ISSN 1330-9862
(FTB-3329)

scientific note

Quality and Composition of Red Wine Fermented with *Schizosaccharomyces pombe* as Sole Fermentative Yeast, and in Mixed and Sequential Fermentations with *Saccharomyces cerevisiae*

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Received: February 6, 2013
Accepted: March 21, 2014

Summary

This work examines the physiology of *Schizosaccharomyces pombe* (represented by strain 938) in the production of red wine, as the sole fermentative yeast, and in mixed and sequential fermentations with *Saccharomyces cerevisiae* 796. For further comparison, fermentations in which *Saccharomyces cerevisiae* was the sole fermentative yeast were also performed; in these fermentations a commercial lactic acid bacterium was used to perform malolactic fermentation once alcoholic fermentation was complete (unlike *S. cerevisiae*, the *Sc. pombe* performs maloalcoholic fermentation and therefore removes malic acid without such help). Relative density, acetic, malic and pyruvic acid concentrations, primary amino nitrogen and urea concentrations, and pH of the musts were measured over the entire fermentation period. In all fermentations in which *Sc. pombe* 938 was involved, nearly all the malic acid was consumed from an initial concentration of 5.5 g/L, and moderate acetic acid concentrations below 0.4 g/L were formed. The urea content of these wines was notably lower, showing a tenfold reduction when compared with those that were made with *S. cerevisiae* 796 alone. The sensorial properties of the different final wines varied widely. The wines fermented with *Sc. pombe* 938 had maximum aroma intensity and quality, and they were preferred by the tasters.

Key words: Schizosaccharomyces pombe, *Saccharomyces cerevisiae*, malic acid, pyruvic acid, urea

Introduction

Yeasts of the genus *Schizosaccharomyces* have interesting metabolic properties that could be of use in winemaking. However, their use has been limited by what some authors have described as their scant oenological aptitude – the consequence of certain strains producing secondary metabolites associated with unwanted sensorial deviations (*1–3*). Nonetheless, members of this genus possess adequate fermentative power for winemaking (*4,5*), although it is probably their capacity to consume malic acid that awakens most interest (*6–8*).

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The maloalcoholic fermentation undertaken by *Schizosaccharomyces* spp. in winemaking renders the use of lactic acid bacteria, the microorganisms normally used for this purpose, unnecessary (*9*). This has the added advantage of limiting the appearance of biogenic amines – unwanted molecules that these bacteria produce in low nutrient environments (*10*). *Schizosaccharomyces* spp. also have urease activity (*11*). Urea is the main precursor of ethyl carbamate, an undesirable molecule owing to its toxicity; this urease activity could be harnessed to reduce urea concentrations and thus improve the food safety status of wine (*9,12*). Using *Schizosaccharomyces* spp. immobilised in alginate (*6,8,13,14*), or in mixed or sequential fermentations with *Saccharomyces* spp. (*15*) to mitigate what has been seen as their reduced oenological aptitude (*16*), has proved successful in biological deacidification of wine under laboratory conditions (*8,17*).

Numerous studies have examined the impact of *Schizosaccharomyces* spp. on the volatile compound composition and sensorial profile of wine, although some of the obtained results have been contradictory (*17–20*).

The use of *Schizosaccharomyces* spp. alone, or with other yeast species in mixed or sequential fermentations might help reduce the 'standardisation' of wines on the market (*21–23*), improving their complexity and aroma profile (*24*). In addition, their rapid autolytic release of cell wall polysaccharides after death could reduce the time required to complete ageing over lees (*25*). The present work examines the potential of the metabolism of *Schizosaccharomyces pombe* 938 in winemaking.

Materials and Methods

Microorganisms

The yeast strains used in this study were *Schizosaccharomyces pombe* 938 from the type collection of the Institute for Industrial Fermentations (IFI, CSIC, Madrid, Spain) selected for its aptitude in red wines (*9*) and *Saccharomyces cerevisiae* 796 selected by the Australian Wine Research Institute (Maurivin, Melbourne, Australia), whose genome sequence is currently known (*26*). The strain of lactic acid bacteria used was *Oenococcus oeni* Alpha (Lallemand, Montreal, Canada).

Fermentations

All fermentations were undertaken using the must of *Vitis vinifera* L. cultivar Garnacha grapes grown in San Martín de Valdeiglesias (Madrid, Spain). Using a method similar to that described by Sampaio *et al*. (*27*), 3.5 kg of unpasteurised crushed grapes were placed in 4.9-litre glass fermentation vessels, leaving enough space for the emission of carbon dioxide. Sulphur dioxide (60 mg/kg) was added to each vessel, along with 3 g/L of L-malic acid (Panreac, Barcelona, Spain) to arrive at a final concentration of 5.5 g/L. Sugar concentration was 246 g/L, relative density 1106.5 g/L at 15 °C, and pH=3.1.

Four assays were performed (all in triplicate): (*i*) inoculation of the must with *Sc. pombe* 938 alone ($1.42 \cdot 10^4$ CFU/g); (*ii*) inoculation of the must with *S. cerevisiae* 796 alone (10^6 CFU/g); (*iii*) inoculation of the must with *Sc. pombe* 938 ($1.42 \cdot 10^4$ CFU/g) and *S. cerevisiae* 796 (10^6 CFU/g) together (mixed fermentation); and (*iv*) inoculation of the must with *Sc. pombe* 938 ($1.42 \cdot 10^4$ CFU/g) followed by *S. cerevisiae* 796 (10^6 CFU/g) 48 h later (sequential fermentation). *Schizosaccharomyces* inocula were obtained using 50 mL of sterilized must with 1 mL of yeast extract, dextrose and peptone (YEDP) liquid medium (*28*) containing 10^6 CFU/mL (determined using a Thomas chamber). To reach this population, 100 μL of each yeast suspension were cultivated in 5 mL of YEPD at 25 °C for 24 h. This procedure was repeated three successive times before the final inoculation of 1 mL in the inocula. All inocula were prepared in 100-mL flasks sealed with a Müller valve filled with 98 % H_2SO_4 (Panreac), which allowed the release of CO_2 while avoiding microbial contamination (*29*). The temperature was maintained at 25 °C for 48 h. The development of inocula proceeded without aeration, oxygen injection or agitation. All fermentations were performed in triplicate. A concentration of 25 g/hL of *Saccharomyces cerevisiae* 796 was added to all fermentations in which this yeast was involved, following the manufacturer's recommendations.

All fermentation processes were carried out at 25 °C. Once the fermentation of sugars was complete (deemed to be represented by a remaining glucose and fructose concentration of 2 g/L), the wines fermented with *Schizosaccharomyces* were racked and stabilized during 7 days at 4 °C and the final product was bottled. A concentration of 50 mg/L of sulphur dioxide in potassium metabisulfite form was added. Corked bottles were placed horizontally in a climate chamber TR2V120 (La Sommelière, Saint-Saturnin, France) under constant temperature and humidity (18 °C and 70 % relative humidity). These conditions were maintained for seven weeks until the sensory evaluation took place. The wines fermented with *Saccharomyces* were racked and stabilized in the same way since they finished malolactic fermentation by *Oenococcus oeni* Alpha (20 g/hL). Then they remained under the same final storage conditions described above for one month before tasting sessions took place.

Analytical determinations: non-volatile compounds

Glucose and fructose, malic, lactic, acetic, pyruvic and citric acids, glycerol, primary amino nitrogen, urea and colour intensity were all determined using a Y15 enzymatic autoanalyzer (Biosystems S.A., Barcelona, Spain). These analyses were performed using the appropriate kits from Biosystems, except for pyruvic acid, which was determined using a kit from Megazyme (Bray, Ireland).

The pH, dry extract, total acidity, alcohol content and relative density were determined following the International Methods of Analysis of Wines and Musts (*30*).

Analytical determinations: volatile compounds

The concentration of 18 volatile compounds (acetaldehyde, methanol, *n*-propanol, diacetyl, ethyl acetate, isobutanol, *n*-butanol, 2-butanol, amylic alcohol, isoamylic alcohol, isobutyl acetate, ethyl butyrate, ethyl lactate, *n*-hexanol, isoamyl acetate, 2-phenylethyl alcohol, 2-phenylethyl acetate and 2,3-butanediol), all of which influence wine quality, were measured at the end of alcoholic and malolactic fermentations by gas chromatography using an Agilent Technologies 6850 gas chromatograph with a flame ionisation detector (Hewlett-Packard, Palo Alto, CA, USA). The apparatus was calibrated with a 4-methyl-2-pentanol internal standard. Gas chromatography quality compounds (Fluka, Sigma-Aldrich Corp., Buchs

SG, Switzerland) were used to provide standard patterns. Higher alcohols were separated as described in the International Methods of the Analysis of Musts and Wines (*30*). The detection limit was 0.1 mg/L.

Sensorial analysis

The final wines were assessed (blind test) by a panel of 10 experienced wine tasters, all members of the staff of the Food Technology Department of the Technical University of Madrid, Spain. Assessments took place in standard sensory analysis chambers with separate booths. Following the generation of a consistent terminology by consensus, two visual descriptors, five aromas and four taste attributes were chosen to describe the wines. Formal assessment consisted of two sessions held on different days where wine tasters tasted all fermented triplicates. The panellists used a 10-cm unstructured scale, from 0 (no defect) to 100 (very strong defect perceptible), to rate the intensity of 11 attributes.

Statistical analysis

Mean and standard deviations were calculated and ANOVA was performed using PC Statgraphics v. 5 software (Graphics Software Systems, Rockville, MD, USA). Significance was set at $p<0.05$ for the ANOVA matrix F-value. The multiple range test was used to compare the mean values.

Results and Discussion

Fermentative kinetics, relative densities and primary amino nitrogen concentrations

Fig. 1 shows the change in the relative density over the fermentation period. The fermentation kinetics was acceptable in all fermentations; it ended around day 10 in all of them, and all produced wines had a residual sugar content of <2 g/L (Table 1) and relative density of around 991 g/L at 20 °C (Fig. 1). The data show that *Sc. pombe*, whether alone or in mixed or sequential fermentations, used sugar in a fashion similar to that shown by *S. cerevisiae* alone. *Schizosaccharomyces* spp. are of high fermentative power (*5*), although under some conditions they may show slower fermentation kinetics (*9*).

Fig. 2 shows the change in primary amino nitrogen (PAN) consumption in the four fermentation systems.

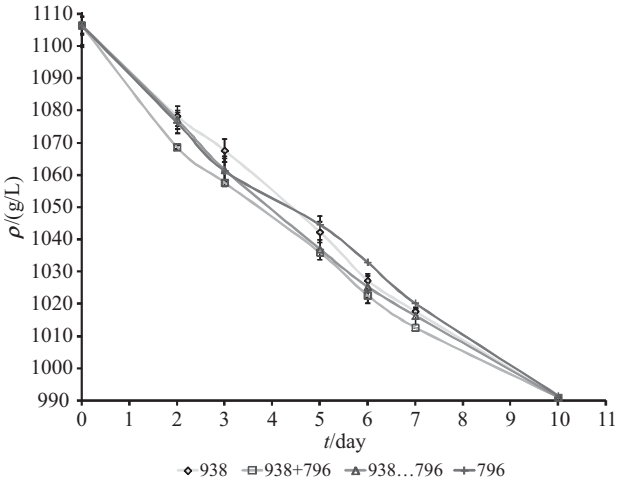

Fig. 1. Change in relative density of red Garnacha wines during fermentation. In this and all other figures, the following nomenclature is used: 938=fermentation with *Schizosaccharomyces pombe* 938 alone, 938+796=mixed fermentation with *Sc. pombe* 938 and *Saccharomyces cerevisiae* 796, 938…796=sequential fermentation with *Sc. pombe* 938 followed by *S. cerevisiae* 796, 796=fermentation with *S. cerevisiae* 796 alone before malolactic fermentation with *Oenococcus oeni* Alpha

Consumption is progressive and follows a linear pattern over the first 72 h, *i.e.* until a concentration of 15 mg/L is reached, when the trend changes. The start of proteolytic activity and the autolytic release of amino acids and low molecular mass peptides may explain the increase seen in primary amino nitrogen from this moment (*31,32*).

Biological deacidification

The potential of *Schizosaccharomyces* spp. as biological deacidifier can be seen in Figs. 3–5, which show the changes in the must acetic and malic acid contents and pH. Fermentations with *S. cerevisiae* 796 alone showed a reduction in malic acid content of 21.28 %, to reach a concentration of 4.33 g/L. This is in agreement with the results reported by other authors (*5,9,33–36*). After allowing malolactic fermentation to proceed in the samples fermented by *S. cerevisiae* 796 alone, the final lactic acid concentration recorded was 2.69 g/L (Table 1). In all fermentations involving *Sc. pombe* 938, however, nearly all

Table 1. Analytical results for the wines produced by different fermentation systems

Assays	γ(lactic acid) g/L	γ(acetic acid) g/L	γ(malic acid) g/L	γ(gluc+fruct) g/L	γ(glycerol) g/L	γ(citric acid) g/L	γ(total acidity) g/L	φ(alcohol) %	pH
938	$(0.04\pm0.01)^a$	$(0.35\pm0.01)^a$	$(0.14\pm0.03)^b$	$(1.42\pm0.47)^b$	$(7.06\pm0.16)^a$	$(0.34\pm0.01)^c$	$(5.11\pm0.05)^a$	$(13.75\pm0.01)^a$	$(3.46\pm0.02)^c$
938+796	$(0.02\pm0.02)^a$	$(0.34\pm0.01)^a$	$(0.19\pm0.04)^b$	$(1.74\pm0.37)^b$	$(6.87\pm0.21)^a$	$(0.33\pm0.02)^c$	$(5.17\pm0.06)^a$	$(14.17\pm0.03)^b$	$(3.43\pm0.03)^c$
938…796	$(0.04\pm0.02)^a$	$(0.35\pm0.01)^a$	$(0.16\pm0.02)^b$	$(1.64\pm0.13)^b$	$(6.98\pm0.16)^a$	$(0.30\pm0.01)^b$	$(5.11\pm0.02)^a$	$(13.77\pm0.01)^a$	$(3.44\pm0.02)^c$
796	$(0.03\pm0.02)^a$	$(0.35\pm0.01)^a$	$(4.33\pm0.02)^c$	$(1.23\pm0.22)^b$	$(6.71\pm0.19)^a$	$(0.31\pm0.01)^{bc}$	$(9.18\pm0.01)^c$	$(14.53\pm0.01)^c$	$(3.11\pm0.01)^a$
796+MLF	$(2.69\pm0.03)^b$	$(0.53\pm0.02)^b$	$(0.09\pm0.02)^a$	$(0.62\pm0.34)^a$	$(6.73\pm0.14)^a$	$(0.08\pm0.01)^a$	$(7.63\pm0.16)^b$	$(14.54\pm0.04)^c$	$(3.28\pm0.03)^b$

Results represent the mean values±standard deviations (S.D.) of three replicates. Mean values in the same column with the same letter in superscript are not significantly different ($p<0.05$). The following nomenclature is used: 938=fermentation with *Schizosaccharomyces pombe* 938 alone, 938+796=mixed fermentation with *Sc. pombe* 938+*S. cerevisiae* 796, 938…796=sequential fermentation with *Sc. pombe* 938 followed by *S. cerevisiae* 796, 796=fermentation with *S. cerevisiae* 796 alone before malolactic fermentation with *Oenococcus oeni* Alpha, 796+MLF= fermentation with *S. cerevisiae* 796 alone after malolactic fermentation with *Oenococcus oeni* Alpha

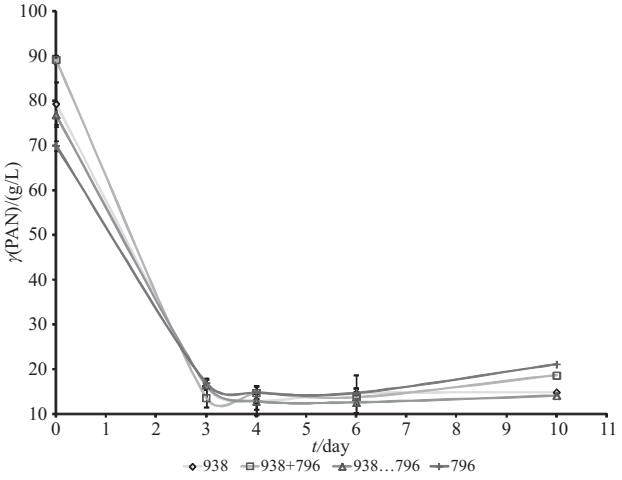

Fig. 2. Change in primary amino nitrogen (PAN) consumption in red Garnacha wines during fermentation. The same nomenclature as in Fig. 1

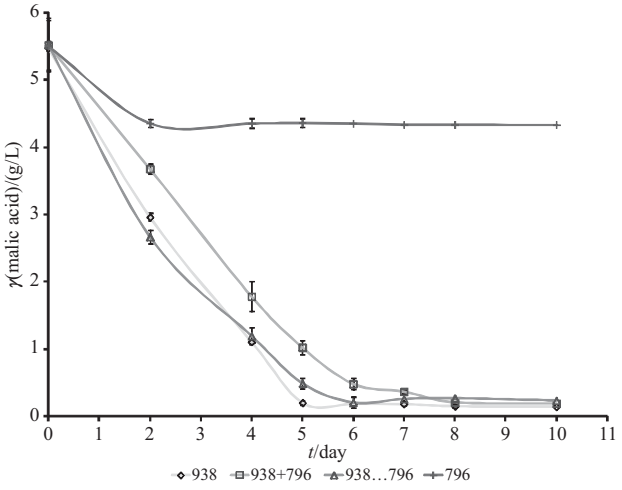

Fig. 3. Change in the consumption of malic acid in red Garnacha wines during fermentation. The same nomenclature as in Fig. 1

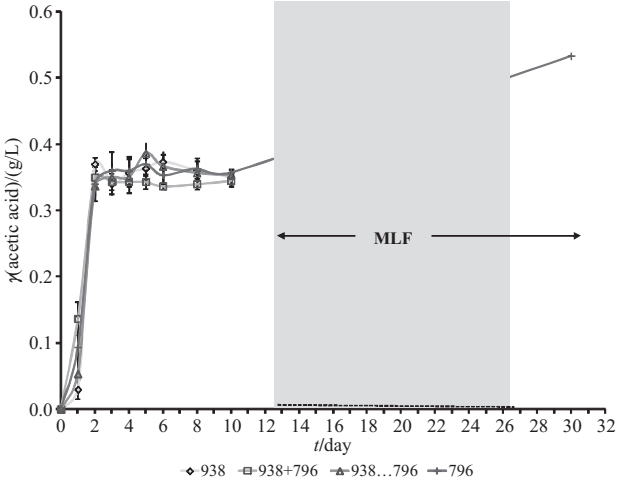

Fig. 4. Change in acetic acid production in red Garnacha wines during fermentation. The same nomenclature as in Fig. 1

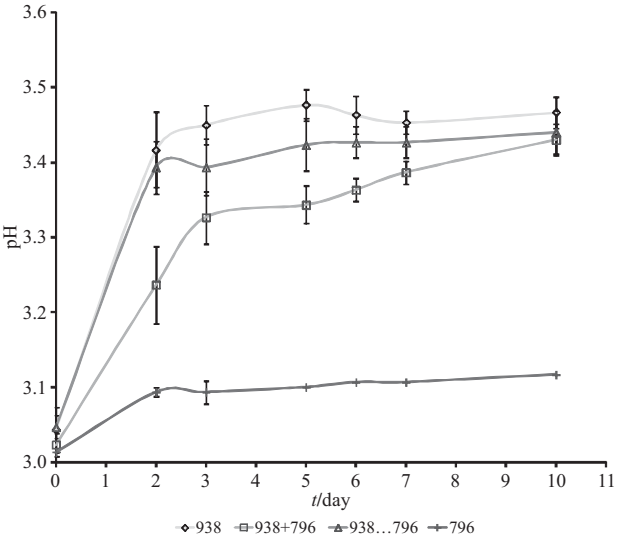

Fig. 5. Change in the pH of red Garnacha wines during fermentation. The same nomenclature as in Fig. 1

the malic acid was consumed (Fig. 3). This is also in agreement with the reports by other authors who recorded 75–100 % reductions in malic acid content depending on the *Schizosaccharomyces* strain used (*6–8,10,17,18,37*). This reduction largely occurred over the first six days of fermentation; at this time the malic acid concentrations recorded were 0.14, 0.19 and 0.16 g/L when using *Sc. pombe* 938 alone, in sequential fermentation, and in mixed fermentation, respectively. This consumption of malic acid translated into a deacidification of some 97 %, made manifest in the pH change (Fig. 5) and total acidity values of the finished wines (Table 1). No significant differences were seen in terms of malic acid content at the end of alcoholic fermentation in any of the fermentation assays involving *Sc. pombe* 938, which showed an increase in pH of 0.30–0.35 compared to the fermentations with *S. cerevisiae* 796 alone before allowing the malolactic fermentation step.

One of the main oenological problems associated with *Schizosaccharomyces* spp. is their significant production of acetic acid in fermentations at laboratory scale (*9*). However, many authors have recorded satisfactory results of mixed fermentations (*i.e.* with *Saccharomyces* spp.), obtaining wines with no obvious olfactory defects (*8,17*). In the present work, the acetic acid content stabilised on the third day of fermentation. In all fermentations involving *Sc. pombe* 938, the concentration of acetic acid remained at around 0.35 g/L from that moment until the end of fermentation (Fig. 4). With *S. cerevisiae* 796 alone, however, the acetic acid concentration increased to 0.53 g/L following the malolactic fermentation step. This increase might be explained by the observed consumption of citric acid (Table 1).

Potential applications of Schizosaccharomyces pombe

Fig. 6 shows the change in pyruvic acid concentration during fermentation. The maximum concentration was reached on the third day in all fermentations, followed by its reduction. The maximum values reached when using *Sc. pombe* 938 alone, in sequential fermentation with *S. cerevisiae* 796, in mixed fermentation, and

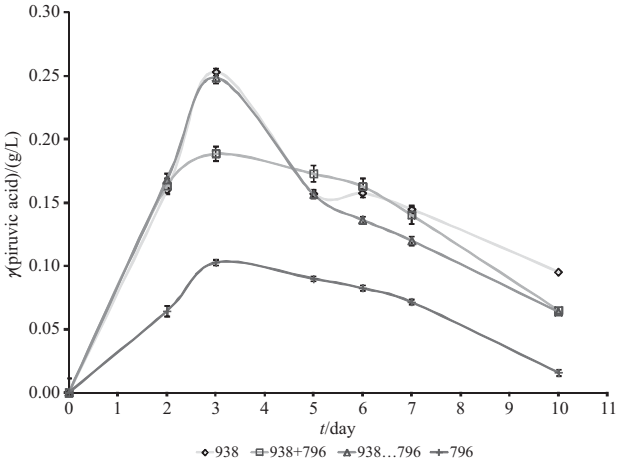

Fig. 6. Change in the production of pyruvic acid in red Garnacha wines during fermentation. The same nomenclature as in Fig. 1

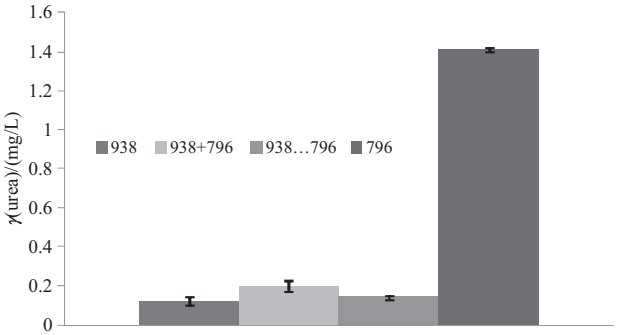

Fig. 7. Urea concentration (mean values±S.D.) in the final wines. The same nomenclature as in Fig. 1

with *S. cerevisiae* 796 alone were 0.25, 0.24, 0.18 and 0.10 g/L, respectively. In other studies involving fermentations with other *Schizosaccharomyces* strains alone, values of up to 0.39 g/L have been recorded (*9*). The pyruvic acid concentrations reached here in fermentations with *Sc. pombe* were high compared to those recorded in earlier work with *S. cerevisiae* 7VA and 9CV selected for their ability to produce pyruvic acid; these produced only between 0.06 and 0.13 g/L of pyruvic acid (*38*). The formation of highly stable pigments such as vitisin A in fermentations with *Sc. pombe* 938, either alone or combined with *S. cerevisiae* 796 (sequential or mixed), would probably be stronger than with *S. cerevisiae* 796 alone (Fig. 6). This could improve the chromatic characteristics of wines, especially during long ageing processes (*38*) when stable pigment forms start to be important over unstable forms.

The urea content of the finished wines (Fig. 7) was 0.12 mg/L in that made with *Sc. pombe* 938 alone, 0.20 mg/L in that made with the mixed fermentation system, 0.14 mg/L in that made with the sequential fermentation system, and 1.41 mg/L in that made with *S. cerevisiae* 796 alone. The differences between the fermentations with either of the yeast strains used alone were significant ($p<0.05$). These differences are easily explained given the greater urease activity by *Sc. pombe* (*11*). A reduction in urea ought to lead to less ethyl carbamate (an undesirable molecule) formation (*39,40*); the use of *Sc. pombe* 938 could, therefore, improve the safety of wines.

Glycerol content in the wines made in fermentations in which *Sc. pombe* 938 was higher, although the difference was not significant compared to the amounts in wine made with *S. cerevisiae* 796 alone (Table 1). Some authors report *Sc. pombe* to have a well developed glyceropyruvic pathway, which might explain its greater production of pyruvic acid and glycerol compared to other yeasts (*5*).

The final alcohol content in the wines made in fermentations involving *Sc. pombe* 938 was lower than that when *S. cerevisiae* 796 acted alone (Table 1). This is in agreement with the reports by other authors who observed *Sc. pombe* to be a relatively poor producer of alcohol (*41*). Lower alcohol content might be the reflection of greater autolytic release of cell wall polysaccharides by *Sc. pombe* (of use in ageing over lees) (*25*); the sugars consumed appear to be used to make compounds other than ethanol or to increase the yeast's biomass.

Volatile compounds

Table 2 shows the production of volatile compounds in the different fermentations. No significant differences were seen in acetaldehyde production, with all fermentations returning values of 7–9 mg/L. This could be beneficial for red wines, since, along with malvidin-3-glucoside, acetaldehyde acts as a precursor of vitisins B, which help stabilise wine colour (*42*). Nonetheless, significant differences were recorded after the final malolactic fermentation step in fermentations with *S. cerevisiae* alone (final value 14.34 mg/L).

Methanol production (13.07–21.42 mg/L) never exceeded the legal limit of 120 mg/L for red wines. At the end of alcoholic fermentation, no significant differences were seen between the different fermentations in terms of ethyl acetate concentration. Nonetheless, following the malolactic fermentation step after fermentation with *S. cerevisiae* 796 alone, a value of 42.30 mg/L was reached, which is significantly higher than those recorded in other fermentations. Even so, although ethyl acetate is one of the most negative of wine esters, being responsible for a glue odour, all values of <70 mg/L can be considered acceptable (*43*).

Higher alcohols (isobutanol, 2-methyl-1-butanol and 3-methyl-1-butanol) were produced in moderate quantities in all fermentations (slightly more with *S. cerevisiae* 796 alone, both before and after the malolactic fermentation step). A total higher alcohol concentration of <350 mg/L is recommended since, above this, wine takes on a disagreeable alcoholic flavour.

The formation of 2-phenylethanol was moderate, although slightly more was made in the fermentation involving *S. cerevisiae* 796 alone (Table 2); this may have increased its floral aroma. The main descriptor for this compound is rose petals. Significantly more isoamyl acetate was produced in the fermentation involving *S. cerevisiae* 796 alone (Table 2). Ethyl lactate production was moderate in all fermentations (9.11–11.15 mg/L), except when *S. cerevisiae* 796 was used alone followed by malolactic fermentation undertaken by the added bacteria (34.06 mg/L).

Table 2. Volatile compounds detected in different fermentations

Compounds	γ/(mg/L)				
	938	938+796	938…796	796	796+MLF
Acetaldehyde	(7.28±0.70)^{a}	(7.15±0.96)^{a}	(7.37±1.73)^{a}	(9.21±1.35)^{a}	(14.34±2.07)^{b}
Methanol	(13.07±1.61)^{a}	(15.50±4.19)^{ab}	(15.83±4.20)^{ab}	(17.11±5.27)^{ab}	(21.42±6.40)^{b}
1-Propanol	(13.37±0.20)^{a}	(15.42±2.15)^{ab}	(13.34±0.64)^{a}	(17.23±3.19)^{b}	(18.52±1.82)^{b}
Diacetyl	(1.22±1.05)^{a}	(2.36±0.40)^{ab}	(2.14±0.14)^{ab}	(2.96±0.53)^{b}	(5.08±0.26)^{c}
Ethyl acetate	(24.26±5.23)^{a}	(26.28±5.40)^{a}	(21.14±1.94)^{a}	(25.83±3.88)^{a}	(42.30±1.93)^{b}
Isobutanol	(39.81±1.88)^{b}	(33.11±2.53)^{a}	(38.14±3.057)^{b}	(49.92±2.84)^{c}	(52.61±2.38)^{c}
1-Butanol	(4.39±0.04)^{b}	(4.60±0.09)^{c}	(4.20±0.05)^{a}	(4.39±0.08)^{b}	(4.56±0.07)^{c}
2-Methyl-1-butanol	(85.80±4.39)^{b}	(80.05±2.91)^{ab}	(72.97±8.73)^{a}	(99.56±6.94)^{c}	(102.66±3.53)^{c}
3-Methyl-1-butanol	(50.09±4.77)^{ab}	(50.18±1.46)^{ab}	(44.38±5.04)^{a}	(55.34±6.74)^{bc}	(62.37±0.95)^{c}
Isobutyl acetate	n.d.	n.d.	n.d.	n.d.	(0.419±0.72)
Ethyl butyrate	n.d.	n.d.	n.d.	n.d.	(0.714±1.23)
Ethyl lactate	(9.11±1.00)^{a}	(10.36±2.20)^{a}	(11.15±4.64)^{a}	(11.10±1.57)^{a}	(34.06±6.18)^{b}
2–3-Butanediol	(824.12±58.73)^{a}	(782.51±85.63)^{a}	(791.36±75.95)^{a}	(776.37±63.11)^{a}	(902.97±64.03)^{a}
Isoamyl acetate	(1.93±1.84)^{ab}	(7.18±4.88)^{b}	(4.45±3.59)^{ab}	(15.14±7.98)^{c}	(0.46±0.21)^{a}
Hexanol	(4.89±0.01)^{ab}	(4.68±0.13)^{a}	(4.68±0.06)^{a}	(5.22±0.12)^{c}	(5.09±0.18)^{bc}
2-Phenylethanol	(15.37±2.55)^{a}	(19.33±2.11)^{b}	(18.85±0.99)^{ab}	(22.55±2.62)^{b}	(21.83±3.19)^{b}
Phenylethyl acetate	(3.49±3.03)^{a}	(5.34±0.05)^{a}	(3.51±3.04)^{a}	(5.59±0.47)^{a}	(5.79±0.18)^{a}

All fermentations were performed at 25 °C with an initial sugar concentration of 246 g/L
Results represent mean values±S.D. of three replicates, n.d.= not detected. Mean values in the same row with the same letter in superscript are not significantly different ($p<0.05$). Nomenclature is the same as in Table 1

Sensorial evaluation

Fig. 8 shows a spider web diagram of the average scores of some olfactory and taste attributes. Large differences in the perception of acidity were recorded (explained above). Fermentation with *S. cerevisiae* 796 alone (followed by the malolactic fermentation step) produced the strongest sensations of oxidation, acetic acidity and bitterness. None of the wines produced by fermentation with *Sc. pombe* 938 had any perceptible organoleptic problems; indeed, they received the best scores from all tasters. The above data show that all fermentations with *Sc. pombe* achieved the main goals related to total malic acid and urea degradation, higher glycerol production and lower alcohol levels. However, the preferred fermentation strategy was the use of *Sc. pombe* 938 alone, probably because in this case the fermented must was less standardized and more different than of the regular wines.

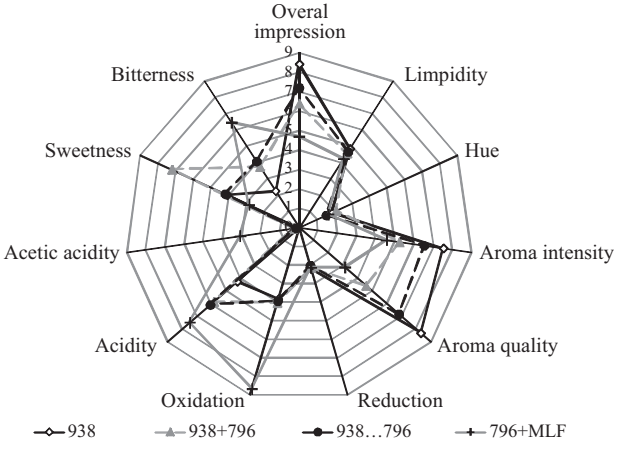

Fig. 8. Taste and olfactory attribute scores of the final wines. Legend as for Fig. 1

Conclusions

The presented results show that *Schizosaccharomyces pombe* 938 meets all the basic requirements of a red-winemaking yeast. Other strains can be investigated to see which might be selected in terms of the ability to deacidify acidic wine, their associated volatile compound profile, and the ability to prevent the production of bacterial toxins. *Schizosaccharomyces* spp. appears to be a real alternative to *Saccharomyces cerevisiae* and other members of the *Saccharomyces* genus, and thus provide a potential for the production of new, less standardised wines.

Acknowledgements

This work was funded by the Ministry of Economy and Competitiveness (MEeC), Spain (Project AGL2013--47706-R). The authors are very grateful for the help received from Biosystems S.A. Special thanks are owed to Pablo Rodríguez Plaza for the donation of the enzyme kits used in this work.

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