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## Comparison of Wine Aroma Compounds Produced by *Saccharomyces paradoxus* and *Saccharomyces cerevisiae* Strains

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### Summary

The aim of this study is to determine specific enological characteristics of *Saccharomyces paradoxus* species, potential differences in production of volatile components between *Saccharomyces paradoxus* and *Saccharomyces cerevisiae* strains and their influence on final wine quality. Samples of young wine were analysed for higher alcohols, fatty acids and volatile esters. At the same time wines were subjected to sensory evaluation. The results showed a notable influence of *Saccharomyces paradoxus* strain RO88 on chemical and sensory properties of Gewürtztraminer wine and indicated some differences between *Saccharomyces paradoxus* and *Saccharomyces cerevisiae* species.

*Key words:* *Saccharomyces paradoxus*, wine volatile compounds, sensory characteristics

### Introduction

The final product of grape must fermentation is the result of a combined action of different yeast species which contribute in different ways to the sensory properties of wine (1). According to modern classification, three biological species: *Saccharomyces cerevisiae* Hansen, *Saccharomyces bayanus* Saccardo (syn. *S. uvarum*) and *Saccharomyces paradoxus* Bachinska as well as the hybrid taxon *Saccharomyces pastorianus* Hansen (syn. *S. carlsbergensis*) are identified within the *Saccharomyces sensu stricto* complex (2). Up to the present research, studies have been largely limited to the selection and use of *S. cerevisiae* or *S. bayanus* as starter culture for must fermentation. These two species were selected according to their positive enological characteristics. *S. paradoxus* is

usually found in exudates of broad-leaved trees, insects and uncultivated soils (2) and little has been done for the application of *S. paradoxus* as a starter culture in practical winemaking.

The influence of the yeast species that ensure fermentation has been studied in wine, and many experiments have been performed to select the yeast strain that will improve wine quality (3–6). The majority of important wine aroma compounds (higher alcohols, aldehydes, fatty acid esters, acetates) are formed by yeast during alcoholic fermentation (7). Some authors have reported more or less pronounced differences among strains of the same species (8–10).

Variety and composition of the yeast flora in the must can vary according to geographic locality, climatic

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conditions and/or grape variety (11–14). Some researchers believe that each microclimate, such as the vineyard, is characterised by a specific *S. cerevisiae* yeast flora, where some strains can remain for many years and become representative of an ecological area (15). Consequently, in this paper we have compared the strains of two different species, *Saccharomyces cerevisiae* and *Saccharomyces paradoxus* with the native yeast flora from the vineyard where these strains were isolated. In a previous study (16), *S. cerevisiae* strain RO64 and *S. paradoxus* strain RO88 showed a good fermentation rate that resulted in dry wines, high alcohol and low acetic acid production. *S. paradoxus* strain RO88 formed higher glycerol concentration than *S. cerevisiae* RO64. At the same time strain RO88 decomposed up to 40 % of malic acid whereas strain RO64 did not show that ability.

The main aim of this study was to determine specific enological characteristics of *Saccharomyces paradoxus* species, potential differences in volatile compounds production between *Saccharomyces paradoxus* and *Saccharomyces cerevisiae* strains and their influence on final Gewürtztraminer wine quality.

## Material and Methods

### Must preparation

The work was carried out in the wine region of continental Croatia, Zagreb subregion, using Gewürtztraminer grape. Grapes from 1998 and 1999 harvest years were first crushed and then pressed with Vaslin press, set at 70 % yield. The juice was treated with 50 mg/L SO<sub>2</sub> and held overnight in the cellar to settle. The following day the juice was racked and the resulting must was divided into 10-litre portions in 12 glass vats. Four vats were fermented with their indigenous yeasts. The remainder were inoculated, four with *S. paradoxus* strain RO88 and four with *S. cerevisiae* strain RO64.

### Yeast cultures

From the group *Saccharomyces sensu stricto*, *S. paradoxus* strain RO88 and *S. cerevisiae* strain RO64 were isolated from Zagreb wine region, location Jazbina and they make a part of the collection of the Department of Microbiology, Faculty of Agriculture. All strains were kept on YEPG agar (1 % yeast agar, 1 % peptone, 2 % glucose and 1.5 % agar) at 4 °C. Identification of strains was performed by standard physiological and molecular (PCR – RFLP) methods (17).

### Vinification

Starters for inoculation were prepared 72 hours before use from sterile portion of the same must with inoculation dosage of 10 %. Must sugar concentration was determined every three days from the beginning of the fermentation. Just after the fermentation, wines were racked and sulfured with 30 mg/L SO<sub>2</sub> and the samples were taken for chemical analysis. After the second racking in February, wines were bottled and kept cold until sensory evaluation was carried out six months later.

### Chemical analysis

The analyses of volatile compounds were performed on Hewlett Packard model 5890 Gas chromatograph fitted with flame ionization detector. Higher alcohols and ethyl acetate were analysed on distillate of wine using HP101 (Hewlett Packard) column of 50 m x 0.32mm and 0.3 µm film thickness. Temperature programming was as follows: 6 min isothermal at 40 °C, then linear temperature rise of 15 °C min<sup>-1</sup> to 200 °C. Injector and detector temperatures were 220 and 250 °C, respectively. Nitrogen was used as carrier gas at 30 mL/min. 1-butanol was used as internal standard.

To determine volatile fatty acids, their ethyl esters, higher alcohol acetates and other volatile compounds, the wine (500 mL), to which 1-heptanol was added as internal standard, was continuously extracted (10 h) by dichloromethane. The extract was dried over anhydrous sodium sulfate, concentrated to 10 mL and stored prior to GC analysis. The extract (1 µL) was injected (split 1:50) into a FFAP – HP (Hewlett Packard) column of 50 m x 0.32 mm and 0.5 µm phase thickness. Temperature programming was: 5 min isothermal at 60 °C, then linear temperature rise of 2.5 °C min<sup>-1</sup> to 190 °C and 20 min isothermal at 190 °C. Injector and detector temperatures were 220 and 260 °C, respectively. The carrier gas used was nitrogen (30mL/min).

### Statistical analysis

One-way analysis of variance and Least Significant Difference (LSD) comparison test were used to statistically interpret mean differences in mean values, if any, at 95 and 99 % accuracy level.

### Sensory analysis

The wines from the 1999 harvest season were subjected to sensory evaluation by a panel comprising 7 members of the Croatian Enological Society, all of them highly experienced in wine sensory testing, especially Gewürtztraminer.

Wines of each yeast treatment were compared by a paired sample test to determine aroma differences among different varieties. Determination of statistical significance for the paired sample test results was done according to literature (18).

## Results and Discussion

### Chemical analysis

#### Concentration of higher alcohols

Experiments by Wagener and Wagener (19) showed that although higher alcohols constitute a relatively lesser quantity of the total substances contained in wine, they may undoubtedly influence certain sensory qualities of white wines. Compared to *S. cerevisiae* strain RO64, strain *S. paradoxus* RO88 produced significantly lower concentrations (below 300 mg/L) of total higher alcohols in both years (Table 1.). According to Rapp and Versini (20) these concentrations in higher alcohols contribute to desirable aroma complexity of wine, but when their concentrations exceed 400 mg/L, these compounds are regarded as a negative quality factor. Total concentration of higher alcohols can be affected by numerous

Table 1. Concentration of higher alcohol (mg/L) in Gewürtztraminer wines

Compounds	Year	Indigenous yeasts	<i>S. paradoxus</i> RO88	<i>S. cerevisiae</i> RO64	LSD
1-Propanol	1998	35.50 <sup>a</sup>	21.50 <sup>b</sup>	29.25 <sup>c</sup>	5 % = 2.8 1 % = 3.98
	1999	16.25 <sup>a</sup>	14.50 <sup>a</sup>	15.25 <sup>a</sup>	5 % = n.s. 1 % = n.s.
Hexanol	1998	0.39 <sup>a</sup>	0.43 <sup>a</sup>	0.52 <sup>b</sup>	5 % = 0.05 1 % = 0.07
	1999	0.52 <sup>a</sup>	0.54 <sup>a</sup>	0.55 <sup>a</sup>	5 % = n.s. 1 % = n.s.
Isobutanol	1998	10.00 <sup>a</sup>	18.75 <sup>b</sup>	48.00 <sup>c</sup>	5 % = 3.22 1 % = 4.56
	1999	31.25 <sup>a</sup>	28.25 <sup>a</sup>	27.50 <sup>a</sup>	5 % = n.s. 1 % = n.s.
Isoamyl alcohol	1998	72.25 <sup>a</sup>	124.50 <sup>b</sup>	174.75 <sup>c</sup>	5 % = 14.39 1 % = 20.34
	1999	197.25 <sup>aBC</sup>	169.00 <sup>bB</sup>	203.75 <sup>cC</sup>	5 % = 22.4 1 % = 31.7
2-Phenyl ethanol	1998	15.50 <sup>a</sup>	17.00 <sup>a</sup>	17.25 <sup>a</sup>	5 % = n.s. 1 % = n.s.
	1999	35.25 <sup>a</sup>	50.00 <sup>b</sup>	47.00 <sup>b</sup>	5 % = 6.15 1 % = 8.69
Σ Higher alcohol	1998	133.50 <sup>a</sup>	182.00 <sup>b</sup>	269.77 <sup>c</sup>	5 % = 18.2 1 % = 25.7
	1999	276.48 <sup>AB</sup>	260.66 <sup>A</sup>	292.39 <sup>B</sup>	5 % = 27.2 1 % = n.s.

Note: Different letters beside the mean of a compound denote a significant difference among treatments (A, B, C for 5 %; a,b,c for 1 %). The same letter beside the mean of a compound denotes an insignificant difference among treatments (A, B, C for 5 %; a,b,c for 1 %).

factors (climate conditions, must composition, juice turbidity, temperature, fermentation procedure).

According to our results during the investigation period the greatest difference in total higher alcohol content was detected in the wines made by indigenous yeasts, whereas inoculated strains showed a notable stability. Phenyl ethanol is an aromatic alcohol whose bouquet resembles roses and is also believed to play a sensory role in the perception of wine aroma. Sponholz and Dittrich (21) found that epiphyte microorganisms produced higher concentrations of 2-phenyl ethanol. On the contrary, Bertolini *et al.* (22) found significant differences in concentrations of 2-phenyl ethanol in the fermentations with inoculated strains. This is partly confirmed by our results because it can be noticed that pure yeast strains can affect the final concentrations of phenyl ethanol. In the 1999 research, inoculated strains produced higher concentrations of this alcohol, but that was not the case in 1998. Between examined strains RO88 and RO64 no difference was noticed.

Isoamyl alcohol is the most abundant higher alcohol making more than 50 % of total higher alcohol content. According to Müller *et al.* (23) isoamyl alcohol is the predominant fragrant component of higher alcohols. In both research years significantly lower amounts of this alcohol were present in *S. paradoxus* strain RO88 wines than in *S. cerevisiae* strain RO64 wines. Indigenous yeasts showed the greatest differences in the isoamyl alcohol produced concentrations between the investigated

years. Experiments carried out in the continental region of Croatia with Traminer cultivar showed the content of 16–45 mg/L 1-propanol, 25–80 mg/L isobutanol and 24–173 mg/L isoamyl alcohol (24). Our results from the fermentations with strains RO88, RO64 and indigenous yeasts correspond entirely to these data.

#### Concentration of fatty acids

According to Cavazza and Grando (25) yeast strains produced significantly different concentrations of butyric, capric, caprylic, caproic and isovaleric acid but our results did not confirm that. There were also no significant differences in total concentrations of fatty acids in both research years (Table 2.). Yeasts synthesize much the same fatty acids irrespective of the nature of the raw materials used. However, the fatty acid composition of yeasts is highly variable; changes in growth substrate and minor alternations in growth conditions (pH, temperature, presence of nutrients) as well as the growth rate of the organism itself may affect the relative proportions of the individual components (26). Our research has indicated that *S. paradoxus* strain RO88 has the potential to produce almost equal concentrations of fatty acids as *S. cerevisiae* strain RO64 and indigenous yeasts.

#### Concentration of volatile esters

During the alcoholic fermentation many esters can be formed, but the most significant ones are acetate esters of higher alcohols (ethyl acetate, isoamyl acetate,

Table 2. Concentration of fatty acids (mg/L) in Gewürtztraminer wines

Compounds	Year	Indigenous yeasts	<i>S. paradoxus</i> RO88	<i>S. cerevisiae</i> RO64	LSD
Butyric acid	1998	0.82	0.66	0.64	5 % = n.s. 1 % = n.s.
	1999	1.01 <sup>Aa</sup>	0.94 <sup>Bb</sup>	0.80 <sup>Bc</sup>	5 % = 0.11 1 % = 0.15
Isobutyric acid	1998	0.31	0.36	0.33	5 % = n.s. 1 % = n.s.
	1999	0.36 <sup>a</sup>	0.22 <sup>b</sup>	0.23 <sup>b</sup>	5 % = 0.04 1 % = 0.06
Isovaleric acid	1998	0.11 <sup>a</sup>	0.18 <sup>b</sup>	0.11 <sup>a</sup>	5 % = 0.04 1 % = 0.05
	1999	0.30 <sup>a</sup>	0.20 <sup>b</sup>	0.23 <sup>b</sup>	5 % = 0.04 1 % = 0.06
Caproic acid	1998	3.33	3.10	2.15	5 % = n.s. 1 % = n.s.
	1999	5.12	4.70	4.80	5 % = n.s. 1 % = n.s.
Caprylic acid	1998	3.90	2.85	2.93	5 % = n.s. 1 % = n.s.
	1999	5.80	5.00	5.00	5 % = n.s. 1 % = n.s.
Capric acid	1998	2.53	2.23	1.95	5 % = n.s. 1 % = n.s.
	1999	3.12	3.27	2.80	5 % = n.s. 1 % = n.s.
Σ Fatty acids	1998	11.59	9.37	8.11	5 % = n.s. 1 % = n.s.
	1999	15.73	14.34	14.04	5 % = n.s. 1 % = n.s.

Note: Different letters beside the mean of a compound denote a significant difference among treatments (A, B, C for 5 %; a,b,c for 1 %). The same letter beside the mean of a compound denotes an insignificant difference among treatments (A, B, C for 5 %; a,b,c for 1 %).

isobutyl acetate, and 2-phenyl-ethyl acetate) and ethyl esters of fatty acids (ethyl butyrate, ethyl lactate, ethyl caprylate, ethyl caprylate and ethyl capronate) (27).

Total concentrations of volatile esters presented in Table 3 indicate *S. paradoxus* strain RO88 as a greater volatile ester producer than *S. cerevisiae* strain RO64. Compared to the indigenous yeasts, significantly higher amount was detected only in 1999.

Ethyl acetate is the main ester occurring in wine with concentrations from 50 to 200 mg/L (28). Concentrations of ethyl acetate contribute significantly to the volatile character of «acetic nose» and levels of 150 to 200 mg/L impart spoilage character to wine. But in very low concentrations (50–80 mg/L) ethyl acetate contributes to the olfactory complexity and has a significant influence on the quality of wine (29). In investigated years inoculated strains RO88, RO64 and also indigenous yeasts synthesized low concentrations of ethyl acetate. In 1998 strain RO64 produced significantly lower concentrations and in 1999 the lowest amount was produced by indigenous yeasts.

Soles *et al.* (28) reported differences in production of 2-phenyl ethyl acetate, isoamyl acetate and hexyl acetate as a function of 14 *Saccharomyces cerevisiae* strains used.

According to Cavazza and Grando (25), acetate esters contribute to the formation of fruity wine aroma. Our results with the acetate esters concentrations are in accordance with the above mentioned.

Concentrations of ethyl caprylate found in wine vary from 0.2 to 1.5 mg/L, in some cases up to 3.4 mg/L. The threshold value is 0.08 mg/L, and it is characterised by green apple, banana and violet fragrance. Ethyl caprylate concentrations in wine can vary from 0.05 to 3.8 mg/L and its fragrance is reminiscent of pineapple and pear, with the threshold value of 0.58 mg/L, whereas ethyl capronate can be found in concentrations from trace to 2.1 mg/L, and has a floral fragrance; threshold value is 0.5 mg/L (30, 28). Our results showed relatively high concentrations of ethyl esters of fatty acids. *S. paradoxus* strain RO88 produced significantly higher amounts of ethyl caprylate than the other tested yeasts. Differences in the production of ethyl caprylate and capronate among examined yeasts also existed but were not significant in neither of the research years.

#### Sensory analysis

The results of sensory evaluation of wines are shown in Table 4, and even without significant differ-

Table 3. Concentration of volatile esters (mg/L) in Gewürtztraminer wines

Compounds	Year	Indigenous yeasts	<i>S. paradoxus</i> RO88	<i>S. cerevisiae</i> RO64	LSD
Ethyl acetate	1998	48.50 <sup>a</sup>	56.25 <sup>a</sup>	31.00 <sup>b</sup>	5 % = 12.05 1 % = 17.00
	1999	50.50 <sup>a</sup>	78.50 <sup>b</sup>	71.00 <sup>b</sup>	5 % = 11.70 1 % = 16.63
Isobutyl acetate	1998	0.07	0.10	0.11	5 % = n.s. 1 % = n.s.
	1999	0.11	0.08	0.08	5 % = n.s. 1 % = n.s.
Isoamyl acetate	1998	1.17 <sup>aA</sup>	1.77 <sup>bB</sup>	1.39 <sup>cB</sup>	5 % = 0.31 1 % = 0.43
	1999	1.36 <sup>aA</sup>	1.06 <sup>bB</sup>	1.20 <sup>bA</sup>	5 % = 0.15 1 % = 0.21
Hexyl acetate	1998	0.07 <sup>a</sup>	0.10 <sup>b</sup>	0.05 <sup>c</sup>	5 % = 0.01 1 % = 0.02
	1999	0.08	0.06	0.08	5 % = n.s. 1 % = n.s.
Phenyl ethyl acetate	1998	0.36 <sup>a</sup>	0.25 <sup>b</sup>	0.14 <sup>c</sup>	5 % = 0.01 1 % = 0.02
	1999	0.44 <sup>aA</sup>	0.47 <sup>aA</sup>	1.02 <sup>bB</sup>	5 % = 0.15 1 % = 0.21
Ethyl butyrate	1998	0.22	0.29	0.25	5 % = n.s. 1 % = n.s.
	1999	0.30	0.21	0.30	5 % = n.s. 1 % = n.s.
Ethyl lactate	1998	1.06 <sup>aA</sup>	1.24 <sup>aA</sup>	1.95 <sup>bB</sup>	5 % = 0.26 1 % = 0.36
	1999	0.86	0.90	0.85	5 % = n.s. 1 % = n.s.
Ethyl caprylate	1998	1.22 <sup>A</sup>	1.86 <sup>B</sup>	1.13 <sup>A</sup>	5 % = 0.63 1 % = n.s.
	1999	0.61 <sup>aA</sup>	0.72 <sup>bB</sup>	0.52 <sup>aA</sup>	5 % = 0.09 1 % = 0.13
Ethyl caprylate	1998	1.63	1.99	1.87	5 % = n.s. 1 % = n.s.
	1999	0.78 <sup>aA</sup>	0.54 <sup>bB</sup>	0.54 <sup>bB</sup>	5 % = 0.13 1 % = 0.19
Ethyl capronate	1998	0.52	0.77	0.53	5 % = n.s. 1 % = n.s.
	1999	0.29 <sup>aA</sup>	0.19 <sup>bB</sup>	0.14 <sup>cB</sup>	5 % = 0.04 1 % = 0.06
Diethyl succinate	1998	0.72	0.65	0.92	5 % = n.s. 1 % = n.s.
	1999	0.06	n.d.	n.d.	5 % = n.s. 1 % = n.s.
Σ Volatile esters	1998	53.43 <sup>aA</sup>	65.31 <sup>aA</sup>	39.25 <sup>bB</sup>	5 % = 13.8 1 % = 19.5
	1999	56.38 <sup>aA</sup>	82.77 <sup>bB</sup>	75.78 <sup>bBC</sup>	5 % = 11.8 1 % = 16.7

Note: Different letters beside the mean of a compound denote a significant difference among treatments (A, B, C for 5 %; a,b,c for 1 %). The same letter beside the mean of a compound denotes an insignificant difference among treatments (A, B, C for 5 %; a,b,c for 1 %).

ences they indicate a substantial effect on the aroma of Gewürtztraminer wine as a result of fermentation with different yeast strains, which is in accordance with literature data (31,32). *S. paradoxus* strain RO88 wines had the most intense aroma, whereas aroma intensity of *S.*

*cerevisiae* strain RO64 wines was not so typical. According to our results, more intense aroma of *S. paradoxus* strain RO88 wines was due to numerous factors, and we suppose that one of them could be a lower amount of total higher alcohols. The aroma differences among pro-



Table 4. Results of sensory evaluation of wines by a paired sample method

Judges	Treatment					
	Indigenous yeasts	Strain RO88	Indigenous yeasts	Strain RO64	Strain RO88	Strain RO64
1		+		+	+	
2		+	+		+	
3		+		+	+	
4		+	+		+	
5		+	+			+
6		+	+		+	
7	+		+		+	
Total	1	6	5	2	6	1

duced wines could also be linked to a different yeast  $\beta$ -glucosidase activity. Laffort *et al.* (33) suggested that specific yeast strain  $\beta$ -glycosidases can affect the aroma of wines.

## Conclusion

The current study reports the use of *S. paradoxus* strain in winemaking, and compared with *S. cerevisiae* RO64 and indigenous yeasts, our results indicated interesting enological properties of *S. paradoxus* RO88 strain.

*S. paradoxus* strain RO88 and *S. cerevisiae* strain RO64 used to ferment musts from Gewürtztraminer grapes were found to produce wines with different amounts of volatile compounds. Wines made with *S. paradoxus* RO88 contained a smaller amount of higher alcohols than those made with *S. cerevisiae* strain RO64. On the contrary, the concentrations of some volatile esters were higher in *S. paradoxus* RO88 wines. Both examined strains produced much the same concentrations of fatty acids.

Sensory evaluation of the wines showed that the judges noticed quality and aroma differences between strain RO88 and strain RO64 wines. The strongest aroma was detected in wines made with *S. paradoxus* strain RO88.

Finally, this work shows that *S. paradoxus* provides good enological properties and that its strains can influence the production of certain volatile compounds and the final quality of wine. In the future this yeast should be better studied and selected.

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## Usporedba aromatskih komponenti vina dobivenih fermentacijom *Saccharomyces paradoxus* i *Saccharomyces cerevisiae* sojeva

### Sažetak

Cilj je ovog istraživanja utvrditi specifične enološke značajke vrste *Saccharomyces paradoxus*, potencijalne različitosti između vrste *Saccharomyces paradoxus* i *Saccharomyces cerevisiae* u sintezi hlapljivih komponenti te njihov utjecaj na kakvoću vina. Analizirane su koncentracije viših alkohola, masnih kiselina i hlapljivih estera, a vina su i senzorno ocijenjena. Rezultati su pokazali bitan utjecaj soja *Saccharomyces paradoxus* RO88 na kemijski sastav i senzorna svojstva vina Traminac te su upozorili i na razlike između vrsta *Saccharomyces paradoxus* i *Saccharomyces cerevisiae*.