Influence of Indigenous *Saccharomyces cerevisiae* Strains on Higher Alcohol Content in Malvazija Istarska Wines

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SUMMARY

Istria is one of the most remarkable wine growing regions in Croatia. The main white grape variety is Malvasia from Istria (Malvazija istarska). Commercial yeast inocula, generally *Saccharomyces cerevisiae*, are widely used as starters today, however it might be preferable to use selected indigenous strains, which may have optimum abilities and may be better adapted to ferment the must of each area. Production and final concentration of higher alcohols is one of the strains most important oenological properties.

In this research we extensively studied three indigenous *S. cerevisiae* strains during three years, isolated from the Istra wine region. Content of higher alcohols (1-propanol, 1-hexanol, Isobutanol, 1-butanol, Isoamyl alcohol and 2-phenyl ethanol were analyzed using GC-SHS. Compared to the control strain all tested indigenous yeast strains produced significantly lower concentration of 1 propanol while only the strain RO 1203 produced higher concentration of Hexanol, Isobutanol and 2-phenyl ethanol in all investigated years. In the 2000 and 2001. wines produced with strain RO1172 had the lowest total higher alcohol amount while in the year 2002 there were no difference between tested strains. Sensory evaluation carried out by Buxbaum method pointed out good enological properties and positive influence on final wine quality of all three tested *S. cerevisae* indigenous strains.

KEY WORDS

Saccharomyces cerevisiae; higher alcohols, wine quality

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Received: April 18, 2005

ACKNOWLEDGEMENTS

This work was supported by grant 0178047 from the Croatian Ministry of Science, Education and Sports.

INTRODUCTION

The most important characteristic in wine is the flavour (Pretorius et al., 2003). The dominant and major compounds contributing to wine aroma are formed during yeast fermentation (Antonelli et al., 1999). The formation of volatile compounds during the fermentation of a must is a complex phenomenon involving a number of factors. In particular, it depends on the nature and concentration of the metabolites initially present in the must (their proportions differ from one grape variety to another) (Usseglio – Tomaset, 1992; Cavazza and Grando, 1998), on the capacity of the yeast to transform them and the conditions used in wine making (Delfini et al., 2001).

Malvazija istarska, a native grape variety of *Vitis vinifera* L., is grown in the Istra wine region of Croatia and gives good quality wines. Limited studies have been carried out out to improve the flavour potential of cv. Malvazija istarska wines by using selected local and commercial strains of *S.cerevisiae*. The majority of important wine aroma compounds (higher alcohols, aldehydes, fatty acid esters, acetates) are formed by yeast during alcoholic fermentation (Rankine, 1967; 2000; Herjavec et al., 2003). Commercial yeast inocula is widely used as starter today, however it might be preferable to use selected indigenous strains, which may be better adapted to ferment the must of each area (Romano, 1997). Some researchers believe that a specific *S. cerevisiae* yeast flora with some strains remaining there for many years and becoming representative of an enological area can characterized each microclimat, such as the vineyards (Romano, 1997, Redzepovic et al., 2002). Consequently, in this research we have compared the difference in the higher alcohol production between three indigenous *S. cerevisiae* strains during three years, isolated from the Istra wine region.

MATERIAL AND METHODS

Yeast

We used three *S. cerevisiae* strains (RO540, RO1172, RO1204) from the wine yeast collection of the Department of microbiology, Faculty of Agriculture, Zagreb and one commercial wine yeast starter culture strain (*S. cerevisiae* VB1) from Gist-brocades, France.

Fermentation

All fermentation experiments were carried out in four repetitions by using 100 L of cold settled and enzymatic treated (X-press, Gist-brocades, France) must. For the inoculum of *S. cerevisiae* strains and the commercial strain yeast culture were preincubated in sterilised grape must for 48 h at 25°C and inoculated at a final level of $5x10^6$ cells ml^{-1}. Must was cold settled on 15°C for 24 hours. After the inoculation of selected strains fermentations was carried out at the temperature between 16 – 18 °C and lasted 20 days. There was no difference in the fermentation rate between tested stains.

Chemical analysis

Alcohol, total and volatile acidity, residual sugar and pH were determinate using methods proposed by O.I.V. (1995). Higher alcohol analysis was performed by gas chromatography (GS-SHS) on a Hewlett Packard model 5890 (Kovačević Ganić et al., 2003).

Sensory analysis

At the end of fermentation all the samples were chemical analysis, and the sensory evaluation was conducted by using the Buxbaum model of positive rating. The model was developed on four sensory characteristics (colour, clearness, odour and taste) with the maximum of 20 points.

Statistical analysis

One-way analysis of variance (ANOVA) and Least Significant Difference (LSD) comparison test of SAS (SAS Institute, Cary, NC, USA) were used to interpret differences in means, if any, at the 95% and 99% confidence level.

RESULTS AND DISCUSSION

Chemical composition of the must and wine

The chemical composition of Malvazija Istarska must is presented in the table 1. There were no marked differences in suger content and total acidity content between investigated years showing good ability of Malvazija Istarska variety to accumulate grape sugar. The table 2 shows the chemical composition of Malvazija Istarska wines. Results were within the normal range of values expected. The total acidity was between 5,17- 7,03 g/L, and the total volatile acidity values oscillate between 0,47-0,66 g/L. All *S. cerevisiae* strains showed high ethanol production and good fermentation vigor. In all research years significantly lower amounts of total acidity were presented in *S. cerevisiae* strain RO 540 wines. We can presume that this strain has the ability for biological deacidification (Redžepović et al, 2003).

Table 1. Must chemical composition of Malvazija istarska

Compound	Year		
	2000	2001	2002
Sugar °Oe	91	90	88
Total acidity (as tartaric) g/L	7.2	6.4	7.3
pH	3.4	3.4	3.3

Table 2. Chemical compositon of Malvazija istarska wines

Compound	Year	Yeast strain				LSD	
		Control	540	1172	1203	5%	1%
Alcohol (vol. %)	2000	13.05^{a}	12.70^{b}	12.73^{b}	12.73^{b}	0.22	0.31
	2001	12.80^{a}	12.60^{a}	12.70^{a}	12.77^{a}	0.36	0.51
	2002	12.13^{a}	11.93^{a}	11.98^{a}	11.95^{a}	0.23	0.33
Total acidity (as tartaric) g/L	2000	6.55^{Aa}	5.97^{Bb}	6.40^{ac}	6.30^{ac}	0.32	0.46
	2001	5.47^{a}	5.17^{Bb}	5.50^{a}	5.58^{A}	0.26	0.38
	2002	7.05^{a}	6.22^{b}	7.03^{a}	6.98^{a}	0.64	0.92
Volatile acidity (as acetic)g/L	2000	0.47^{b}	0.54^{ab}	0.57^{a}	0.49^{b}	0.09	0.13
	2001	0.56^{a}	0.53^{a}	0.63^{a}	0.57^{a}	0.13	0.19
	2002	0.49^{b}	0.52^{b}	0.66^{a}	0.60^{ab}	0.14	0.20
Total dry extract (g/L)	2000	20.53^{ab}	20.55^{ab}	19.85^{b}	21.00^{a}	0.96	1.38
	2001	18.88^{a}	19.15^{a}	18.83^{a}	19.50^{a}	1.10	1.58
	2002	23.40^{b}	25.00^{a}	23.30^{b}	24.33^{ab}	1.56	2.24
Reducing sugar (g/L)	2000	1.58^{a}	1.43^{b}	1.83^{a}	1.93^{a}	0.39	0.57
	2001	1.55^{a}	1.78^{a}	1.68^{a}	1.58^{a}	0.43	0.63
	2002	2.2^{b}	3.18^{a}	2.33^{a}	2.68^{ab}	0.69	0.99
Ash (g/L)	2000	2.38^{a}	2.48^{a}	2.30^{a}	2.45^{a}	0.19	0.28
	2001	2.98^{a}	2.63^{a}	2.78^{a}	2.80^{a}	0.57	0.82
	2002	2.85^{b}	3.53^{a}	3.28^{a}	3.55^{a}	0.31	0.44
pH	2000	3.32^{BC}	3.39^{Bd}	3.55^{Aa}	3.48^{Ab}	0.057	0.08
	2001	3.40^{a}	3.40^{a}	3.53^{a}	3.55^{a}	0.16	0.22
	2002	3.30^{b}	3.43^{a}	3.33^{b}	3.40^{a}	0.05	0.07

Table 3. Higher alcohol concentrations in Malvazija Istarska wines (mg/L).

Compound	Year	Yeast strain				LSD	
		Control	RO 540	RO 1172	RO 1203	5%	1%
1 – propanol	2000	37.18^{A}	24.95^{B}	24.35^{B}	26.38^{B}	4.03	5.79
mg/L	2001	15.09^{a}	11.39^{b}	10.84^{b}	13.41^{ab}	3.69	5.30
	2002	25.44^{a}	19.32^{ab}	18.84^{b}	17.81^{b}	7.14	10.25
Hexanol	2000	3.60^{a}	4.55^{a}	3.90^{a}	4.15^{a}	1.19	1.72
mg/L	2001	4.57^{a}	4.79^{a}	5.05^{a}	6.05^{a}	1.53	2.21
	2002	1.95^{Bb}	2.75^{a}	2.83^{a}	3.06^{A}	0.76	1.08
Isobutanol	2000	25.55^{a}	35.58^{a}	33.75^{a}	34.88^{a}	19.75	28.38
mg/L	2001	23.71^{Bb}	26.07^{ABb}	25.19^{Bb}	31.90^{Aa}	4.51	6.49
	2002	19.98^{a}	26.82^{a}	26.54^{a}	27.22^{a}	11.50	16.53
1 – butanol	2000	0.89^{b}	1.60^{a}	0.94^{ab}	1.20^{ab}	0.67	0.96
mg/L	2001	0.36^{a}	0.19^{a}	0.32^{a}	n.d.	0.59	0.85
	2002	1.08^{a}	1.13^{a}	1.18^{a}	0.57^{b}	0.50	0.73
Isoamil alcohol	2000	224.98^{a}	247.18^{a}	194.63^{a}	206.70^{a}	67.86	97.50
mg/L	2001	204.59^{b}	211.88^{ab}	189.03^{Bb}	236.61^{Aa}	24.94	35.83
	2002	132.13^{a}	139.08^{a}	149.83^{a}	146.56^{a}	37.26	53.53
2 – phenil etanol	2000	30.30^{a}	34.26^{a}	20.40^{a}	35.30^{a}	15.10	21.10
mg/L	2001	39.36^{ab}	50.93^{ab}	36.41^{b}	56.93^{a}	18.07	25..97
	2002	6.51^{b}	11.19^{ab}	13.41^{a}	10.74^{ab}	6.70	9.57
Total higher alcohol	2000	322.17^{a}	348.08^{a}	277.96^{a}	308.60^{a}	101.20	145.30
mg/L	2001	287.67^{Bbc}	305.24^{b}	266.86^{Bc}	344.90^{Aa}	38.24	54.97
	2002	187.08^{a}	200.21^{a}	212.63^{a}	205.95^{a}	60.49	86.91

Concentration of higher alcohols

According to Rapp and Versini (1991) concentrations of total higher alcohols below 300 mg/L certainly contribute to desirable aroma complexity of wine. However, when concentrations exceed 400 mg/L, these compounds are regarded as a negative quality factor. All wines produced with tested strains had relativly low amount of total higher alcohols. More notable difference was noticed between investigated years than between tested strains as shown in table 3.

The results of Isobutanol, Hexanol and 2-phenyl ethanol have indicated that indigenous *S. cerevisiae* strains had the potential to produce higher concentrations of these alcohols than control strain. At the contrary during three investigation years comercial *S. cerevisiae* strain VB1 produced the highest concentrations of 1-propanol (Table 3). All indigenous strains tested sintetized lower and similar concentrations of this alcohol. There were no regularity between tested strains in the production of 1-butanol .

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 (Herjavec et al., 2003). The concentrations of Isoamyl alcohol varied according to the vintage year (Tupajić et al., 1996). The lowest amount of this compound was detected in the year 2002 and it was not conected with the yeast strain used (Table 3). In general, indigenous strain RO1172 produced lower concentration of Isoamyl alcohol and the difference was especially pronunced in the 2000 and 2001 year.

Sensory analysis

The results of sensory evaluation of wines are shown in table 4, and even without significant differences they indicate a substantial effect on the quality of Malvazija Istarska wines as a result of fermentation with different yeast strains (Lurton et al., 1995; Villa et al., 1998; Nurgel et al., 2002). Our results pointed out that all *S. cerevisae* wines were of similar or better quality compared to wines made with commercial strain according to organoleptic valuation. Also, we only considered the higher alcohol concentrations, although yeast strain could influence the levels of varietal or pre-fermentation volatile compounds.

Table 4. Sensory analysis of Malvazija istarska wines

Year	Control	RO 540	RO 1203	RO 1172
2000	17.9	18.2	17.7	17.9
2001	17.7	18.2	17.9	18.0
2002	17.7	17.9	17.5	18.2

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acs70_09