Original article

# Influence of indigenous *Saccharomyces paradoxus* strains on Chardonnay wine fermentation aroma

**Sandy Orlic[1]*, Sulejman Redzepovic[1], Ana Jeromel[2], Stanka Herjavec[2] & Lucilla Iacumin[3]**

1 Department of Microbiology, Faculty of Agriculture, University of Zagreb, Svetosimunska 25, 10000 Zagreb, Croatia
2 Department for Viticulture and Enology, Faculty of Agriculture, University of Zagreb, Svetosimunska 25, 10000 Zagreb, Croatia
3 Department of Food Science, Faculty of Agriculture, University of Udine, Via Marangoni 97, 33100 Udine, Italy

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**Summary** Seven indigenous yeast strains of *Saccharomyces paradoxus* previously isolated and identified using molecular and physiological methods were tested for their fermentation abilities. Chemical analyses of produced wines showed differences compared with the *Saccharomyces cerevisiae* strain used as a control. The examined *S. paradoxus* strains showed good fermentation vigour, ethanol tolerance and difference in the fermentation rate. Four of seven analysed *S. paradoxus* strains (RO66, RO54, RO11 and RO134) metabolised sugar up to 1 g $L^{-1}$. The total amount of higher alcohol was lower compared with *S. cerevisiae* wines. Strain RO83 was able to degrade up to 40% malic acid and can be used for biological deacidification. Sensory evaluation of dry and semidry wines underlined good enological properties and positive influence of tested wine strains on final wine quality. All these properties of *S. paradoxus* strains pointed out the possibility of their application in wine industry.

**Keywords** Chardonnay, *Saccharomyces paradoxus*, volatile compounds.

## Introduction

Flavour is the most important distinguishing characteristic (Lambrechts & Pretorius, 2000) of wine. Wine flavour is classified according to the sources of the different compounds contributing to it. This includes varietal flavour (compounds originating from the grapes), prefermentative flavour (compounds formed during extraction and conditioning of must), fermentative flavour (produced by yeast and bacteria during alcoholic and malolactic fermentation) and postfermentative flavour (compounds that appear during the ageing process as a result of enzymatic or physicochemical actions in wood or in the bottle) (Schreier, 1979; Boulton *et al.*, 1995; Rapp, 1998). The formation of volatile compounds during the fermentation of must is a complex phenomenon involving a number of factors. In particular, it depends on the nature and concentration of the compounds initially present in the must (their proportions differ from one grape variety to another), the capacity of the yeast to transform them and the conditions used in winemaking (Lurton *et al.*, 1995). Commercial yeast inocula, generally *Saccharomyces cerevisiae*, are widely used as a starter today; however, it might be preferable to use selected indigenous strains that may be better adapted to ferment the must of each area (Regodon *et al.*, 1997, Perez-Coello *et al.*, 1999). Before selecting such strains, it is necessary to isolate the yeast species present in the fermenting must, identify these species and select the most suitable strains on the basis of established enological criteria (Rainieri & Pretorius, 2000). In the present work, we have extensively studied seven indigenous *Saccharomyces paradoxus* strains from the same area of production (Zagreb sub region, position Jazbina, Croatia), which were selected from a previous ecological survey (Redzepovic *et al.*, 2002). *Saccharomyces paradoxus* is usually found in exudates of broad-leaved trees, insects and uncultivated soils (Naumov, 1996) and little has been done to evaluate the application of *S. paradoxus* as a starter culture in enology.

In our previous study (Majdak *et al.*, 2002), we determined specific enological characteristics of one strain of *S. paradoxus* and the potential differences in volatile compound production between *S. paradoxus* and *S. cerevisiae* strains and their influence on final Gewürztraminer wine quality.

The aim of this work was to understand whether the different strains of *S. paradoxus* from the natural yeast population could affect the quality of wine produced in the same region.

*Correspondent: Fax: +385-1-2393881;
e-mail: sorlic@agr.hr

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## Materials and methods

### Must

The must was obtained from Chardonnay grapes (with a sugar content of 220 g $L^{-1}$, a total acidity of 8.1 g $L^{-1}$, malic acid content of 3.4 g $L^{-1}$ and pH 3.2; vintage 1999; Zagreb wine region – Croatia).

### Yeast

We used seven *S. paradoxus* strains (RO11, RO21, RO54, RO66, RO83, RO102, RO134) from the wine yeast collection of our laboratory (Redzepovic *et al.*, 2002) and a commercial wine yeast starter culture strain (*S. cerevisiae* CS2, Montreal, Canada) from Lallemand.

### Fermentation

The grapes were first crushed and then pressed with pneumatic press (Vaslin-Bucher, France), set at 70% yield. The must was sterilised by filtration through a 0.45-μm Seitz-Supra EK filter (Seitz, Bad Kreuznach, Germany). All the equipment used in this experiment was sterilised with steam for 15 min, and all the samples were taken aseptically, so no contamination could occur. The juice was treated with 50 mg $L^{-1}$ $SO_2$ and held overnight in the cellar for settling. All fermentation experiments were carried out in triplicate using 15 L of must at 15 °C. For the inoculum of *S. paradoxus* strains and the commercial strain, yeast culture was preincubated in sterilised grape must for 48 h at 25 °C and inoculated at a final level of $5 \times 10^6$ cells $mL^{-1}$ . Chemical and sensory analyses were performed at the end of fermentation in all the samples.

### Analysis

Alcohol, total and volatile acidity, residual sugar and pH were determined using methods proposed by OIV (1995). Glycerol, malic and lactic acids were determined enzymatically with specific kits following the procedures specified by the manufacturer (Boehringer, Mannheim, Germany).

### Gas chromatography

Volatile compound analysis was performed on a Hewlett Packard Model 5890 Gas Chromatograph (Hewlett Packard, Palo Alto, CA, USA) fitted with a flame ionisation detector. For data treatment, a Hewlett Packard Model 3396 Series II Integrator was used. Higher alcohols and ethyl acetate were analysed on wine distillate using an HP 101 (Hewlett Packard) column of 50 m × 0.32 mm and 0.3 μm film thickness. Temperature programming was as follows: 6 min isothermal at 40 °C, then a linear temperature rise of 15 °C $min^{-1}$ to 200 °C. Injector and detector temperatures were 220 and 250 °C, respectively. Carrier gas was nitrogen at 30 mL $min^{-1}$. 1-Butanol was used as an internal standard.

For aroma compound analysis, the wine samples were subjected to 10-h liquid–liquid extraction using pentane–dichloromethane (2/1, v/v) and analysed by GC (Drawert & Rapp, 1968; Margheri & Versini, 1979). 1-Heptanol was used as an internal standard.

### Statistics

ANOVA and l.s.d. comparison test of SAS (SAS Institute, Cary, NC, USA) were used to interpret differences in means, if any, at the 95% and 99% confidence levels. The duo-trio difference test and ranking method results were interpreted using binominal probability tables where the null hypothesis was $P = 0.5$, with one-tailed alternative hypothesis of $P > 0.5$ (Amerine & Roessler, 1976).

### Sensory analysis

Wines were sensory-evaluated by an experienced panel of eleven judges. Initially wines were compared by duo-trio testing (Amerine & Roessler, 1976) to determine whether there were any differences between repetitions of each yeast treatment. Ranking method was used in order to determine quality difference between tested wines.

Sensory descriptive analysis was used to describe and define the extent of any differences in aroma attributes of the wines as a result of the inoculation treatment. The panel was previously trained to evaluate the individual characteristics of Chardonnay wine and to define characteristic aroma descriptors used in sensory analysis. Each sample of wine was judged for intensity of apple, citrus, floral, fruity, herbaceous/vegetative, spicy, sweety and yeasty aromas using a 10-point scale where 0 was 'not detectable', 1 was 'just detectable' and 9 was 'of high intensity'.

For the sensory analysis, tested wines were separated into two groups according to the residual sugar values and tested three times in random order.

## Results

### Fermentation rate

All inoculated strains started fermentation simultaneously, the day after the inoculation but showed different fermentation abilities. *S. paradoxus* strains RO54, RO66 and *S. cerevisiae* strain CS2 yielded prompt fermentation, and after 16 days and at low fermentation temperature (15 °C) wines reached dryness. Fermentation with *S. paradoxus* strains, RO11 and

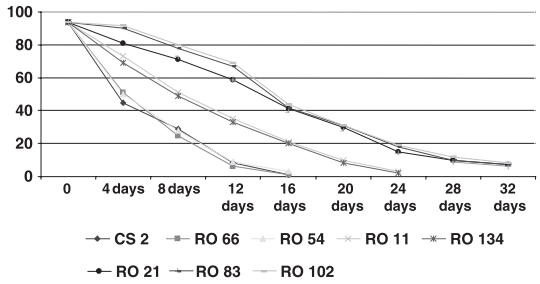


**Figure 1** Degradation of sugar (°Oe) during fermentation of Chardonnay must.

RO134, lasted 1 week longer (24 days) but wines also reached dryness. Compared with these two groups of strains, fermentation with *S. paradoxus* strains RO21, RO83 and RO102 lasted longer (32 days), and at the end wines contained 7–8 g $L^{-1}$ of residual sugar (Fig. 1).

## Chemical composition of wine

Table 1 shows the chemical composition of Chardonnay wines. Results were within the normal range of values expected. The total acidity was low in all the wines between 5.7 and 6.8 g $L^{-1}$, and the total volatile acidity values oscillate between 0.22 and 0.45 g $L^{-1}$ . All *S. paradoxus* strains showed high ethanol production although there were some significant differences between them, mainly due to some incomplete fermentations resulting in different amounts of residual sugar (Table 1). The strains studied demonstrated low volatile acidity production and some strains had a capacity for malic acid reduction. For this reason, *S. paradoxus* strain RO83 may be useful for biological deacidification in the presence of high malic acid concentrations (Redzepovic *et al*., 2003). Glycerol is typically produced at levels ranging from 4 to 10 g $L^{-1}$, and apart from ethanol and carbon dioxide it is the most abundant product of grape juice fermentation (Barros Lopes *et al*., 2000). In this research, glycerol content varied from 5.20 to 9.17 g $L^{-1}$ . It is well established that differences exist in the amount of glycerol formed by various yeast strains during fermentation (Radler & Schutz, 1982; Pretorius, 2000) (Table 1).

## Concentration of higher alcohols

According to Rapp & Versini (1991), concentrations of total higher alcohols below 300 mg $L^{-1}$ certainly contribute to desirable aroma complexity of wine. However, when concentrations exceed 400 mg $L^{-1}$, these compounds are regarded as a negative quality factor. All *S. paradoxus* strains, except the strains RO83 and RO134, produced significantly less total higher alcohols compared with *S. cerevisiae* strain CS2 (Table 2). Isoamyl alcohol is the most abundant higher alcohol, representing more than 50% of the total higher alcohol amount and is the predominant odorous component of the higher alcohol fraction (Majdak *et al*., 2002). All examined *S. paradoxus* strains, except the strains RO83 and RO134, produced significantly lower concentrations of this alcohol compared with *S. cerevisiae* strain CS2 (Table 2). The results of isobutanol and 2-phenyl ethanol have indicated that *S. cerevisiae* strain CS2 has the potential to produce higher concentrations of these alcohols than *S. paradoxus* strains. The exception was again *S. paradoxus* strain RO83 that produced the highest amount of 2-phenyl ethanol. Concentrations of hexanol can vary from 0.3 to 12 mg $L^{-1}$ (Lambrechts & Pretorius, 2000). All examined *S. paradoxus* strains produced significantly higher concentrations of hexanol compared with *S. cerevisiae* strain CS2 (Table 2). 1-Propanol was not detected in excessive quantities in the Chardonnay wines, although the content varied notably between the different samples, 12.00–17.33 mg $L^{-1}$ .

**Table 1** Means of Chardonnay wine chemical components

| Compounds | *Saccharomyces cerevisiae CS 2* | *Saccharomyces paradoxus* | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | RO11 | RO21 | RO54 | RO66 | RO83 | RO102 | RO134 | l.s.d. |
| Alcohol (vol%) | $12.70^{ab}$ | $12.51^{c}$ | $12.34^{d}$ | $12.48^{cd}$ | $12.78^{a}$ | $12.37^{cd}$ | $12.35^{d}$ | $12.60^{bc}$ | 0.15 |
| Glycerol (g $L^{-1}$) | $5.20^{e}$ | $6.16^{d}$ | $5.63^{de}$ | $9.17^{a}$ | $5.49^{de}$ | $7.00^{c}$ | $7.13^{bc}$ | $7.90^{b}$ | 0.8 |
| Reducing sugar (g $L^{-1}$) | $<1^{c}$ | $3^{b}$ | $7^{a}$ | $3^{b}$ | $<1^{c}$ | $7^{a}$ | $8^{a}$ | $2^{bc}$ | 1.2 |
| Total acidity (g $L^{-1}$)* | $6.8^{a}$ | $6.2^{c}$ | $6.6^{b}$ | $6.6^{b}$ | $6.6^{b}$ | $5.7^{e}$ | $6.1^{cd}$ | $6^{d}$ | 0.1 |
| Volatile acidity (g $L^{-1}$)† | $0.45^{a}$ | $0.35^{b}$ | $0.22^{c}$ | $0.33^{b}$ | $0.35^{b}$ | $0.24^{c}$ | $0.36^{b}$ | $0.31^{b}$ | 0.05 |
| Malic acid (g $L^{-1}$) | $3.17^{a}$ | $2.82^{b}$ | $3.10^{a}$ | $3.05^{a}$ | $3.05^{a}$ | $2.10^{c}$ | $2.72^{b}$ | $2.80^{b}$ | 0.22 |
| Lactic acid (g $L^{-1}$) | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.2 | 0.2 | 0.1 | n.s. |
| pH | 3.25 | 3.30 | 3.20 | 3.22 | 3.20 | 3.32 | 3.30 | 3.31 | |

Different letters beside the mean of compound denote a significant difference among treatments (a, b, c for 5%). The same letter beside the mean of a compound denotes an insignificant difference among treatments (a, b, c for 5%); n.s., not significant.

* tartaric acid.

† acetic acid.

**Table 2** Means of Chardonnay wines higher alcohol (mg $L^{-1}$) concentrations

| Compounds | *Saccharomyces cerevisiae CS 2* | *Saccharomyces paradoxus* | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | RO11 | RO21 | RO54 | RO66 | RO83 | RO102 | RO134 | l.s.d. |
| 1-Propanol | 12.00$^{e}$ | 12.33$^{de}$ | 13.67$^{de}$ | 13.67$^{de}$ | 14.67$^{bc}$ | 17.33$^{a}$ | 16.67$^{ab}$ | 14.33$^{cd}$ | 2.25 |
| Hexanol | 0.83$^{e}$ | 0.93$^{cd}$ | 0.89$^{d}$ | 1.06$^{b}$ | 1.08$^{b}$ | 1.15$^{a}$ | 1.09$^{b}$ | 0.95$^{c}$ | 0.04 |
| Isobutanol | 37.67$^{a}$ | 30.67$^{b}$ | 19.67$^{de}$ | 16.33$^{f}$ | 20.33$^{d}$ | 24.33$^{c}$ | 18.67$^{def}$ | 17.00$^{ef}$ | 3.29 |
| Isoamyl alcohol | 247.00$^{b}$ | 219.00$^{c}$ | 159.33$^{e}$ | 152.67$^{e}$ | 184.33$^{d}$ | 242.33$^{b}$ | 159.00$^{e}$ | 286.67$^{a}$ | 10.18 |
| 2-Phenyl ethanol | 60.04$^{b}$ | 50.48$^{c}$ | 34.60$^{e}$ | 37.78$^{de}$ | 54.08$^{bc}$ | 86.95$^{a}$ | 43.04$^{d}$ | 53.72$^{c}$ | 6.68 |
| $\sum$ Higher alcohol | 357.54$^{b}$ | 313.41$^{c}$ | 229.16$^{ef}$ | 221.51$^{f}$ | 274.49$^{d}$ | 372.09$^{a}$ | 238.47$^{e}$ | 372.67$^{a}$ | 10.25 |

Different letters beside the mean of compound denote a significant difference among treatments (a, b, c for 5%). The same letter beside the mean of a compound denotes an insignificant difference among treatments (a, b, c for 5 %); n.s., not significant.

The wine produced by fermentation with *S. cerevisiae* CS2 yeast showed a minimum content of this alcohol.

## Concentration of fatty acids

Medium-chain volatile fatty acids are produced by yeasts as intermediates in the biosynthesis of long-chain fatty acids. According to various authors (Majdak *et al.*, 2002; Nurgel *et al.*, 2002), yeast strains can produce significantly different amounts of butyric, capric, caprylic, caproic and isovaleric acids. Our results (Table 3) are in accordance with these findings. Compared with *S. cerevisiae* strain CS2, all *S. paradoxus* strains produced significantly lower concentrations of capric acid. *S. paradoxus* strains RO102 and RO134 produced significantly higher concentrations of butyric, isovaleric, caproic and caprylic acids than *S. cerevisiae* strain CS2. All other *S. paradoxus* strains tested produced lower or similar concentrations of these fatty acids. In accordance with these results, we can separate *S. paradoxus* strains into three groups: (1) strains RO11 and RO21 producing significantly lower total fatty acids than *S. cerevisiae* CS2, (2) strains RO54 and RO66 producing fatty acids in different but not significant amount compared with *S. cerevisiae* CS2 and (3) strains RO83, RO102 and RO134 producing significantly higher amounts of fatty acids.

## Concentration of volatile esters

The acetates of higher alcohols and ethyl esters of fatty acids are the most desirable compounds in young white wine (Soles *et al.*, 1982). In agreement with Majdak *et al.* (2002), all *S. paradoxus* strains produced significantly lower concentrations of ethyl acetate, isoamyl acetate and total volatile esters compared with *S. cerevisiae* strain CS2 (Table 4). Ethyl acetate is the main ester occurring in wine and in concentrations between 50 and 80 mg $L^{-1}$ contributes to olfactory complexity and has significant influence on the quality of wine (Soden *et al.*, 2000). Significantly higher concentrations of isobutyl acetate and hexyl acetate compared with *S. cerevisiae* strain CS2 were produced by *S. paradoxus* strain RO134, while *S. paradoxus* strain RO21 synthesised low amounts of isobutyl acetate. Soles *et al.* (1982) reported differences in the production of 2-phenyl ethyl acetate, isoamyl acetate and hexyl acetate as a function of the fourteen *S. cerevisiae* strains used. Compared with the *S. paradoxus* wines, our results showed relatively higher concentrations of acetate esters in the *S. cerevisiae* CS2 wines, while the difference between *S. paradoxus* strains was minimal.

The difference in the production of ethyl esters of fatty acids existed but was greater between examined

**Table 3** Means of Chardonnay wines fatty acids (mg $L^{-1}$) concentrations

| Compounds | *Saccharomyces cerevisiae CS 2* | *Saccharomyces paradoxus* | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | RO11 | RO21 | RO54 | RO66 | RO83 | RO102 | RO134 | l.s.d. |
| Butyric acid | 0.74$^{d}$ | 0.67$^{d}$ | 0.79$^{d}$ | 0.71$^{d}$ | 0.77$^{d}$ | 1.67$^{a}$ | 1.05$^{c}$ | 1.36$^{b}$ | 0.14 |
| Isovaleric acid | 0.93$^{c}$ | 0.65$^{d}$ | 0.39$^{c}$ | 0.63$^{d}$ | 0.61$^{d}$ | 1.88$^{a}$ | 1.23$^{b}$ | 1.19$^{b}$ | 0.15 |
| Caproic acid | 4.88$^{cd}$ | 4.80$^{cd}$ | 4.27$^{d}$ | 6.23$^{b}$ | 5.64$^{bc}$ | 7.83$^{a}$ | 6.18$^{b}$ | 6.31$^{b}$ | 1.13 |
| Caprylic acid | 4.97$^{c}$ | 2.52$^{e}$ | 2.38$^{e}$ | 4.08$^{d}$ | 5.02$^{c}$ | 5.09$^{c}$ | 6.72$^{a}$ | 5.58$^{b}$ | 0.43 |
| Capric acid | 0.32$^{a}$ | 0.09$^{de}$ | 0.05$^{e}$ | 0.12$^{d}$ | 0.18$^{c}$ | 0.23$^{b}$ | 0.25$^{b}$ | 0.25$^{b}$ | 0.04 |
| $\sum$ Fatty acids | 11.87$^{c}$ | 8.74$^{d}$ | 7.92$^{d}$ | 11.74$^{c}$ | 12.21$^{c}$ | 16.67$^{a}$ | 15.39$^{ab}$ | 14.62$^{b}$ | 1.69 |

Different letters beside the mean of compound denote a significant difference among treatments (a, b, c for 5%). The same letter beside the mean of a compound denotes an insignificant difference among treatments (a, b, c for 5 %); n.s., not significant.

**Table 4** Means of Chardonnay wines volatile esters (mg $L^{-1}$) concentrations

| Compounds | *Saccharomyces cerevisiae* CS2 | Saccharomyces paradoxus | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | RO11 | RO21 | RO54 | RO66 | RO83 | RO102 | RO134 | l.s.d. |
| Ethyl acetate | 31.00$^{a}$ | 18.00$^{d}$ | 20.67$^{c}$ | 23.67$^{b}$ | 22.33$^{bc}$ | 17.67$^{d}$ | 20.67$^{c}$ | 18.33$^{d}$ | 1.79 |
| Isobutyl acetate | 0.04$^{bc}$ | 0.05$^{bc}$ | 0.01$^{d}$ | 0.04$^{bc}$ | 0.04$^{bc}$ | 0.03$^{c}$ | 0.06$^{ab}$ | 0.08$^{a}$ | 0.02 |
| Isoamyl acetate | 2.49$^{a}$ | 1.82$^{bc}$ | 1.13$^{d}$ | 1.66$^{c}$ | 1.35$^{cd}$ | 2.10$^{b}$ | 1.71$^{c}$ | 1.75$^{bc}$ | 0.36 |
| Hexyl acetate | 0.08$^{cd}$ | 0.11$^{b}$ | 0.05$^{d}$ | 0.07$^{cd}$ | 0.07$^{cd}$ | 0.08$^{cd}$ | 0.10$^{bc}$ | 0.15$^{a}$ | 0.03 |
| Phenyl ethyl acetate | 0.58$^{a}$ | 0.42$^{abc}$ | 0.45$^{abc}$ | 0.38$^{bc}$ | 0.30$^{bc}$ | 0.55$^{ab}$ | 0.45$^{abc}$ | 0.54$^{ab}$ | 0.17 |
| Ethyl butyrate | 0.12$^{b}$ | 0.14$^{b}$ | 0.11$^{bc}$ | 0.13$^{b}$ | 0.24$^{a}$ | 0.16$^{b}$ | 0.14$^{b}$ | 0.06$^{c}$ | 0.05 |
| Ethyl lactate | 0.97$^{d}$ | 0.90$^{e}$ | 0.96$^{d}$ | 0.96$^{d}$ | 1.07$^{c}$ | 1.27$^{b}$ | 1.53$^{a}$ | 1.01$^{d}$ | 0.05 |
| Ethyl caproate | 0.84$^{ab}$ | 0.59$^{abc}$ | 0.63$^{abc}$ | 0.55$^{abc}$ | 0.87$^{a}$ | 0.40$^{c}$ | 0.52$^{bc}$ | 0.52$^{bc}$ | 0.32 |
| Ethyl caprylate | 0.25$^{bcd}$ | 0.33$^{ab}$ | 0.21$^{cd}$ | 0.20$^{d}$ | 0.29$^{abc}$ | 0.33$^{ab}$ | 0.19$^{d}$ | 0.37$^{a}$ | 0.08 |
| Ethyl caprate | 0.04$^{c}$ | 0.02$^{d}$ | 0.02$^{d}$ | 0.02$^{d}$ | 0.02$^{d}$ | 0.03$^{cd}$ | 0.08$^{b}$ | 0.10$^{a}$ | 0.01 |
| Diethyl succinate | 0.11$^{b}$ | 0.18$^{b}$ | 0.19$^{b}$ | 0.14$^{b}$ | 0.40$^{a}$ | 0.24$^{b}$ | 0.12$^{b}$ | 0.24$^{b}$ | 0.14 |
| $\sum$ Volatile esters | 36.52$^{a}$ | 22.55$^{c}$ | 24.40$^{bc}$ | 27.80$^{b}$ | 27.00$^{b}$ | 22.85$^{c}$ | 25.57$^{bc}$ | 23.14$^{c}$ | 3.84 |

Different letters beside the mean of compound denote a significant difference among treatments (a, b, c for 5%). The same letter beside the mean of a compound denotes an insignificant difference among treatments (a, b, c for 5 %); n.s., not significant.

*S. cerevisiae* CS2 strain and *S. paradoxus* strains than among *S. paradoxus* strains (Table 4).

## Sensory analysis

The results of duo-trio test showed no differences in aroma among the treatment fermentation triplicates. The wines of each treatment were blended and evaluated by descriptive analysis and ranking method. For the elimination of reducing sugar influence on the overall wine quality, the samples were divided into two groups: the first was dry wine and the second semidry wine. Figures 2 and 3 show the mean aroma attribute intensity scores for the eight inoculation treatments while the results of the ranking method are presented in Table 5.

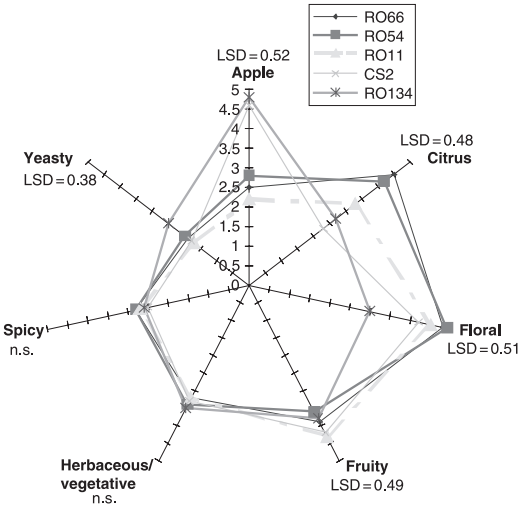


**Figure 2** Mean ratings for attributes in dry Chardonnay wines.

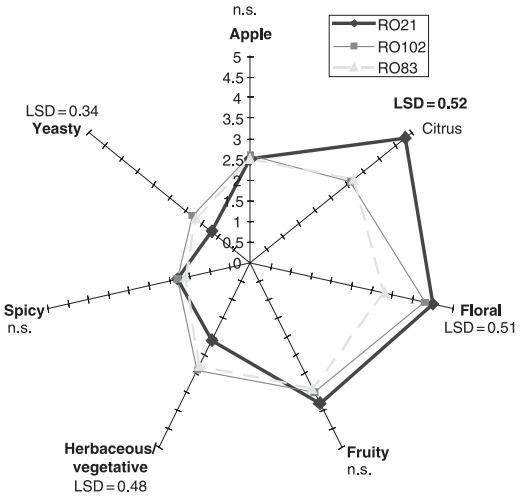


**Figure 3** Mean ratings for attributes in semidry Chardonnay wines.

**Table 5** Results of sensory evaluation of dry and semidry Chardonnay wines

| | Rank totals |
|---|---|
| Dry wine treatments | |
| RO66 | 10* |
| RO54 | 11 |
| RO11 | 23 |
| CS2 | 24 |
| RO134 | 33* |
| Semidry wine treatments | |
| RO21 | 13 |
| RO102 | 19 |
| RO83 | 27† |

* Any rank total outside the range 11–31 is significant at $P < 1\%$.
† Any rank total outside the range 10–25 is significant at $P < 1\%$.

Generally, sensory evaluation of the dry wine group showed better overall quality of strains RO66 and RO54 wines, even only the RO66 wines resulted significantly better on the level at $P < 5\%$ (Table 5). The results of evaluation indicate that the fermentation by RO134 strain resulted in wines of the most inferior overall quality. The RO66- and RO54-treated wines were characterised by stronger floral and citrus aromas, probably because of considerably lower total concentration of higher alcohol and somewhat higher content of volatile esters.

In the group of semidry wines, the judges also noticed quality differences between the samples: significantly on the level at $P < 5\%$ the best was wine fermented with strain RO21, whereas the quality of the wine produced with RO83 strain was of the most inferior quality. The RO21 wines had more intense citrus, floral and fruity aroma, whereas aroma intensity of the other two wines was not so pronounced, as their total higher alcohol amount was significantly higher. Importantly, these aroma-related differences did not overwhelm the typical varietal characteristic of Chardonnay wines and results given confirmed the importance of indigenous strains in aroma formation and wine quality (Lurton *et al*., 1995).

## Discussion

The ability of *S. paradoxus* strains to influence the wine aroma must was investigated. Their fermentation vigour is good, levels of volatile acidity production are not high and all strains showed good tolerance to alcohol. The possibility of some strains to degrade malic acid by up to 40% can be used for biological wine deacidification (Redzepovic *et al*., 2003). The *S. paradoxus* strains studied possess other traits of enological importance; namely the ability to produce elevated amounts of glycerol and lower amounts of total higher alcohols. The ability to enhance or lower glycerol production through the strategic use of *S. cerevisiae* strains in a genetic improvement experiment has been demonstrated (Rainieri *et al*., 1998). The described *S. paradoxus* strains provide an alternative approach to Chardonnay fermentation, like *Saccharomyces bayanus* in some cases (Eglinton *et al*., 2000). The characteristic fruity odours of wine are primarily due to a mixture of hexyl acetate, ethyl caproate and caprylate (apple-like aroma), isoamyl acetate (banana-like aroma) and 2-phenylethyl acetate (fruity, flowery flavour) (Pretorius, 2000). Suomalainen and Lehtonen (1979) showed that *S. cerevisiae* produces significantly more isoamyl acetate, ethyl caproate, ethyl caprylate and ethyl caprate than does *Saccharomyces uvarum*. Delteil and Jarry (1992) studied the characteristic effects of two commercial strains on Chardonnay wine volatiles and determined different concentrations of isoamyl acetate, ethyl laureate and total esters. According to our results, significantly greater difference was found between the tested *S. cerevisiae* and *S. paradoxus* strains than among the different *S. paradoxus* strains (Table 4). Most volatile compounds produced by yeast during must fermentation are not well identified (Vila *et al*., 1998), and several investigations have pointed out the outstanding influence of some of them on specific flavours such as the fruity aroma of Pinotage (Wyk van *et al*., 1979) or the guava-like flavour of some South African wines (van Royen *et al*., 1982). Our results pointed out that all *S. paradoxus* wines were of similar or of better quality compared with wines made with *S. cerevisiae* CS2 strain according to organoleptic evaluation (Table 5), although *S. cerevisiae* CS2 strain produced wines with more acetates and fatty acid esters.

The outcomes clearly indicated some differences between *S. cerevisiae* and *S. paradoxus* strains. All the investigated *S. paradoxus* strains produced higher concentration of 1-propanol, hexanol and lower concentration of isobutanol, capric acid, ethyl acetate, isoamyl acetate and total volatile esters when compared with *S. cerevisiae* CS2. There is a strong correlation between higher concentration of 1-propanol and lower concentration of isobutanol (Estévez *et al*., 2004).

Finally, it must be stated that this work constitutes a preliminary approach to the more complete study of how *S. paradoxus* strains influence the production of certain volatile compounds and wine quality.

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