Use of *Schizosaccharomyces* strains for wine fermentation—Effect on the wine composition and food safety

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A B S T R A C T

Schizosaccharomyces was initially considered as a spoilage yeast because of the production of undesirable metabolites such as acetic acid, hydrogen sulfide, or acetaldehyde, but it currently seems to be of great value in enology.o ced Nevertheless, *Schizosaccharomyces* can reduce all of the malic acid in must, leading to malolactic fermentation. Malolactic fermentation is a highly complicated process in enology and leads to a higher concentration of biogenic amines, so the use of *Schizosaccharomyces pombe* can be an excellent tool for assuring wine safety. *Schizosaccharomyces* also has much more potential than only reducing the malic acid content, such as increasing the level of pyruvic acid and thus the vinylphenolic pyranoanthocyanin content. Until now, few commercial strains have been available and little research on the selection of appropriate yeast strains with such potential has been conducted. In this study, selected and wild *Sc. pombe* strains were used along with a *Saccharomyces cerevisiae* strain to ferment red grape must. The results showed significant differences in several parameters including non-volatile and volatile compounds, anthocyanins, biogenic amines and sensory parameters.

1. Introduction

The world wine market is experiencing increasing interest in new yeast strains that can produce unique wines with novel properties (Benito et al., 2015a; Carrau et al., 2015; Englezos et al., 2015; Esteve-Zarzoso et al., 1998; Fleet, 2008; Jolly et al., 2014, 2006; Medina et al., 2013; Pretorius, 2000; Uthurry et al., 2004). This is the reason that certain non-*Saccharomyces* yeast, such as *Schizosaccharomyces*, which have the ability to lower the malic acid content of wine, could prove to be an excellent alternative to lactic acid bacteria and are currently viewed with much interest (Benito et al., 2015b, 2014a; Fleet, 1999; Suárez-Lepe et al., 2012).

Schizosaccharomyces was initially considered as a spoilage yeast because of the production of undesirable metabolites that produced a negative sensory impact, but it has been used successfully at an industrial level in cane sugar fermentation during rum making, palm wine production and cocoa fermentation (Fleet, 1999), so there are high hopes for its use in the wine-making industry (Suárez-Lepe et al., 2012). Malolactic fermentation performed by *Oenococcus oeni* is usually used to reduce the malic acid content of musts and wines, mostly in red wine-making. However, malolactic fermentation is sometimes a highly complicated process in enology because of the growth requirements of the bacteria employed (Terrade and Mira de Orduña, 2009). The possible use of maloalcoholic fermentation could be an excellent way to preserve young aroma characteristics while reducing the harsh "green apple sourness" attributed to malic acid. It is evident that the use of *Sc. pombe* could become an invaluable new tool for grapes from the northern viticultural regions, where malic acid is present in excessive concentrations. Although the International Organization of Vine and Wine (OIV) approved "Deacidification by *Schizosaccharomyces*" (OIV, 2013), few commercial strains are available (Benito et al., 2014b; Suárez-Lepe et al., 2012) as a consequence of its high acetic acid production rate of approximately 1 g/L (Benito et al., 2012, 2014b). A high acetic acid content is inconsistent with quality wine. Mixed and sequential cultures with *Saccharomyces* have been used to reduce the negative effects of the currently available *Sc. s*pp. strains (Benito et al., 2012, 2014c). The selection of more appropriate *Sc.* strains is therefore of great enological interest.

Nevertheless, *Sc. pombe* has much greater potential than just its ability to lower the malic acid content and ferment sugar. Some researchers are using *Sc. pombe* to lower gluconic acid (Peinado et al., 2004a, 2004b, 2005, 2007). Another application is aging over lees because of the stronger autolytic release of polysaccharides than with *Saccharomyces* (Palomero et al., 2009). The ability of *Sc. pombe* to reduce 4-ethylphenol in wine due to its high adsorption ability has also been studied (Palomero et al., 2011). Furthermore the urease activity (Deák, 2008; Lubbers et al., 1996) is also of much interest concerning food safety. Urea is the primary precursor of ethyl carbamate (Uthurry et al., 2004) so reducing the urea content could lower ethyl carbamate, which is one of the principal food safety problems

in modern enology (Benito, 2015c; Uthurry et al., 2006). Additionally, the use of *Schizosaccharomyces* could limit the risk of biogenic amines (Alcaide-Hidalgo et al., 2007; Benito, 2015c), which are notorious for causing physiological problems in humans (Moreno-Arribas and Polo, 2008). Moreover, *Schizosaccharomyces* produces a high quantity of pyruvic acid (Benito et al., 2014a), and the significant hydroxycinnamate decarboxylase activity of *Schizosaccharomyces* favors the formation of vinylphenolic pyranoanthocyanins (Morata et al., 2012).

The aim of this study was to identify suitable *Sc. pombe* strains for winemaking that enhance wine quality and food safety by studying their performance in red must fermentation.

2. Materials and methods

2.1. Microorganisms

The yeast used in this study were the wild *Schizosaccharomyces pombe* strains 2139 and 938 from the type collection of the Institute for Industrial Fermentations (IFI, CSIC, Madrid, Spain) as well as V1 and 4.2, which were selected for their low production of volatile acids (Benito et al., 2014b). The *Saccharomyces cerevisiae* strain selected was 7VA from the "Laboratorio de Tecnología de Alimentos" of E.T.S.I. Agronomos in the Madrid collection because of its high pyruvic acid and acetaldehyde production (Morata et al., 2006). The lactic acid bacterial strain used was *O. oeni* 217 (Spanish Type Culture Collection, Valencia, Spain) and was selected for its good deacidification properties and its high resistance to low pH.

2.2. Vinification

All fermentations were undertaken using must from *Vitis vinifera* L. cultivar Tempranillo grapes grown at Socuéllamos, Ciudad Real in Castilla la Mancha, Spain. The must was pasteurized in an autoclave for 1 min at 105 °C in 24-L tanks. Sugars were amended up to 207 g/L and malic acid up to 4.87 g/L, and the final pH was 3.14. Citric acid was 0.24 g/L, and lactic and acetic acids were less than 0.1 g/L. To facilitate the fermentation, nutrients were added at a concentration of 0.4 g/L (Nutrient Vit—Lallemand, Montreal, Canada). Six experiments were performed in triplicate: (i) inoculation of the must with *Sc. Pombe* 2139, (ii) inoculation of the must with *Sc. Pombe* 938, (iii) inoculation of the must with *Sc. pombe* V1, (iv) inoculation of the must with *Sc. Pombe* 4.2, (v) inoculation of the must with *S. cerevisiae* 7VA, and (vi) inoculation of the must with *S. cerevisiae* 7VA, and when alcoholic fermentation was finished, the must was inoculated with *O. oeni* 217. The fermentations were carried out in 1.8-L vessels with 1.5 L of must and 50 mL of a single yeast suspension grown in Yeast Extract, Peptone, Dextrose (YEPD) liquid medium. To reach a sufficient and equal yeast population, the *Sc. pombe* suspensions were cultivated at 24 °C for 72 h and the *S. cerevisiae* suspensions were cultivated at 24 °C for 48 h. The fermentation vessel was sealed with a Müller valve (Alamo, Madrid, Spain) filled with H_2SO_4 (Panreac, Barcelona, Spain), which allowed the release of CO_2 while avoiding microbial contamination (Martini and Vaughan-Martini, 1998). The initial yeast population was calculated as approximately 10^6 CFU/mL (Fig. 1). Fermentation was accomplished at a stabilized room temperature of 25 °C. Samples of approximately 1.5 mL were taken every few days to monitor the fermentation and other compounds as explained later. At the end of the fermentation, the wine was transferred into 750-mL clear glass bottles, sealed with a natural cork and placed in a cold room at 4 °C to settle until the sensory analysis session (2 months).

2.3. Microvinification growth kinetics

During fermentation, aliquots were taken periodically under aseptic conditions and further serial ten-fold dilutions were made. Every time a sample was taken, the vessel was stirred manually to obtain a representative sample. Growth kinetics were monitored by plating 20 μL of the appropriate dilution on Petri dishes containing Yeast Extract Peptone Dextrose agar medium. Colonies were counted after incubation at 30 °C for 48–72 h. *Sc. pombe* colonies were differentiated from *S. cerevisiae* colonies by diameter, size and microscopic observation.

2.4. Analytical determinations of non-volatile compounds

Glucose and fructose, malic, lactic, acetic, pyruvic and citric acids, glycerol, and acetaldehyde were determined with a Y15 enzymatic autoanalyzer (Biosystems S.A., Barcelona, Spain). These analyses were performed using the appropriate kits from Biosystems (http://www.biosystems.es). The Y15 equipment was calibrated with the external standards that are provided in every kit by Biosystems. The alcohol content was determined following the International Methods of Analysis of Wines and Musts (OIV, 2015).

2.5. Analytical determination of anthocyanins

The following anthocyanins and pyronoanthocyanins, all of which influence wine quality, were determined at the end of the alcoholic and malolactic fermentations: delphinidin-3-O-glucoside (D3G). cyanidin-3-O-glucoside (C3G), petunidin-3-O-glucoside (Pt3G), peonidin-3-O-glucoside (Pn3G), malvidin-3-O-glucoside (M3G), Vitisine B (Vit B), Vitisine A (VitA), delphinidin-3-O-(6″-acetylglucoside) (D3G Ac), cyanidin-3-O-(6″-acetylglucoside) (C3G Ac), petunidin-3-O-(6″-acetylglucoside) (Pt3G Ac), peonidin-3-O-(6″-acetylglucoside) (Pn3G Ac), malvidin-3-O-(6″-acetylglucoside) (M3G Ac), cyanidin-3-O-(6″-p-coumaroylglucoside) (C3G Cm), petunidin-3-O-(6″-p-coumaroylglucoside) (Pt3G Cm), malvidin-3-O-(6″-p-coumaroylglucoside) (M3G Cm), malvidin-3-O-glucoside-4-vinylphenol (M3G Vph), Vinifera anthocyanin (Vinf), malvidin-3-O-glucoside-4-vinylguaiacol (M3G GVG), and malvidin-3-O-(6″-p-coumaroylglucoside)-vinylphenol (M3Gcm Vph). All substances were monitored by high-performance liquid chromatography using an Agilent Technologies series 1200 infinity series with a diode array detector (Hewlett-Packard, Palo Alto, CA, USA). Gradients of solvent C (water/formic acid, 95:5, v/v) and D (methanol/formic acid, 95:5, v/v) were used in a reverse-phase Poroshell 120 (Hewlett-Packard, Palo Alto, CA, USA) (5 cm; particle size 2.7 μm) as follows: a linear gradient of 85% C and 15% D (1 mL/min) from 0 to 2 min, a linear gradient of 85–50% C and 15–20% D (1 mL/min) from 2 to 10 min, a linear gradient of 50% C and 50% D (1 mL/min) from 10–12 min and re-equilibration of the column from 12 to 13 min to 15 min. Detection was performed by scanning in the 250–600 nm range. Calibration was performed using malvidin-3-O-glucoside (Extrasynthese, Geney, France) as an external standard. The R2 value obtained was greater than 0.999. The sensitivity was higher than 0.1 mg/L. Malvidin-3-O-glucoside controls were used to verify the calibration in each sequence. Wine samples (20 μL) of previously filtered (0.45-μm membrane filters made of cellulose methylic esters (Teckorama, Barcelona, Spain)) were injected into the HPLC apparatus. The different anthocyanins were identified by their retention times with respect to the majority anthocyanin malvidin-3-O-glucoside and by comparing the UV–visible spectra with literature data (Heier et al., 2002; Morata et al., 2007).

2.6. Analytical determinations of volatile compounds

The concentrations of 18 volatile compounds (methanol, n-propanol, diacetyl, ethyl acetate, iso-butanol, n-butanol, 2-butanol, amylic alcohol, isoamylic alcohol, isobutyl acetate, ethyl butyrate, ethyl lactate, n-hexanol, isoamyl acetate, 2-phenylethyl alcohol, 2-phe-nylethyl acetate and 2,3-butanediol), all of which influence wine quality, were measured at the end of the alcoholic and malolactic fermentations by gas chromatography using an Agilent Technologies

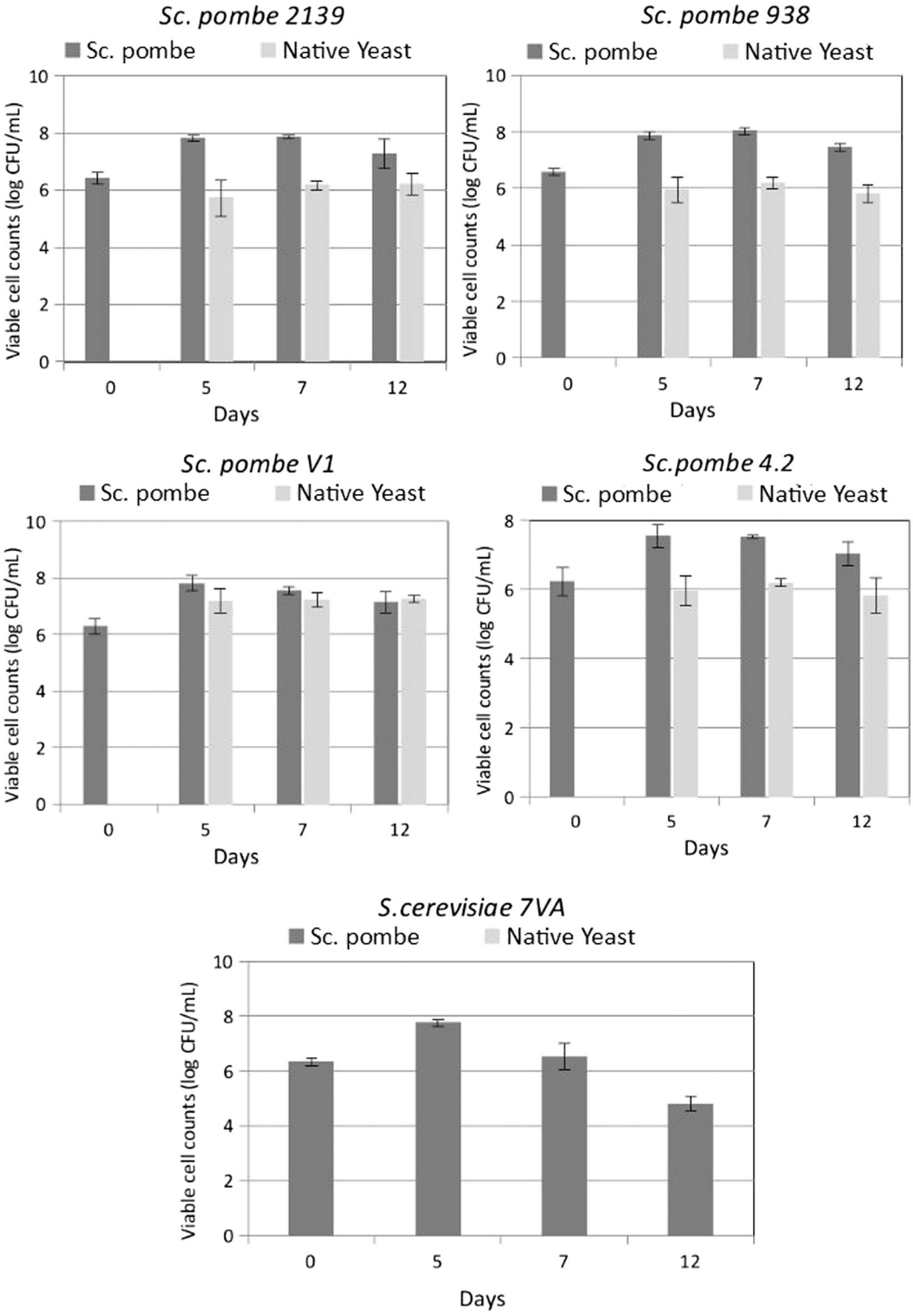

Fig. 1. Change in the population of *S. pombe* and *S. cerevisiae* 7VA during fermentation (log CFU/mL). Values are means ± standard deviations for three independent fermentations. Native yeast were counted in all fermentations.

6850 gas chromatograph with a flame ionization detector (Hewlett-Packard, Palo Alto, CA, USA). The apparatus was calibrated with a 4-meth-yl-2-pentanol internal standard at 50 mg/L. Gas chromatography quality reagents (Fluka, Sigma-Aldrich Corp., Buchs SG, Switzerland) were used as standards. Higher alcohols were separated as described in the International Methods of the Analysis of Musts and Wines (OIV, 2015). An individual calibration for each volatile compound was accomplished using an external standard at six concentrations ranging from 1 to 500 mg/L, except for 2,3-butanediol, which ranged from 100 to 1000 mg/L in accordance with its mean concentration in wine. The R2 values were greater than 0.999 for all compounds tested. The detection limit of the method was 0.1 mg/L.

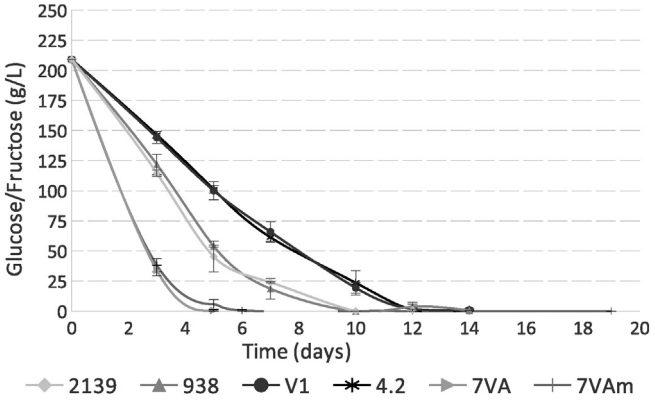

Fig. 2. Change in the glucose/fructose content of Tempranillo must during fermentation. The following nomenclature is used: Assays fermented with wild *Sc. pombe* strains 2139 and 938, selected *Sc. pombe* strains V1 and 4.2, *S. cerevisiae* 7VA, and the letter m indicates assays in which malolactic fermentation with *Oenococcus oeoni* 217 occurred.

2.7. Analytical determination of biogenic amines

Biogenic amines were determined using a Jasco (Tokyo, Japan) UHPLC chromatograph series X-LCTM, equipped with a 3120-FP fluorescence detector. Gradients of solvent A (methanol/acetonitrile, 50:50, v/v) and B (sodium acetate /tetrahydrofuran, 99:1, v/v) were used in a C18 (HALO, USA) column (100 mm × 2.1 mm; particle size 2.7 μm) as follows: 60% B (0.25 mL/min) from 0 to 5 min, a linear gradient of 60–50% B (0.25 mL/min) from 5 to 8 min, 50% B from 8 to 9 min, a linear gradient of 50–20% B (0.2 mL/min) from 9 to 12 min, 20% B (0.2 mL/min) from 12 to 13 min, a linear gradient of 20–60% B (0.2 mL/min) from 13 to 14.5 min, and re-equilibration of the column from 14.5 to 17 min. Detection was performed by scanning in the 340–420 nm range. Quantification was performed by comparison against external standards. Different biogenic amines were identified by their retention times.

2.8. Color measurements

An Agilent 8453 UV–Visible ChemStation diode array spectrophotometer (Santa Clara, USA) was used for the analysis. Samples were analyzed in a quartz cuvette with a 1-mm path length and a range of 200 to 1100 nm, following the procedure of Glories (Glories, 1984a, 1984b). Absorbance at 420 nm, 520 nm, and 620 nm was measured. Color intensity was calculated as the sum of absorbance at the three wavelengths, while the tonality (hue) was calculated as the ratio between the absorbance at 420 nm and 520 nm.

2.9. Sensory analysis

The experimental wines were evaluated by a team of (12) experienced wine tasters (7 females and 5 males), 6 employees of the Chemistry and Food Technology Department (Madrid, Spain) and 6 students. Five visual descriptors, eight taste parameters and 10 aromas were used to evaluate the final fermentations. No specific training was carried out prior to the tasting session. Six wines were evaluated in a randomized order. The wines were presented in clear tasting glasses identified with numbers from one to six in an air-conditioned (20 °C) tasting room. Twenty five milliliters of each wine was served at 14 °C. The panelists were asked to rate the wines for 23 attributes after tasting on an unstructured scale of 0 to 5, with 0 (absent) to 5 (very intense). Additionally, the panelists were asked to name descriptors as free comments for each wine.

2.10. Statistical analysis

All statistical analyses were performed using the Rstudio program software (Version 0.98.501—© 2009–2013 Rstudio, Inc.). The significance was set to $p < 0.05$ for the ANOVA matrix F value. Tukey's Studentized Range (HSD) test was used to compare the means.

3. Results and discussion

3.1. Fermentation microbiology

The results of the yeast count of the Tempranillo must prior to inoculation were 10^5 CFU/mL for the refrigerated must and $1.6 \cdot 10^2$ CFU/mL for the pasteurized must. The results of the yeast counts during fermentation are shown in Fig. 1. The *Sc. pombe* cell population varied from 10^6 to 10^8 during fermentation, while the native yeast remained 2 log scale units below the inoculated population (Fig. 1), which was directly related to the malic acid degradation by *Sc. Pombe*, as explained below.

3.2. Fermentation kinetics

3.2.1. Sugars and malic acid

Fig. 2 shows the change in the glucose/fructose concentration over the fermentation period. The fermentation kinetics was acceptable for every fermentation; fermentations were concluded at approximately the 6th day in the *S. cerevisiae* assays and at approximately the 14th day for *Sc. pombe* 2139, 938, V1 and 4.2. Previous studies have also reported slower fermentation by *Schizosaccharomyces* (Benito et al., 2012, 2013). In those cases, the fermentation times for *Sc. pombe* varied from 10 to 19 days, while the *Saccharomyces* controls varied from 6 to 10 days. The difference between *Sc. pombe* V1 and 4.2 and the wild *Sc. pombe* 2139 and 938 was evident because strains V1 and 4.2 consumed the sugar in the must more slowly than the wild strains. Even the sugar consumption kinetics are slower for *S. pombe*; the wines do not need to perform the malolactic fermentation, which usually takes longer than the alcoholic one. The data showed that the fermentation kinetics of the selected *Sc. pombe* strains was more nearly linear than those of the wild strains and that the wild strains had more similar fermentation kinetics to *S. cerevisiae* even though they were slightly slower. In each

Table 1
Analytical results for the wines produced by the different fermentation assays. The following nomenclature is used: The following nomenclature is used: Assays fermented with wild *Sc. pombe* strains 2139 and 938, selected *Sc. pombe* strains V1 and 4.2, *S. cerevisiae* 7VA, and the letter m indicates assays in which malolactic fermentation with *Oenococcus oeoni* 217 occurred.

Compounds	2139	938	V1	4.2	7VA	7VAm
Pyruvic Acid (mg/L)	352.33 ± 6.66^{a}	330.67 ± 8.50^{a}	191.00 ± 5.00^{b}	193.00 ± 2.65^{b}	219.67 ± 57.20^{b}	36.33 ± 5.51^{c}
L-Lactic Acid (g/L)	0.16 ± 0.06^{a}	0.14 ± 0.14^{a}	0.00 ± 0.00^{a}	0.14 ± 0.14^{a}	0.10 ± 0.09^{a}	1.79 ± 0.26^{b}
Glucose/Fructose (g/L)	0.88 ± 0.84^{a}	0.54 ± 0.23^{a}	0.86 ± 0.45^{a}	1.22 ± 1.18^{a}	0.89 ± 0.18^{a}	0.05 ± 0.09^{a}
Glycerol (g/L)	6.18 ± 0.38^{bc}	5.99 ± 0.58^{bc}	$6.52 \pm 0,62^{ab}$	7.55 ± 0.43^{a}	5.30 ± 0.17^{c}	5.35 ± 0.25^{bc}
Citric Acid (mg/L)	401.67 ± 7.02^{c}	465.00 ± 5.57^{abc}	500.67 ± 25.15^{ab}	508.00 ± 45.51^{a}	513.00 ± 47.00^{a}	423.00 ± 6.08^{bc}
L-Malic Acid (g/L)	0.04 ± 0.07^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.08 ± 0.14^{a}	3.76 ± 0.50^{b}	0.17 ± 0.06^{a}
Acetic Acid (g/L)	1.01 ± 0.24^{a}	1.12 ± 0.11^{a}	0.35 ± 0.02^{c}	0.31 ± 0.08^{c}	0.51 ± 0.06^{b}	0.59 ± 0.10^{b}
Acetaldehyde (mg/L)	29.33 ± 1.53^{b}	27.00 ± 3.00^{b}	76.00 ± 1.00^{a}	69.67 ± 7.64^{a}	85.00 ± 16.00^{a}	3.33 ± 0.58^{c}
pH	3.49 ± 0.03^{c}	3.50 ± 0.02^{c}	3.47 ± 0.03^{c}	3.49 ± 0.02^{c}	3.14 ± 0.02^{a}	3.29 ± 0.03^{b}

Results represent the mean ± SD for three replicates. Means in the same row with the same letter are not significantly different ($p < 0.05$).

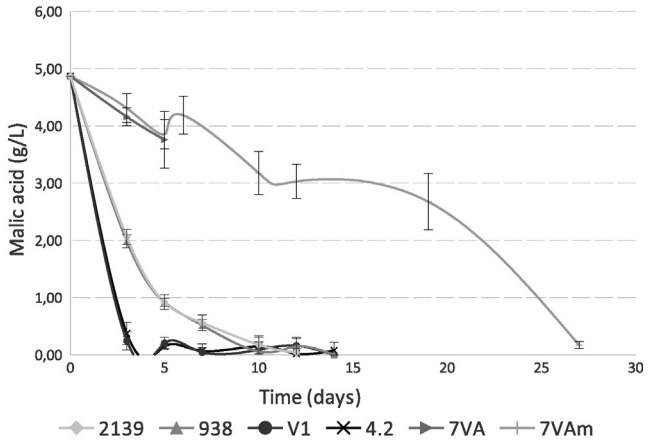

Fig. 3. Change in the malic acid content of Tempranillo must during fermentation. The following nomenclature is used: Assays fermented with wild *Sc. pombe* strains 2139 and 938, selected *Sc. pombe* strains V1 and 4.2, *S. cerevisiae* 7VA, and the letter m indicates assays in which malolactic fermentation with *Oenococcus oeoni* 217 occurred.

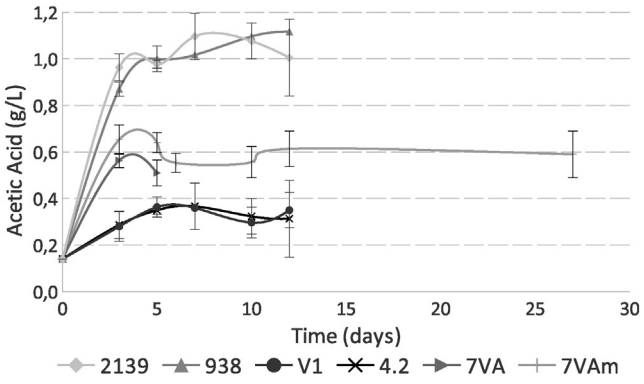

Fig. 4. Change in the acetic acid content of Tempranillo must during fermentation. The following nomenclature is used: Assays fermented with wild *Sc. pombe* strains 2139 and 938, selected *Sc. pombe* strains V1 and 4.2, *S. cerevisiae* 7VA, and the letter m indicates assays in which malolactic fermentation with *Oenococcus oeoni* 217 occurred. **a**. Acetic acid concentrations in the final Tempranillo wines. The following nomenclature is used: Assays fermented with wild *Sc. Pombe* strains 2139 and 938, selected *Sc. Pombe* strains V1 and 4.2, *S. cerevisiae* 7VA, and the letter m indicates assays in which malolactic fermentation with *Oenococcus oeoni* 217 occurred.

fermentation, all of the wine produced had a residual sugar content of less than 2 g/L (Table 1) and an alcohol content of approximately 12% by volume. In contrast, other authors have shown that some non-*Saccharomyces* yeast produced a lower yield of ethanol than *Saccharomyces* (Kutyna et al., 2010; Gobbi et al., 2014; Contreras et al., 2014). Previous studies with *S. pombe* have showed slight reductions up to 0.5 g/L when compared to the *Saccharomyces* control (Benito et al., 2013) in some instances. Other authors observed a higher final ethanol reduction over 1% by volume using other non-*Saccharomyces* species under specific conditions of high aeration (Contreras et al., 2015; Morales et al., 2015).

Malic acid has a "harsh" mouthfeel and can reduce wine quality in high concentrations. Fig. 3 shows the potential of *Sc. pombe* as a biological deacidifier, which can be easily seen by the changes in the malic acid content in the under fermentation must. It is evident that the assays with *Sc. pombe* strains V1 and 4.2 have faster and different kinetics than those of *Sc. pombe* 2139 and 938. For the selected *Sc. pombe*, the malic acid was totally consumed by day 7, but in the case of the wild ones, it was day 10, indicating that the selected *Sc. pombe* preferentially consumed malic acid. This is also in agreement with previous studies in which 75-100% reductions in the malic acid content were noted depending on the *Schizosaccharomyces* strain used (Benito et al., 2014c; De Fatima et al., 2007; Gao and Fleet, 1995; Magyar and Panyik, 1989; Silva et al., 2003; Snow and Gallander, 1979; Taillandier et al., 1995; Thornton and Rodriguez, 1996). In those studies, malic acid degradation varied from 5 to 10 days depending on the strain. After allowing malolactic fermentation to proceed in the assay fermented with *S. cerevisiae* 7VA, the final lactic acid concentration recorded was 1.79 g/L (Table 1). The level of citric acid diminished by approximately 0.1 g/L after the malolactic fermentation. Other authors have reported reductions up to 0.3 g/L of citric acid by lactic acid bacteria during a malolactic fermentation; in such cases, the citric acid metabolism was accompanied by increases in the acetic acid, acetoin and diacetyl concentrations that decreased the wine quality (Shimazu et al., 1985). No significant differences were seen in the malic acid content at the end of the maloalcoholic and malolactic fermentations in any of the experiments (Table 1). The final pH was higher for the *Sc. pombe* fermentations due to the consumption of malic acid without the production of lactic acid (Table 1). However, the pH remained lower in both of the *S. cerevisiae* fermentations. In one experiment, a malolactic fermentation did not occur as evidenced by a final malic acid concentration of 3.76 g/L, and in another experiment, a malolactic fermentation did occur, as evidenced by the conversion of malic acid into 1.79 g/L (Table 1) of lactic acid by the lactic acid bacteria.

3.2.2. Acetic acid

As previously mentioned, a primary enological concern associated with *Schizosaccharomyces* spp. is its significant production of greater than 1 g/L of acetic acid in a laboratory-scale fermentation (Benito et al., 2012, 2014b). Acetic acid has a negative impact on wine flavor, and low levels usually produce better sensory profiles. Levels over 0.8 g/L can generate wine quality problems. In the present study, the acetic acid content became relatively stable on approximately day 8 of the fermentation (Fig. 4). Fig. 4a shows the final acetic acid concentration. In the fermentations involving *Sc. pombe* V1 and 4.2, the concentration of acetic acid remained at approximately 0.30 g/L from day 5 until the end of fermentation and remained approximately at 1 g/L for *Sc. pombe* 2139 and 938 (Fig. 4). Other authors have reported a final acetic acid value of approximately 0.4 g/L for a few selected *Schizosaccharomyces* strains in small-scale trials (Benito et al., 2014b). Acetic acid production is a strain-dependent characteristic of *Schizosaccharomyces* species that varied from 0.4 to 1.3 g/L in previous studies, commonly averaging between 0.6 and 0.9 g/L (Benito et al., 2014b). However, *S. cerevisiae* alone increased the acetic acid concentration (Fig. 4a) greater than strains V1 and 4.2, and it was even higher following the malolactic fermentation step, reaching a value of 0.59 g/L of acetic acid. That finding confirms that a well-chosen *Sc. pombe* strain can have results as good as *S. cerevisiae* for low acetic acid production.

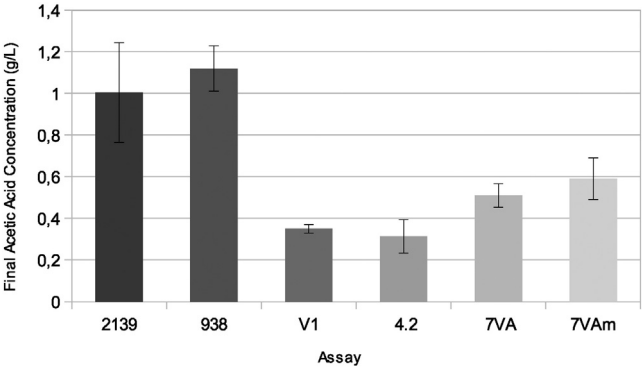

Fig. 5. Change in the pyruvic acid content of Tempranillo must during fermentation. The following nomenclature is used: Assays fermented with wild *Sc. Pombe* strains 2139 and 938, selected *Sc. Pombe* strains V1 and 4.2, *S. cerevisiae* 7VA, and the letter m indicates assays in which malolactic fermentation with *Oenococcus oeoni* 217 occurred.

Table 2
Volatile compounds detected in the wines produced by the different fermentation assays. The following nomenclature is used: Assays fermented with wild *Sc. pombe* strains 2139 and 938, selected *Sc. pombe* strains V1 and 4.2, *S. cerevisiae* 7VA, and the letter m indicates assays in which malolactic fermentation with *Oenococcus oeoni* 217 occurred.

Compounds (mg/L)	2139	938	V1	4.2	7VA	7VAm
Methanol	38.82 ± 1.30^{a}	40.92 ± 0.50^{a}	41.64 ± 1.09^{a}	41.62 ± 1.74^{a}	40.78 ± 1.66^{a}	41.57 ± 2.48^{a}
1-propanol	28.48 ± 2.82^{ab}	29.41 ± 1.21^{a}	23.72 ± 0.72^{c}	24.85 ± 0.02^{bc}	28.21 ± 0.91^{ab}	28.86 ± 1.44^{ab}
Diacetyl	3.98 ± 0.23^{d}	4.00 ± 0.31^{d}	5.26 ± 0.01^{c}	5.50 ± 0.33^{c}	3.72 ± 0.28^{d}	14.89 ± 3.19^{b}
Ethyl acetate	76.87 ± 9.49^{a}	74.36 ± 7.02^{a}	43.19 ± 2.35^{b}	44.17 ± 5.61^{b}	63.26 ± 2.76^{a}	77.23 ± 3.19^{a}
2-butanol	2.77 ± 0.88	–	–	–	–	–
Isobutanol	8.02 ± 1.53^{c}	8.51 ± 0.88^{c}	31.47 ± 0.91^{b}	30.43 ± 0.12^{b}	120.60 ± 11.27^{a}	115.25 ± 12.78^{a}
1-butanol	22.16 ± 6.44^{a}	15.49 ± 4.92^{ab}	13.51 ± 7.55^{abc}	8.09 ± 0.69^{bc}	18.09 ± 2.30^{ab}	5.20 ± 1.41^{bc}
Acetoin	103.34 ± 9.33^{a}	93.26 ± 6.69^{a}	51.41 ± 0.45^{b}	42.93 ± 5.84^{b}	11.60 ± 0.25^{c}	23.50 ± 1.31^{c}
2-methyl-1-butanol	25.84 ± 3.77c	28.02 ± 1.67^{c}	111.48 ± 0.74^{b}	111.62 ± 1.78^{b}	257.40 ± 25.64^{a}	277.30 ± 17.08^{a}
3-methyl-1-butanol	14.47 ± 3.47^{c}	13.63 ± 0.36^{c}	28.13 ± 2.89^{b}	27.92 ± 1.49^{b}	59.20 ± 5.15^{a}	65.08 ± 0.87^{a}
Isobutyl acetate	–	–	–	–	–	–
Ethyl butyrate	–	–	–	–	2.22 ± 0.12	–
Ethyl lactate	9.78 ± 0.20^{c}	11.37 ± 3.32^{c}	9.80 ± 0.69^{c}	8.83 ± 1.03^{c}	18.43 ± 0.75^{b}	76.50 ± 2.61^{a}
2-3 butanodiol	216.20 ± 0.45^{c}	237.56 ± 19.08^{c}	305.25 ± 9.32^{b}	322.43 ± 25.76^{b}	451.01 ± 18.21^{a}	476.64 ± 42.74^{a}
Isoamyl acetate	3.72 ± 0.13^{e}	13.63 ± 0.36^{b}	7.66 ± 0.77^{d}	7.42 ± 0.03^{d}	10.81 ± 0.75^{c}	9.40 ± 1.49^{cd}
Hexanol	4.22 ± 0.13^{a}	3.79 ± 0.01^{a}	–	3.98 ± 0.28^{a}	4.10 ± 0.31^{a}	–
2-phenylethanol	34.81 ± 2.29^{b}	37.34 ± 2.57^{b}	39.00 ± 0.43^{b}	40.26 ± 0.49^{b}	78.46 ± 0.18^{a}	86.26 ± 7.60^{a}
Phenylethyl acetate	8.50 ± 0.14^{a}	8.02 ± 0.19^{ab}	7.11 ± 0.17^{b}	6.98 ± 0.18^{b}	7.80 ± 0.08^{ab}	9.03 ± 0.33^{a}

Results represent the mean ± SD for three replicates. Means in the same row with the same letter are not significantly different ($p < 0.05$).

3.2.3. Pyruvic acid

Fig. 5 shows the change in the pyruvic acid concentration during fermentation. High levels of pyruvic acid are related to the formation of highly stable pigments such as vitisin A, which improves the chromatic characteristics of wines, especially during a long aging processes (Morata et al., 2003) when stable pigment forms become more important than unstable forms. The maximum concentration was reached on day 3 for *S. cerevisiae* and on day 5 for the *Sc. pombe* strains, followed by its reduction. The maximum concentration was achieved with the wild *Sc. pombe*. *S. cerevisiae* fermentation without a malolactic fermentation (MLF), which showed a higher than usual concentration of pyruvic acid that could be explained by the higher than usual speed of the fermentation process. *S. cerevisiae* showed much lower values when an MLF was subsequently performed. In other studies involving fermentations with other *Schizosaccharomyces* strains, values up to 0.39 g/L have been recorded (Benito et al., 2012). The pyruvic acid concentrations reached in fermentations with wild *Sc. pombe* were almost double that with the selected *Sc. pombe* but were also higher than that with *S. cerevisiae* 7VA, which was chosen for its ability to produce a high amount of pyruvic acid. The formation of highly stable pigments such as vitisin A in fermentations with wild *Sc. pombe* strains should probably be higher than the rest.

3.2.4. Glycerol

The final glycerol content of wine produced by different yeast was higher in the *Sc. pombe* fermentations. The highest value detected was 7.55 g/L for *Sc. pombe* 4.2, while the lowest was 5.30 g/L for *S. cerevisiae* 7VA (Table 1). *Schizosaccharomyces* species have previously been reported to produce more glycerol than *Saccharomyces* species, reaching values up to 10 g/L and approximately 1 g/L higher than the *Saccharomyces* control (Benito et al., 2014b). Some authors have reported that some *Sc. pombe* strains have a well-developed glyceropyruvic pathway, which might explain the slightly higher pyruvic acid and glycerol production compared to other yeast (Suárez-Lepe and Leal, 2004). An increased glycerol content has been described as one of the primary contributions of non-*Saccharomyces* strains to wine quality (Jolly et al., 2006) because it enriches the mouth-feel. Other authors have reported higher glycerol production for other non-*Saccharomyces* species (Comitini et al., 2011; Gobbi et al., 2013). Although other yeast species such as *Candida stellata* have been described as higher producers of glycerol up to 14 g/L (Jolly et al., 2014), the use of *Schizosaccharomyces* to improve this quality parameter could be interesting.

3.2.5. Acetaldehyde

The experiments with the selected *Sc. pombe* V1 and 4.2 strains and *S. cerevisiae* without malolactic fermentation had the highest final acetaldehyde content, while the experiment with the *S. cerevisiae* with MLF had the lowest (Table 1). High acetaldehyde values could be beneficial for red wine because, along with malvidin-3-glucoside, it acts as a precursor of vitisin B, which helps stabilize wine color (Benito et al., 2011).

3.3. Volatile aromas

Table 2 shows the production of volatile compounds in the different fermentations. Methanol production (approximately 40 mg/L) never exceeded the legal limit of 120 mg/L for red wine. At the end of all fermentations, the ethyl acetate that was produced was approximately 77 mg/L, which is considered acceptable because it is below the threshold of 150 mg/L considered to be undesirable (Rapp et al., 1992; Lambrechts and Pretorius, 2000); higher levels could be responsible for an undesirable glue odor. The highest values were achieved with the wild *Sc. pombe* strains but also with the *S. cerevisiae* with or without subsequent MLF. The lowest values were achieved for the selected V1 and 4.2 *Sc. Pombe* that produced concentrations 20 mg/L lower than the others, which could influence the wine quality in a positive way. MLF did not seem to have an effect on ethyl acetate production, but it was highly strain-dependent in *Sc. Pombe* fermentations.

Higher alcohols (isobutanol, 2-methyl-1-butanol and 3-methyl-1-butanol) were produced in the highest quantities in the *S. cerevisiae* fermentations, both before and after the malolactic fermentation step. The lowest values were produced with the wild *Sc. pombe* strains 2139 and 938. Other authors have reported that other non-*Saccharomyces* yeast produced lower amounts of higher alcohols than *Saccharomyces cerevisiae* (Benito et al., 2015a; Clemente-Jimenez et al., 2004; Gobbi et al., 2013; Parapouli et al., 2010; Romano and Suzzi, 1993; Romano, 2003; Zironi et al., 1993). Sometimes low levels of higher alcohols increased the perception the aroma of varietal grape varieties, which was considered a positive effect (Belda et al., 2015). A total higher alcohol concentration of less than 350 mg/L is recommended because a higher concentration gives the wine a disagreeable alcoholic flavor. In this study, *S. cerevisiae* exceeded the recommended value. Nevertheless,

Table 3
Anthocyanins detected in the wines produced by the different fermentation assays. The following nomenclature is used: Assays fermented with wild *Sc. pombe* strains 2139 and 938, selected *Sc. pombe* strains V1 and 4.2, *S. cerevisiae* 7VA, and the letter m indicates assays in which malolactic fermentation with *Oenococcus oeoni* 217 occurred.

Compounds (mg/L)	2139	938	V1	4.2	7VA	7VAm
D3G	1.30 ± 0.09^{ab}	1.13 ± 0.10^{b}	0.86 ± 0.12^{c}	0.88 ± 0.05^{c}	1.47 ± 0.04^{a}	0.47 ± 0.04^{d}
C3G	0.62 ± 0.20^{a}	0.49 ± 0.05^{ab}	0.28 ± 0.12^{b}	0.31 ± 0.08^{b}	0.34 ± 0.09^{ab}	–
Pt3G	1.19 ± 0.04^{b}	1.12 ± 0.13^{b}	0.80 ± 0.10^{c}	0.88 ± 0.04^{c}	1.6 ± 0.04^{a}	0.49 ± 0.02^{d}
Pn3G	4.42 ± 0.02^{a}	4.10 ± 0.13^{a}	2.42 ± 0.39^{b}	2.62 ± 0.32^{b}	4.10 ± 0.03^{a}	1.23 ± 0.14^{c}
M3G	13.23 ± 0.79^{b}	12.28 ± 0.09^{a}	10.45 ± 0.65^{c}	11.09 ± 1.23^{c}	17.47 ± 0.21^{a}	5.74 ± 0.72^{d}
VitB	0.25 ± 0.00^{a}	0.23 ± 0.03^{a}	0.22 ± 0.01^{a}	0.22 ± 0.03^{a}	0.15 ± 0.04^{b}	–
VitA	1.32 ± 0.01^{a}	1.28 ± 0.05^{a}	1.16 ± 0.08^{a}	1.23 ± 0.10^{a}	1.25 ± 0.06^{a}	0.81 ± 0.03^{b}
D3G Ac	0.25 ± 0.02^{a}	0.22 ± 0.04^{a}	0.20 ± 0.03^{a}	0.19 ± 0.05^{a}	0.30 ± 0.06^{a}	0.07 ± 0.00^{b}
C3G Ac	0.49 ± 0.02^{a}	0.43 ± 0.06^{a}	0.44 ± 0.03^{a}	0.44 ± 0.06^{a}	0.42 ± 0.05^{a}	0.32 ± 0.00^{b}
Pt3G Ac	0.47 ± 0.04^{b}	0.45 ± 0.05^{b}	0.34 ± 0.05^{bc}	0.38 ± 0.01^{c}	0.63 ± 0.04^{a}	0.17 ± 0.01^{d}
Pn3G Ac	0.82 ± 0.07^{b}	0.81 ± 0.12^{b}	0.79 ± 0.04^{b}	0.82 ± 0.07^{b}	1.21 ± 0.16^{a}	0.43 ± 0.02^{c}
M3G Ac	4.02 ± 0.20^{b}	3.74 ± 0.24^{bc}	3.18 ± 0.20^{bc}	3.41 ± 0.31^{c}	4.97 ± 0.17^{a}	1.12 ± 0.17^{d}
C3G Cm	–	–	–	–	0.11 ± 0.01^{a}	–
Pt3G Cm	0.13 ± 0.05^{a}	0.14 ± 0.11^{a}	0.12 ± 0.02^{a}	0.12 ± 0.01^{a}	0.14 ± 0.02^{a}	–
M3G Cm	1.24 ± 0.11^{ab}	1.22 ± 0.11^{ab}	1.11 ± 0.10^{a}	1.22 ± 0.15^{b}	1.48 ± 0.05^{a}	0.48 ± 0.04^{c}
M3G Vph	–	–	–	–	0.40 ± 0.03^{b}	0.55 ± 0.01^{a}
Vinf	–	–	–	–	0.12 ± 0.01^{b}	0.15 ± 0.00^{a}
M3G GVG	–	–	–	–	0.13 ± 0.01^{b}	0.21 ± 0.18^{a}
M3Gcm Vph	–	–	–	–	0.09 ± 0.01^{b}	0.21 ± 0.18^{a}
Total	29.77 ± 1.67^{b}	27.67 ± 1.67^{b}	22.37 ± 2.52^{c}	23.82 ± 1.90^{c}	36.40 ± 1.13^{a}	12.38 ± 1.42^{d}

Results represent the mean ± SD for three replicates. Means in the same row with the same letter are not significantly different ($p < 0.05$).

other authors (Rapp and Mandery, 1986) showed that concentrations less than 300 mg/L for *S. pombe* strains could improve the general complexity of the wine. However, this was not perceived during a sensory analysis. The formation of 2-phenylethanol is connected with floral aromas and is considered positive, and it was over the threshold limit of 7.5 mg/L in every case (Lambrechts and Pretorius, 2000) with higher values in *S. cerevisiae* 7VA fermentations with final values as high as 78 mg/L. Other authors have reported greater production of this compound by other yeast species of as high as 180 mg/L (Lambrechts and Pretorius, 2000; Comitini et al., 2011; Gobbi et al., 2013; Benito et al., in press). Diacetyl was over the threshold of 5 mg/L established as objectionable (Lambrechts and Pretorius, 2000) in a trial of a malolactic fermentation in which the final concentration was approximately 14 mg/L. Isoamyl acetate was detected in every assay at concentrations of less than 14 mg/L but always higher than the threshold of 0.26 mg/L (Lambrechts and Pretorius, 2000), which could contribute a fruity aroma described as banana. Ethyl lactate production was moderate in all fermentations, except the *S. cerevisiae* 7VA fermentation followed by a malolactic fermentation. The acetoin concentration was normal according to other authors who reported values in wine from undetectable levels to 80 mg/L (Romano and Suzzi, 1996; Romano et al., 2003; Shinohara et al., 1979). Acetoin has no negative organoleptic effects at those levels. However, non-selected *Schizosaccharomyces* strains showed levels as high as 100 mg/L. Concentrations higher than 150 mg/L (Gonzalez et al., 2001) in wine can produce an unpleasant buttery flavor.

3.4. Anthocyanins

Table 3 shows the values for anthocyanins in the different fermentations. Vitisine A was detected in similar concentrations in all of the fermentations except for the one with a subsequent MLF. The lower concentration was detected in the *S. cerevisiae* assay with a MLF, probably because of the much lower pyruvic acid concentration (Table 1). Vitisine B was not detected for fermentations in which a subsequent MLF occurred. The *Sc. pombe* experiments had significantly higher values than the *S. cerevisiae* experiments. The formation of highly stable pigments such as Vitisines is connected with improved chromatic characteristics of the wine, especially during a long aging process (Morata et al., 2003) when stable pigment forms become more important than unstable forms. On the other hand, M3G was higher value with *S. cerevisiae* and decreased significantly up to 12 mg/L after the MLF. A similar effect was observed for other anthocyanins. The experiments with the wild *Sc. pombe* strains had significantly higher values (approximately 1 mg/L for M3G) than the other *Sc. pombe* strains. A direct strong connection with the acetaldehyde concentration to explain the Vitisine A content cannot be made. The total anthocyanin concentration was significantly higher for *S. cerevisiae* without the MLF, but it dropped significantly after the MLF. The total anthocyanin content for the experiments with the wild *Sc. pombe* was significantly higher than for the other *Sc. pombe* strains; it was approximately 28 mg/L and 23 mg/L, respectively. For *S. cerevisiae* fermentations, the total anthocyanin concentration was 36 mg/L; however, after MLF, the final concentration was

Table 4
Color measurements in the wines produced by the different fermentation assays. The following nomenclature is used: Assays fermented with wild *Sc. pombe* strains 2139 and 938, selected *Sc. pombe* strains V1 and 4.2, *S. cerevisiae* 7VA, and the letter m indicates assays in which malolactic fermentation with *Oenococcus oeoni* 217 occurred.

Color measurements	2139	938	V1	4.2	7VA	7VAm
420 nm	0.09 ± 0.01^{bc}	$0.09 \pm 0{,}01^{bc}$	0.11 ± 0.00^{ab}	0.10 ± 0.00^{ab}	0.08 ± 0.00^{c}	0.08 ± 0.00^{c}
520 nm	0.01 ± 0.01^{bc}	0.09 ± 0.01^{bc}	0.12 ± 0.00^{a}	0.11 ± 0.01^{ab}	0.10 ± 0.00^{bc}	0.08 ± 0.00^{c}
620 nm	0.00 ± 00^{bc}	0.00 ± 0.00^{bc}	0.01 ± 0.00^{ab}	0.01 ± 0.00^{ab}	0.00 ± 0.00^{c}	0.02 ± 0.00^{a}
dA %	52.32 ± 2.29^{b}	51.50 ± 2.09^{bc}	48.01 ± 0.76^{c}	48.33 ± 1.06^{bc}	56.68 ± 0.51^{a}	38.80 ± 2.28^{c}
CI	0.19 ± 0.02^{bc}	0.19 ± 0.02^{bc}	0.24 ± 0.01^{a}	0.22 ± 0.01^{ab}	0.18 ± 0.01^{c}	0.18 ± 0.01^{c}
Yellow: A420 (%)	47.01 ± 0.97^{a}	47.50 ± 0.51^{a}	46.71 ± 0.67^{a}	46.91 ± 0.40^{a}	46.81 ± 0.46^{a}	46.36 ± 0.56^{a}
Red: A520 (%)	51.21 ± 1.21^{b}	50.83 ± 1.08^{bc}	49.03 ± 0.37^{c}	49.18 ± 0.51^{bc}	53.58 ± 0.29^{a}	44.98 ± 0.93^{c}
Blue: A620 (%)	1.79 ± 2.06^{bc}	1.67 ± 1.59^{bc}	4.26 ± 0.36^{b}	3.91 ± 0.11^{b}	0.00 ± 0.00^{c}	8.67 ± 1.10^{a}
Hue	0.92 ± 0.01^{bc}	0.93 ± 0.01^{bc}	0.95 ± 0.02^{b}	0.95 ± 0.02^{b}	0.87 ± 0.02^{b}	1.03 ± 1.10^{a}

Results represent the mean ± SD for three replicates. Means in the same row with the same letter are not significantly different ($p < 0.05$).

Table 5
Biogenic amines detected in the wines produced by the different fermentation assays. The following nomenclature is used: Assays fermented with wild *Sc. pombe* strains 2139 and 938, selected *Sc. pombe* strains V1 and 4.2, *S. cerevisiae* 7VA, and the letter m indicates assays in which malolactic fermentation with *Oenococcus oeoni* 217 occurred.

Compounds (mg/L)	Must	2139	938	V1	4.2	7VA	7VAm
Histamine	0.42 ± 0.01^{c}	0.30 ± 0.01^{b}	0.29 ± 0.01^{b}	0.30 ± 0.01^{b}	0.31 ± 0.01^{b}	0.31 ± 0.01^{b}	0.92 ± 0.02^{a}
Tiramine	0.26 ± 0.01^{a}	0.19 ± 0.01^{b}	0.20 ± 0.01^{b}	0.20 ± 0.01^{b}	0.19 ± 0.01^{b}	0.19 ± 0.01^{b}	0.25 ± 0.01^{a}
Putrescine	1.38 ± 01^{a}	1.05 ± 01^{c}	1.03 ± 0.02^{c}	1.06 ± 0.01^{c}	0.99 ± 0.02^{d}	1.10 ± 0.02^{b}	1.11 ± 0.02^{b}
Cadaverine	0.64 ± 0.01^{d}	0.40 ± 0.01^{ab}	0.39 ± 0.01^{b}	0.41 ± 0.01^{c}	0.36 ± 0.01^{c}	0.42 ± 0.01^{a}	0.43 ± 0.01^{a}

Results represent the mean ± SD for three replicates. Means in the same row with the same letter are not significantly different ($p < 0.05$).

reduced in 20 mg/L. High anthocyanin levels are connected to a better color quality, improved mouthfeel and a better aging potential.

3.5. Color measurements

Table 4 shows the results for the color measurements of the different fermentations. No significant difference was found for the hue in the different fermentations other than one in which MLF occurred, which was slightly higher. The color intensity (CI) was slightly higher for the assays of the selected *Sc. pombe* (greater for strain V1). The CI was not significantly different before and after the MLF for the *S. cerevisiae* experiments. The *S. cerevisiae* fermentations had a "redder" color, while the selected *Sc. pombe* had a more "bluish" color than the rest, probably indicating a younger wine.

3.6. Biogenic amines

The experiment in which a malolactic fermentation occurred showed the highest concentration of biogenic amines (Table 5). *Schizosaccharomyces* can restrict the activity of the indigenous lactic acid bacteria because it removes malic acid (another nutrient source). Indigenous or non-selected lactic acid bacteria can cause higher biogenic amine concentrations in wine (Alcaide-Hidalgo et al., 2007; Benito, 2015c), so *Schizosaccharomyces* can be used to prevent biogenic amine production. A special reference should be made for histamine. Fig. 6 shows the histamine levels for each fermentation. A value of 2 mg/L for histamine is considered the highest acceptable level in some countries due to food safety legislation (Lehtonen, 1996). The slight reported differences in cadaverine and putrescine could be explained by the different metabolic ability of the yeast to remove biogenic amines because these biogenic amines originate from the grapes. Other authors have reported a higher reduction of biogenic amines of up to 2.2 mg/L with alcoholic fermentation for the non-*Saccharomyces* species *Hanseniaspora vineae* (Medina et al., 2013). The urease enzymatic activity described for *Schizosaccharomyces* (Lubbers et al., 1996) could also reduce the level of ethyl carbamate precursors (Benito et al., 2014a, 2015c).

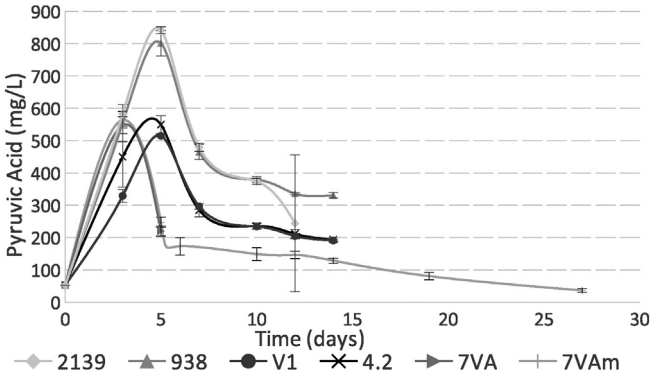

Fig. 6. Histamine concentrations in the final Tempranillo wines. The following nomenclature is used: Assays fermented with wild *Sc. pombe* strains 2139 and 938, selected *Sc. pombe* strains V1 and 4.2, *S. cerevisiae* 7VA, and the letter m indicates assays in which malolactic fermentation with *Oenococcus oeoni* 217 occurred.

3.7. Sensory evaluation

Fig. 7 shows the results obtained in the sensory evaluation. Fermentations involving *Sc. pombe* showed a perception of less general acidity (Fig. 7c), which is correlated with the final malic and lactic acid content (Table 1; Fig. 3). Differences in color intensity were observed (Fig. 7a). The fermentations with the selected *Sc. pombe* strains reached the highest values, which was in accordance with the previously recorded color intensity values (Table 4). On the other hand, experiments in which a malolactic fermentation occurred showed the lowest color intensity level and the highest value of color evolution, both of which are correlated with the low final anthocyanin values reported previously (Table 3). The acetic acid character and the volatile acidity perception were higher in the fermentations with the wild *Sc. pombe* strains 2139 and 938, which was related to the final acetic acid content (Table 1; Fig. 4a). The wild *Sc. pombe* strains 2139 and 938 and the *S. cerevisiae* strain 7VA showed a more fruity character, while the selected *Sc. pombe* strains V1 and 4.2 showed a spicier profile (Fig. 7b).

4. Conclusion

The primary conclusion of this study is that well-chosen *Schizosaccharomyces pombe* strains can contribute to wine quality and food safety. However, further research is necessary to select more appropriate *Sc. pombe* strains to produce a wine with the desired characteristics. *Sc. pombe* strains have great potential to produce less acidic, safer wines with a lower histamine content, a higher anthocyanin content and a higher color intensity.

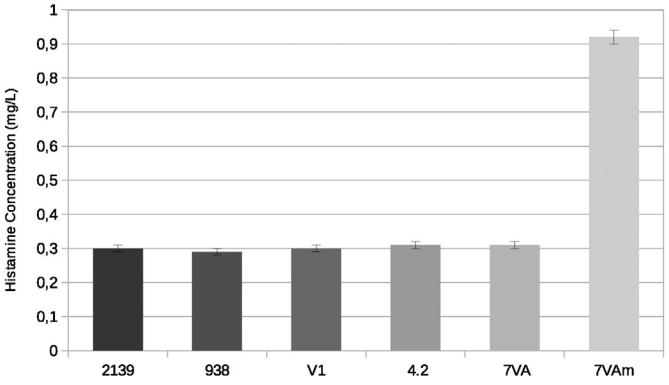

Fig. 7. a. Spider Graph of the color characteristics studied during the sensory analysis. The following nomenclature is used: Assays fermented with wild *Sc. pombe* strains 2139 and 938, selected *Sc. pombe* strains V1 and 4.2, *S. cerevisiae* 7VA, and the letter m indicates assays in which malolactic fermentation with *Oenococcus oeoni* 217 occurred. **b.** Spider Graph of the aroma characteristics studied during the sensory analysis. The following nomenclature is used: Assays fermented with wild *Sc. pombe* strains 2139 and 938, selected *Sc. pombe* strains V1 and 4.2, *S. cerevisiae* 7VA, and the letter m indicates assays in which malolactic fermentation with *Oenococcus oeoni* 217 occurred. **c.** Spider Graph of the retro-olfative characteristics studied during the sensory analysis. The following nomenclature is used: Assays fermented with wild *Sc. pombe* strains 2139 and 938, selected *Sc. pombe* strains V1 and 4.2, *S. cerevisiae* 7VA, and the letter m indicates assays in which malolactic fermentation with *Oenococcus oeoni* 217 occurred.

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