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Exploring the potential of *Hanseniaspora vineae* for quality wines production

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Abstract. The species *Hanseniaspora vineae* has been attracting considerable interest in recent years. In this work. this yeast has been used in Chardonnay wine production. Two different times for sequential inoculation were tested: first inoculation with *Hanseniaspora vineae* and sequential inoculation of *Saccharomyces cerevisiae* when 4% alcohol was reached (CHv4), when 7% alcohol was reached (CHv7); finally. a test with only *S. cerevisiae*, named CSc, was used as control. The fermentations were conducted in duplicate in 5-litre flasks and their kinetics monitored by weight loss. Results showed that the alcoholic degree of the final wines was comparable in all samples. but the volatile and total acidity was slightly lower in all the wines fermented with *Hanseniaspora* whatever the inoculation scenario. The analysis with GC-FID showed that wines fermented by *H. vineae* revealed a higher quantity of volatile compounds. The sensory preference test was statistically significant only for the odour; in particular, the sample CHv7 was more preferred than CSc. and the third sample (CHv4) was intermediate. This study reveals that the use of *H. vineae* with a sequential inoculation of *S. cerevisiae* can be a powerful tool to improve and complexify wine aroma profile.

1. Introduction

The increasing use of non-Saccharomyces yeasts in wine production demonstrates their potential to improve the flavour profiles of wines. These yeasts are commonly used in combination with *Saccharomyces cerevisiae* to ensure thorough fermentation and achieve desired sensory characteristics.

One particular non-S*accharomyces* yeast, *Hanseniaspora vineae*, has been receiving considerable attention in the wine research community due to its unique traits and potential benefits. It has become a popular choice for wine producers looking to enhance the sensory qualities of their wines, leading to a growing number of scientific studies on this yeast in recent years [1,2,3]. This research investigates the impact of sequentially introducing *Hanseniaspora vineae* into Chardonnay must, Differences in the chemical composition and sensory profiles between wines fermented with *H. vineae* and the conventional production using *S. cerevisiae* were compared.

2. Material and method

2.1. Experimental design

Chardonnay must was purchased at Cantina di Nizza (Nizza Monferrato. Italy). Basic parameters on the initial must were analysed using OIV official methods.

Fermentations were carried out in 5 litres flasks filled with 4 L of Chardonnay must.

The experimental design was the following:

- Chardonnay inoculated with *Saccharomyces cerevisiae* LVCB (20g/hl) and Naturferm bright 30 g/hl. indicated as **CSc.**
- Chardonnay inoculated with *Hanseniaspora vinae* added with Tiamin 50 mg/l. and at 4 alcoholic degrees added with *S. cerevisiae* and Naturferm bright. indicated as **CHv4**.
- Chardonnay inoculated with *Hanseniaspora vinae* added with Tiamin 50 mg/l. and at 7 alcoholic degrees added with *S. cerevisiae* and Naturferm bright. indicated as **CHv7**.

Yeasts and additives were from Oenobrands (France). Fermentations were conducted at 18°C in duplicate and they were followed by weight loss.

After AF all samples were racked and aged on fine lees for one month. Battonage was done twice a week.

2.2. Chemical analyses

Residual sugars were quantified by HPLC. using an Agilent 1200 equipped with refractive index detector (RID).

Basic parameters the final wines were analysed using OIV official methods.

2.3. Volatile compounds quantification

Samples were analysed by GC-FID using an HP5890 instrument (Hewlett Packard) according to Ortega et al.

[4] with the following conditions: capillary column HPINNOWAX 30m x 0.25mm I.D. 0.25 µm (JW

scientific. Folsom. CA. USA). carrier gas flow rate 1 ml/min and splitless time 3 min. This method allows to quantify the most important wine fermentation compounds with a single chromatographic acquisition. with a liquid-liquid extraction with dichloromethane (250 µl) using a small wine sample (2ml).

2.4. Sensory analysis

All the wines were evaluated by the CREA-VE panel: 16 judges in the case of the 3 Chardonnay (CScA. CH4A and CH7A) The tests were carried out in an ISO (8589-2007) test room and with ISO (3591-1977) approved glasses . In all the sensory tests the wines. indicated by a 3-digit code. were evaluated by each judge in a different order, following a randomized scheme. The wines were evaluated with **ranking tests**. This test allows establishing an ascending order. as in this case. or a decreasing order among the wines as a function of the following parameters: colour. odour. taste and overall wine preference.

The ranking test allows comparing the samples for a specific characteristic; it is not a test giving a quantitative measure of the examined characteristic.

The results of each ranking test were processed by Friedman test and multiple comparisons (p = 95%) using XLStat ® software (Addinsoft, New York) and they are shown graphically in figures where the Y-axis corresponds to the sum of the ranks for each wine and for each parameter.

3. Results and discussion

3.1. Trend of fermentation

Table 1 reports the results of the basic parameters conducted on the must.

Table 1. Chardonnay must parameters.

	Sugar (Brix)	Potential Alcohol	Total acidity g/L	pH	APA mg/L	free SO2 mg/L	total SO2 mg/L
Raw Must	24.4	14.3	3.5	3.15	150	< 10	< 10

Fermentations were monitored daily. and their weight loss was annotated. Figure 1 shows trends of alcoholic fermentation in all samples.

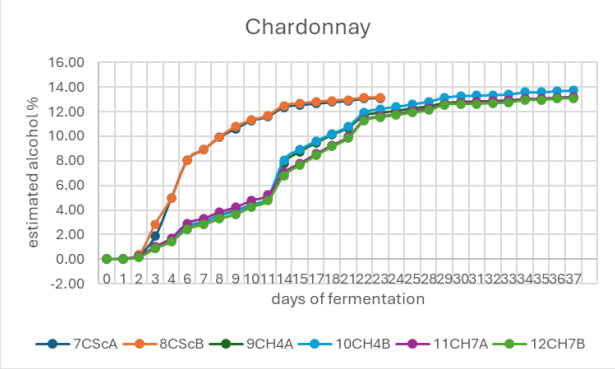

Figure 1. Ethanol % estimated by CO_2 loss during alcoholic fermentation.

The trends of alcoholic fermentation (AF) are very different. As expected, the sample with only *Saccharomyces cerevisiae* was faster than others; in fact, AF was completed in 23 days, while the other samples completed AF in 37 days. Hanseniaspora reached 4 % alcohol after 7 days and 7% after 9 days.

3.2. Chemical parameters of final wines

Table 3 shows chemical analyses made in the sample at the end of aging.

Table 3. Chemical analysis on samples made at the end of aging.

	%ALCOOL		tot ac g/L		vol ac g/l		pH	
CSc	13.39	±0.01	6.38	±0.0	0.62	±0.01	3.08	±0.01
CHv4	13.40	±0.01	5.35	±0.4	0.56	±0.04	3.11	±0.01
CHv7	13.51	±0.03	5.32	±0.4	0.52	±0.04	3.12	±0.01

Data showed that the wines fermented with *Hanseniaspora vineae* gave a lower volatile acidity, while the alcoholic degree was quite similar in all wines.

The main volatile compounds were quantified, and they are shown in table 4.

Table 4. Volatile compounds in Chardonnay samples (mg/L) (lower quantities are in red, higher quantities are in green).

	CSc	CHv4	CHv7
isobutanol	31,69	26,11	29,08
Isoamyl acetate	0,82	2,65	2,62
1-butanol	0,39	1,41	1,07
Isoamyl alcohol	142,76	145,36	124,83
Ethyl caproate	0,71	0,54	0,69
Hexyl acetate	0,17	0,15	0,28
3-OH 2-butanone (acetoin)	0,74	1,38	1,47
Ethyl lactate	3,32	15,38	20,98
Hexanol	1,78	1,92	1,66
cis-3-hexenol	0,01	0,02	0,04
Diacetyl (2,3 butandione)	1,22	1,29	1,32
Ethyl octanoate	0,47	1,90	2,36
Ethyl 3-OH butyrrate	0,17	0,27	0,28
Isobutyric acid	0,09	0,13	0,19
Butyric ac	0,76	0,66	1,13
γ- butyrolactone	0,09	0,04	0,09
Ethyl decanoate	0,75	0,37	0,47
Isovaleric acid	0,87	0,47	0,55
Diethyl succinate	0,17	0,18	0,11
Methionol	0,60	0,50	0,51
Ethyl 4-OH butanoate	4,76	9,77	9,81
Phenylethyl acetate	0,01	0,01	0,01
Hesanoic acid	2,10	1,27	1,35
Benzyl alcohol	0,11	0,06	0,17
2-Phenylethanol	20,86	19,38	16,56
Ethylguaiacol	0,16	0,14	0,11
Caprylic acid	2,92	1,79	1,95
Capric acid	0,05	0,03	0,06
Monoethyl succinate	0,34	0,69	0,65

Statistical significative differences were found for several compounds. In particular, in wines fermented with *Hanseniaspora vineae* isoamyl acetate, ethyl lactate, ethyl octanoate, ethyl octanoate, were significantly higher than in wines fermented with only *Saccharomyces cerevisiae*.

In general, it was observed that wines fermented by *S. cerevisiae* have a lower quantity of volatile compounds.

PCA analysed the matrix composed by the volatile compounds and the results are shown in figure 3.

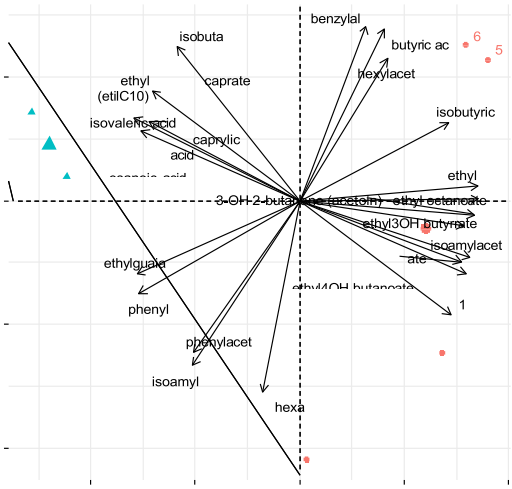

Figure 3. Biplot of PCA analysis. 1 and 2 = Chardonnay fermented by *Saccharomyces (*samples CSc*)*, 3 and 4 = Chardonnay fermented by *Hanseniaspora* at 4 % alcohol (samples CH4) and then *Saccharomyces*; 5 and 6=Chardonnay fermented by *Hanseniaspora* and then *Saccharomyces* at 7 % alcohol (samples (CH7).

PC1 and PC2 accounted for 78.3 % of the total variance. It is possible to observe that samples are separated in the variable space. In particular, the first dimension separates samples fermented by different yeast species. Wines obtained by *Saccharomyces* are characterized by caprylic acid, ethyl butyrate, ethyl octanoate, phenyl ethanol, diethyl succinate and ethyl lactate. Wines obtained by *H. vineae* are distinguished by mono ethyl succinate, isoamyl acetate, ethyl lactate, ethyl octanoate, isobutyric acid, and hexyl acetate.

3.3. .Sensory analysis

The preference test resulted statistically significant (Friedman test and Multiple comparisons Test, p=95%) only for the odour preference, but not for the colour, the taste and the overall preference (Fig.4). In particular, the sample CHv7 resulted more preferred than CSc, the third sample (CHv4) was intermediate

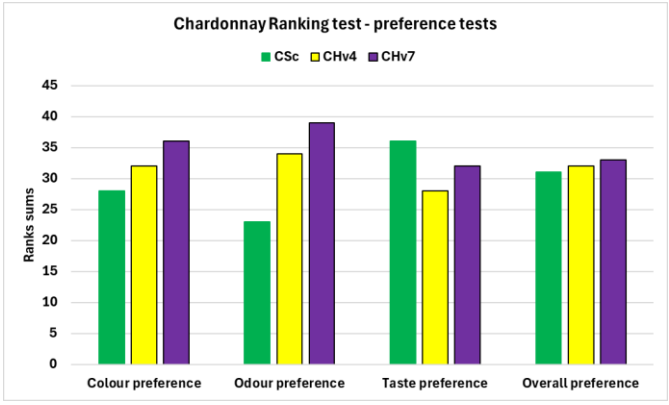

Figure 4. Results of the ranking test for preference. Different letters mean statistically significant differences (Friedman test and Multiple comparisons Test, p=95%).

4. Conclusions

The wines obtained in this project are quite different. In particular, the presence of *Hanseniaspora vineae* in the first stage of fermentation increases the volatile compounds in wines. This has also been highlighted by the panel during the sensory sessions. This study reveals that the use of *H. vineae* with a sequential inoculation of

S. cerevisiae can be a powerful tool to improve and complexify wine aroma profile.

5. References

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