds than spontaneous and inoculated fermentations. Strain Sc5 presented the highest content of C4-C5 acids, and strain Sc11 the highest content of C6-C10 acids. In contrast, spontaneous fermentation yielded the lowest concentration of volatile acids. However, at the individual compound level, significant differences were only observed for caproic acid, for which the concentration was higher for Sc11 (Table 4).

Finally, significant differences were found among wines in other compounds such as acetaldehyde, acetoin and 2,3-butanediol-meso (Table 4). Acetaldehyde, the major carbonyl compound found in wine, contributes to the wine aroma with bruised apple notes, but it can also be a sign of wine oxidation in white wines. In red wines, acetaldehyde, together with other metabolites, is involved in reactions with anthocyanins and other phenolic compounds to produce stable pigments (Swiegers *et al.*, 2005). Wine from Sc24 presented the highest concentration of acetaldehyde but the lowest values of colour intensity, tonality and TPI. In contrast, Sc21 wine had the lowest content of acetaldehyde but the highest values for the aforementioned colour parameters (Tables 3 and 4). Acetaldehyde is converted to diacetyl, which is further metabolized to acetoin and 2,3-butanediol, mainly by lactic acid bacteria during malolactic fermentation, but yeasts are also able to synthesize these compounds during alcoholic fermentation (Romano and Suzzi, 1996; Styger *et al.*, 2011). In this study, Sc24 wines presented the highest concentrations of acetaldehyde, acetoin and 2,3-butanediol, although these compounds have no strong odour and they are not above their threshold value (Romano and Suzzi, 1996).

To evaluate the contribution of individual compounds to the overall sensory properties of Mencía wines made with different yeasts, their odour activity values were calculated. Table 5 includes the OAV of those compounds with concentrations above their odour threshold. In total, 16 out of the 34 quantified volatile compounds presented an OAV >1, and therefore, they could potentially influence the final aroma of these wines. Most of these relevant chemicals, including the higher alcohols, acetaldehyde and some esters and acetates, showed significant differences among wines (Tables 4 and 5), and therefore, they may contribute to distinguishing these wines in sensory analysis.

Taking into account those compounds showing significant differences between strains and/or those chemicals with an OAV >1, principal components analysis was carried out to separate wines fermented

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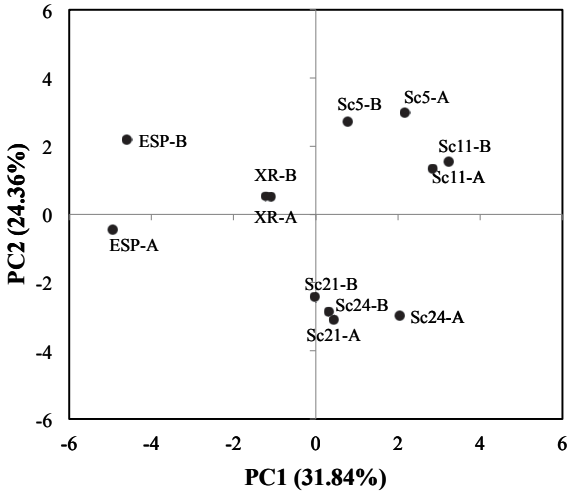


Figure 3. Separation of Mencía wines produced with different *S. cerevisiae* strains according to their volatile composition.
Principal component analysis was performed considering those compounds that showed significant differences between strains and/or with an OAV >1.

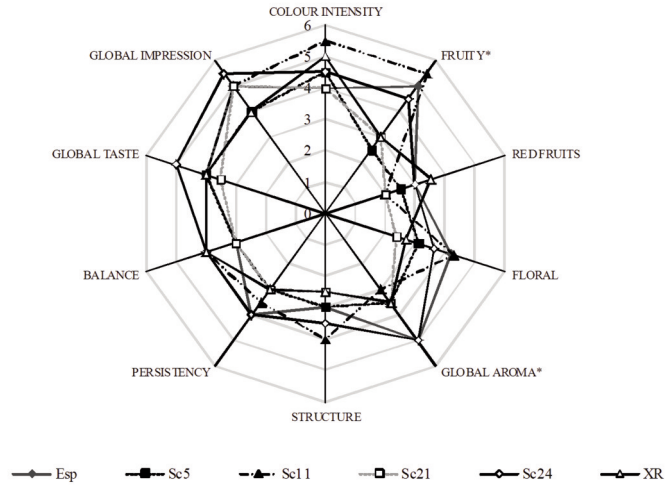


Figure 4. Sensory profile of Mencía wines made with different *S. cerevisiae* strains.
Descriptors with an asterisk showed significant differences among wines at $p<0.05$.

with different yeast strains. The first two components, PC1 and PC2, explained 56.2% of the variance (Figure 3). PC1 was mainly defined by the concentrations of caproic and iso-butyric acid, esters (ethyl octanoate, ethyl acetate, ethyl butyrate and ethyl hexanoate) and 2-phenylethanol, while PC2 was defined by the contents of higher alcohols (2-methyl-1-propanol, 2-methyl-1-butanol and 3-methyl-1-butanol) and 2,3-butanediol-meso. The distribution of samples in the PC1 and PC2 components displayed a clear separation among wines from different yeast strains. Thus, wines from spontaneous fermentation and those made with commercial yeast XR were plotted on the negative side of PC1, whereas wines obtained with indigenous strains were located on its positive side due to their higher content of esters and organic acids (Table 4 and Figure 2). In addition, PC2 allowed us to distinguish between wines made with indigenous yeast strains. Sc5 and Sc11 wines were located on the positive side of PC2, and Sc21 and Sc24 wines on the negative side. Sc5 and Sc11 produced wines with a higher content of fusel alcohols, and Sc21 and Sc24 wines presented a lower content of higher alcohols and a higher amount of 2,3-butanediol-meso (Table 4 and Figure 2).

The differentiation of chemical profiles of wines according to yeast strain has been widely reported for white (Blanco *et al.*, 2006; Torrens *et al.*, 2008; Cortés and Blanco, 2011; Blanco *et al.*, 2013) and red varieties (Callejón *et al.*, 2010; Álvarez-Pérez *et al.*, 2012; Blazquez Rojas *et al.*, 2012). The results exposed here for Mencía red wines confirm those findings. Furthermore, despite the fact that the indigenous strains fermented in co-dominance, they still had a relevant impact on wine chemical composition and the resulting wines were clearly separated by PCA.

The influence of several winemaking techniques on Mencía wine phenolic composition has been tested. Indeed, the application of pre-fermentative maceration and the addition of enzymes, chips or tannins modify the phenolic composition and colour parameters of Mencía wine (Soto Vázquez *et al.*, 2010; Ortega-Heras *et al.*, 2012). In addition, the use of selected yeast strains is one of the simplest options to obtain differentiated wines, as supported by this study. The combination of both adequate technology and yeast starter constitutes a powerful tool to tailor wines that could open new market possibilities.

4. Sensory analysis

Chemical composition differences among Mencía wines were reflected in their organoleptic properties. Sensory analysis showed differences depending on yeast strain related to global impression and certain taste and aroma descriptors of these wines. Panellists did not distinguish wines based on taste attributes like alcohol or acidity, but they did for global taste, balance, structure and persistence. Regarding aroma, attributes like spicy and lactic achieved very low scores compared to floral, fruity and red fruits. These descriptors reached scores >2 for all wines and presented clear differences among strains (Figure 4). Wine from strain Sc24 achieved the best score in global impression, both in taste and aroma. On the

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other hand, Sc11 wine presented the highest scores for colour intensity, structure and fruity and floral aroma. In contrast, wines from Sc5 and Sc21 achieved the lowest score for almost all descriptors, and so these strains would not be appropriate to elaborate wines from Mencía. Under the conditions of this study, commercial strain XR did not stand out in any attribute, except for red fruit aroma, but its wines were well balanced and with acceptable colour intensity. Finally, spontaneous fermentation wines were characterized by fruity and floral aromas, like those from strain Sc11, but with a lack of structure and colour intensity. These results were in agreement with the differences found at the chemical level. Thus, Sc11 wines presented a trend to higher contents in acetates and ethyl esters (Table 4 and Figure 2), which are compounds that contribute fruity and floral notes to wine (Lambrechts and Pretorius, 2000). In addition, spontaneously fermented wines contained the highest content of 2-phenylethanol, which is related to floral (rose) aroma (Aznar *et al.*, 2001). Nevertheless, wine chemical composition and the interaction among compounds and their impact on sensory properties is very complex and still not well known (Escudero *et al.*, 2007; Pineau *et al.*, 2009; Lytra *et al.*, 2012). Sometimes, the balance among wine components is more appreciated than the prevalence of specific compounds. For example, in this study, Sc24 wine did not exhibit the highest scores for any descriptor, in concordance with average values for its chemical components, but it was the most appreciated wine in global taste and aroma impression.

CONCLUSION

The indigenous *S. cerevisiae* strains evaluated in this study presented good fermentative ability, although they fermented in co-dominance with a winery resident strain. Nevertheless, they had an influence on the chemical and sensory characteristics of the resulting wines. From the chemical point of view, certain trends were detected among wines: those made with indigenous strains had a lower content of higher alcohols and higher concentrations of esters and volatile acids than those from spontaneous fermentation and commercial strains. These differences were detected at the sensory level as well. Wines from Sc24 had the best score in global impression, whereas wines from Sc11 stood out in colour intensity, structure and fruity and floral aroma. Therefore, both are suitable strains to be used for Mencía wine elaboration in order to achieve distinctive and favourable sensory characteristics

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