UDC 663.12:663.2
ISSN 1330-9862

original scientific paper

(FTB-1193)

The Influence of Some Commercial *Saccharomyces cerevisiae* Strains on the Quality of Chardonnay Wines

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Received: October 1, 2002
Accepted: January 24, 2003

Summary

Changes in some aroma compounds and sensory properties caused by different commercial *S. cerevisiae* strains and by epiphyte microorganisms in Chardonnay wines were investigated. Wines fermented with the Lalvin-71 B strain contained significantly lower concentrations of alcohol, isoamyl alcohol, isobutanol and total acidity than the wines obtained from spontaneous and other inoculated fermentations. The highest concentrations of 2-phenyl ethanol and 2-phenyl ethyl acetate were found in the spontaneously fermented wines, whereas no essential changes in these compounds were found among the wines of other treatments. Compared to the spontaneous fermentation, Lalvin-71 B strain fermented wines had somewhat higher concentrations of butyric and caproic acid and ethyl butyrate. Lalvin-71 B strain fermented wines were assessed as the best whereas the quality of the wines produced with Lalvin aromatic-2056 strain was the most inferior in quality.

Key words: *S. cerevisiae*, volatile compounds, sensory properties, wine

Introduction

The quality of white wines, especially their olfactory characteristics, largely depends on the concentration and content of volatile compounds that yeast synthesises during alcohol fermentation *(1,2)*. Higher alcohols, volatile esters and fatty acids are the most important wine aroma compounds formed during alcoholic fermentation; their concentration can vary depending on many factors, especially the yeast strain *(3–5)*. Various yeast species and strains present in the epiphyte microflora may yield higher concentrations of higher alcohols and ethyl acetate. Thus spontaneous fermentation may result in inferior wine quality *(6,7)*. Some investigations have reported significant differences in chemical composition beetwen the wines of spontaneous and inoculated fermentations *(8,9)*. The wines obtained through fermentation by starter cultures have lower content of undesirable aroma compounds than the spontaneously fermented wines *(8)*. However, the use of alien starter cultures, which are not adapted for fermentation of must originating in a certain region, may result in wines of very similar aromatic characteristics, which leads to the loss of distinctive features of a wine. Therefore, the use of commercial *S. cerevisiae* strains is justified only when they do not affect the aromatic specificity of produced wines *(10)*. Various commercial strains of *S. cerevisiae*, invariably of alien origin, are present on the Croatian

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market. The selection of a starter culture and its use in wine production have been left to the producer himself, without any knowledge about the influence of a particular variety on the wine quality in a specific viticultural area.

The objective of this study has been to check how and to what extent some commercial strains of *S. cerevisiae* of alien origin and the epiphyte microflora of grapes influence the Chardonnay wine quality under the conditions prevailing in the region of the Daruvar vineyards.

Material and Methods

Vinification

Chardonnay grapes from the 1997 harvest season were grown in the continental wine region of Croatia, subregion Slavonia. Harvested grapes were divided into five lots, destemmed and crushed. Free-run juice of each lot was sulfured with SO_2 (100 mg/L) and settled for 24 hours. The must of each lot was racked and divided into 15-L glass bottles according to the following treatments: the first one included spontaneous fermentation, whereas other must lots were inoculated with commercial *S. cerevisiae* strains, trade marks: Uvaferm-CM, Uvaferm-CS2, Lalvin-71 B and Lalvin-2056. All treatments were done in four repetitions. Lyophilized yeast cultures were reactivated prior to inoculation and added to sulfited musts. Fermentation with commercial strains began one day after inoculation, whereas spontaneous fermentation started 8 days later. Complete sugar degradation in wines of all treatments lasted longer than two months. During alcoholic fermentation the temperature did not exceed 18 °C. Wines of all treatments were chemically analysed just after the first racking in November. After the second racking in February the wines were bottled and stored under the cellar conditions until the sensory evaluation, which was carried out two months later.

Chemical analysis

Ethanol, total and volatile acidity were determined using the methods proposed by O.I.V. (1995). Reducing sugar was determined by the Soxlet method and pH was measured on the pH-meter 538 WTW GmbH.

Analyses of propanol-1, isobutanol, isoamyl alcohol and ethyl acetate were made on the gas chromatograph Carlo Erba, Vega Series 6130 fitted with flame ionization detector. For data treatment the integrator Carlo Erba SP 4290 was used. The compounds were analysed on wine distillate using DB WAX column of 30 m × 0.32 mm × 0.2 μm film thickness. Temperature programming was as follows: 6 min isothermal at 40 °C, then linear temperature rise of 15 °C/min to 190 °C. Injector and detector temperatures were 220 and 230 °C, respectively. The carrier gas was hydrogen. Pentanol–1 was used as the internal standard.

Analyses of 2-phenyl ethanol, hexanol, volatile esters and fatty acids were performed on Hewlett Packard model 5890 Gas chromatograph fitted with flame ionization detector. The wine (500 mL) to which heptanol–1 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 × 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 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 (30 mL/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 evaluation of wines

A panel of nine judges carried out sensory evaluation of wines by applying the ranking method as well as the Buxbaum method after the second racking. The statistical significance was determined according to Amerine and Roessler *(11)*.

Results and Discussion

Chemical composition of wine

Results given in Table 1 show that there were no essential differences among the various wines in respect to reducing sugar and volatile acidity. Compared to the wines of other treatments, the samples fermented by Lalvin-71 B strain had a significantly lower content of alcohol and total acidity. Curschmann *et al. (12)* observed that all commercial starter cultures that we also used in our investigation synthesise different concentrations of ethanol compared to epiphyte microorganisms. Our results confirmed this only for Lalvin-71 B strain.

Table 1. Chemical composition of wines

Compounds	Treatments					
	Spontaneous fermentation	Uvaferm CM	Uvaferm CS2	Lalvin 71 B	Lalvin 2056	LSD***
φ (Alcohol) / %	12.70	12.90	12.90	12.10	12.50	1 % =0.5
γ (Reduc. sugar) /g L^{-1}	3.0	3.0	2.0	2.0	2.0	n.s.
γ (Total acidity) / g L^{-1}*	8.60	8.30	8.40	7.00	8.90	1 % =0.31
γ (Volatile acidity) / g L^{-1}**	0.70	0.62	0.68	0.63	0.61	n.s.****
pH	3.01	3.03	3.02	3.08	3.02	n.s.****

* as tartaric acid ** as acetic acid ***Least Significant Difference **** not significant

According to Paronetto *(13)*, Lalvin-71 B strain can cause a decrease in malic acid of up to 30 %. Consequently, we presume that a decrease in total acidity found in Chardonnay wines fermented by the same strain was also linked to the malic acid degradation by maloethanolic fermentation.

Concentration of volatile compounds

Higher alcohols

The quality of white wines greatly depends on the concentration of higher alcohols. According to Rapp and Versini *(14)*, the concentrations of higher alcohols above 300 mg/L can cause an undesirable aroma in wine. Investigations carried out with Chardonnay wines in the continental region of Croatia have shown a concentration of propanol-1 (11–51 mg/L), isobutanol (22–68 mg/L) and isoamyl alcohol (69–202 mg/L) *(15)*, which is also confirmed by our results. In comparison with other treated wines, fermentation by Lalvin-71 B strain resulted in wines with a considerably lower concentration of higher alcohols. The concentrations of individual higher alcohols differed depending on the yeast strain responsible for fermentation (Table 2), which corresponds to the results obtained by Cabrera *et al. (1)*, Lema *et al. (9)* and Romano *et al. (8)*. In comparison with the spontaneously fermented wines, the wines fermented by both Uvaferm strains had a significantly higher content of isoamyl alcohol, whereas the significantly lower content of isoamyl alcohol was found in Lalvin-71 B treated wines. The concentration of isobutanol in the Lalvin-71 B treated wines was significantly lower than in the spontaneously fermented wines. The concentration of 2-phenyl ethanol in spontaneously fermented wines can be up to 280 mg/L *(16)*. Zeeman *et al.* cited by Rapp and Versini *(14)* reported that excessivelly high concentrations of this alcohol can adversely affect the wine quality. In our investigation the spontaneously fermented wines had a double content of 2-phenyl ethanol than wines obtained by inoculated fermentations. The lowest concentration of this alcohol was established in the wines fermented with Lalvin-2056 strain, whereas among the wines obtained by other commercial strains minor differences were found. Sponholz and Ditrich *(17)* also conclude that epihyte microorganisms syntesise larger concentration of 2-phenyl ethanol than starter cultures. On the contrary, Bertollini *et al.*, cited by Majdak *et al. (18)* found significant differences in concentrations of 2-phenyl ethanol in the fermentations with inoculated strains.

Volatile esters and fatty acids

Our results show that the wines of all treatments had concentrations of ethyl acetate below 50 mg/L. According to Ribereau-Gayon *et al. (19)* this ester has a significant influence on the quality of wine at very low concentrations (50–80 mg/L). Herraiz *et al. (20)* established that commercial *S. cerevisiae* strains produced less ethyl acetate than epiphyte microorganisms. Our inves-

Table 2. Concentration of volatile compounds in Chardonnay wines/(mg/L)

Compounds	Treatments						
	Spontaneous fermentation	Uvaferm-CM	Uvaferm-CS2	Lalvin-71 B	Lalvin-2056	LSD*	
Higher alcohols							
Propanol-1	8.8	7.1	7.3	16.3	9.2	1 % = 2.8	5 % = 2.0
Isobutanol	21.9	25.9	35.9	14.3	22.0	1 % = 8.2	5 % = 5.9
Isoamyl alcohol	229	278	275	166	232	1 % = 41.5	5 % =30.0
Hexanol	0.5	0.5	0.5	0.7	0.6		
2-Phenyl ethanol	108	54	55	50	38		
Σ Higher alcohols	399	365	374	264	301		
Esters							
Ethyl acetate	46.5	28.8	49.2	27.8	28.8	1 % = 13.9	5 % = 10.0
Isoamyl acetate	0.56	0.76	0.58	0.59	0.79		
Isobutyl acetate	n.d.	n.d.	n.d.	n.d.	n.d.		
Hexyl acetate	n.d.	0.05	0.05	0.05	0.03		
Isobutyl acetate	n.d.	n.d.	n.d.	n.d.	n.d.		
Phenyl ethyl acetate	0.42	0.09	0.26	0.16	0.06		
Σ Acetates	0.98	0.90	0.89	0.80	0.88		
Ethyl butyrate	0.15	0.09	0.08	0.23	0.10		
Ethyl caprate	0.12	0.07	0.08	1.15	0.70		
Ethyl caproate	0.27	0.31	0.43	0.47	0.33		
Ethyl caprylate	0.24	0.25	0.27	0.29	0.23		
Σ Ethyl esters of fatty acids	0.78	0.72	0.86	1.15	0.70		
Ethyl lactate	1.95	1.45	0.90	1.15	1.05		
Diethyl succinate	0.80	0.35	0.30	0.35	0.20		
Butyric acid	0.85	0.78	0.75	1.24	0.95		
Caproic acid	3.4	3.4	4.9	5.6	3.1		
Caprylic acid	4.4	3.4	4.7	4.6	3.1		
Capric acid	2.2	1.9	2.5	2.4	1.6		
Σ Fatty acids	10.85	9.48	12.85	13.84	8.75		

* Least Significant Difference

tigations did not confirm this only for commercial Uvaferm CS2 strain. Yeast strains can produce various concentrations of acetates of higher alcohols *(2,20,21)* and fatty acids *(20,22)*. Our results show that the concentrations of acetates of higher alcohols, as well as fatty acids and their ethyl esters were generally low in comparison with the data from literature. In spontaneously fermented wines a somewhat higher concentration of 2-phenylethyl acetate was found (Table 2). Our results regarding higher concentrations of 2-phenyl ethanol and phenyl ethyl acetate are in accordance with Majdak *(23)* and Nykanen *(24)*, who also established positive correlation between the concentration of 2-phenyl ethyl acetate and 2-phenyl ethanol. No essential difference was found in concentrations of other volatile compounds among wines under separate treatments, although the Lalvin-71 B treated wines had a somewhat higher content of butyric and caproic acids, ethyl butyrate, ethyl caproate and ethyl caprate. According to Cavazza and Grando *(22)*, concentrations of butyric, capric, caprylic and caproic acids greatly depend on yeast strain. Lambrechts and Pretorius *(25)* concluded that the fatty acids composition of yeasts is highly variable; relative proportions of the individual components are linked to growth substrate and growth conditions (pH, temperature, presence of nutrients). Due to their esterase activities, yeasts form various esters. Cavazza and Grando *(22)* established that a concentration of ethyl butyrate depends on yeasts strain. Although acetates of higher alcohols are very important, ethyl esters have more interesting aromas *(19)*. The same authors reported that yeast strain Lalvin-71 B produced large quantities of these compounds, which contributed to the fermentation aroma of young wines. Our results also confirmed that. As the synthesis of acetates of higher alcohols and fatty acid ethyl esters, as well as their final concentration in wine depend on numerous factors, such as the yeast strain *(9,20)*, cultivar *(12)*, must composition *(26)*, temperature and the mode of conducting fermentation *(14)*, an explanation of the obtained results is difficult to provide within the framework of this study.

Table 3. Results of Chardonnay wine sensory evaluation by the ranking method

Treatment	Order	Rank total
Lalvin-71 B	1	23
Spontaneous fermentation	2	26
Uvaferm-CS2	3	26
Uvaferm-CM	4	26
Lalvin-2056	5	34

Note: Any rank total outside the range 17–37 is significant at P< 5 %

Table 4. Results of the Chardonnay wine sensory evaluation by the Buxbaum method

Treatment	Spontaneous fermentation	Uvaferm CM	Uvaferm CS2	Lalvin-71 B	Lalvin-2056
Mediana	17.3	17.1	17.1	17.9	16.5

Sensory characteristics of wines

The strain of *S. cerevisiae* has a major influence on the sensory properties of wine *(7,26–30)*. According to Romano *et al. (8)*, wines fermented with starter cultures have better overall quality in comparison with the wines of spontaneous fermentation. Contrary to this, as concluded by Margalit *(31)*, spontaneous fermentation yields wines of a more complex aroma and better quality.

Although there was no significant difference in quality among the wines, the results of evaluation by the ranking and Buxbaum methods indicate that the fermentation by the Lalvin-71 B strain resulted in wines of better quality in comparison with other treated wines. (Tables 3 and 4). The Lalvin-71 B treated wines were characterised by a purer and stronger bouquet, probably due to a considerably lower total concentration of higher alcohols and a somewhat higher content of fatty acids ethyl esters. Furthermore, a better balance between alcohol and acidity resulted in a wine of more harmonious flavour. Wines obtained through fermentation by Lalvin-A-2056 strain were evaluated as the most inferior, whereas among the other wines there were no essential quality variations.

Conclusions

The applied *S. cerevisiae* strains had a varying influence on the composition and sensory properties of Chardonnay wine. In view of the large-scale foreign supply of commercial *S. cerevisiae* strains on the Croatian market, the starter culture should be selected exclusively on the basis of the results of a specific cultivar's mini-vinification within a specific viticultural area. The studies conducted thus far have shown that the best quality of Chardonnay wine originating from the Daruvar vineyards was obtained through fermentation by the commercial Lalvin-71 strain, whereas the most inferior by Lalvin-A-2056 strain. Spontaneous fermentation also resulted in good quality wines, which suggests that the selection of autochthonous yeast strains is entirely justified in Croatia.

Acknowledgements

The authors wish to thank Ms Pavica Tupajić (Department of Viticulture and Enology) for her GC analysis of volatile esters and fatty acids.

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Utjecaj komercijalnih sojeva *Saccharomyces cerevisiae* na kakvoću vina Chardonnay

Sažetak

Istraživane su promjene udjela aromatskih komponenti i senzornih svojstava vina Chardonnay, uvjetovane primjenom različitih komercijalnih sojeva *S. cerevisiae* i epifitnih mikrooganizama. Vina fermentirana sa sojem Lalvin-71 B imala su puno manju koncentraciju etilnog i izoamilnog alkohola, izobutanola, te ukupne kiselosti nego vina dobivena spontanom ili inokuliranom fermentacijom. Najveća koncentracija 2-fenil-etanola i 2-fenil-etil-acetata nađena je u vinima spontane fermentacije, dok u vinima podvrgnutim drukčijim postupcima nisu utvrđene bitne promjene udjela tih spojeva. U usporedbi s rezultatima dobivenim spontatnom fermentacijom, vina fermentirana sa sojem Lalvin-71 B imala su nešto višu koncentraciju maslačne i kapronske kiseline, te etil-butirata. Fermentacijom sa sojem Lalvin-71 B postignuta je najbolja kakvoća vina, a najslabija sa sojem Lalvin aromatic-2056.