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The Influence of Yeast Strains on the Composition and Sensory Quality of Gewürztraminer Wine

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Summary

The aim of this study is to evaluate the influence of yeast strains on the composition and sensory quality of Gewürztraminer wine. Three different commercial yeast strains were examined on the microvinification scale. In the wines, the chemical parameters and the concentration of free volatile monoterpene alcohols were measured and a descriptive sensory analysis was performed. Significantly more geraniol and nerol were detected in the fermentation conducted with reference *Saccharomyces cerevisiae* strain and more citronellol was found in the fermentation conducted with a hybrid of *S. cerevisiae* hybrid and *S. paradoxus*. However, more α-terpineol and linalool were found in the wine fermented with a combination of *Saccharomyces* and *Torulaspora delbrueckii* strains. The best wine flavour of tropical fruits was obtained using a hybrid of *S. cerevisiae* hybrid and *S. paradoxus*, and the best wine quality was achieved with a combination of *Saccharomyces* and *T. delbrueckii* strains. The selection of yeast strains for the fermentation of Gewürztraminer must significantly influenced the concentration of free volatile monoterpene alcohols and the sensory quality of the wine. With the selected hybrid of *S. cerevisiae* hybrid and *S. paradoxus* or the combination of *Saccharomyces* and *T. delbrueckii* strains either a better flavour or overall wine quality than with the reference strain can be achieved.

Key words: Gewürztraminer, monoterpene alcohols, *Saccharomyces* sp., *Torulaspora* sp., wine, yeast strains

Introduction

Wine varietal aroma is an important parameter of its sensory quality and mostly depends on the grapevine variety. The most aromatic compounds in grape must are some of the monoterpene alcohols (monoterpenols), such as linalool, α-terpineol, nerol, geraniol, citronellol and hotrienol, and they are regarded as key odorants of some varieties such as Boal, Gewürztraminer, Godello, Muscat, Malvasia, Riesling, Albarino, Ribolla Gialla and Sercial, to which they impart their characteristic floral, fruity and citrus aroma (*1–5*). Their olfactory perception thresholds are very low, 18–400 μg/L. The most aromatic are citronellol and linalool (*4*). Terpenes are mostly accumulated in grapes in the form of odourless precursors (glycosides) and the aroma is effectively released only after the precursor molecule is transformed (*6*).

Various factors can influence the concentration of free volatile monoterpene alcohols in wine, such as pesticide residues (*7*), pre-fermentative skin contact, fermentation temperature and the addition of functional exogenous β-glucosidases (*6,8,9*). Since certain genera of yeast, especially non-*Saccharomyces* ones, are able to release grape odourless precursors through their β-glucosidase activity, biotransform them or even synthesize new aroma molecules, it can be affirmed that they can enhance wine varietal aroma (*10–12*). Particularly interesting non-*Saccharomyces* yeast in this area is *Torulaspora delbrueckii* as it has a high β-glucosidase activity (*13*), high

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ability to biotransform monoterpene alcohols (*11*) and low production of acetic acid (*14–16*). Various *Saccharomyces* species and their hybrids might influence the amounts of monoterpene alcohols as well (*10,17*). It has been shown that a hybrid of *S. cerevisiae* and *S. paradoxus* can increase the production of certain aroma compounds and decrease the production of hydrogen sulphide (*18*).

Different methods have been used and described for determining free volatile monoterpene alcohols in wine. SPME GC-MS is often employed (*2,3,19*) and it was used in this study as well. The contribution of aromatic compounds to wine aroma is often evaluated by calculating their odour activity value (OAV), which is the ratio between the concentration of aromatic compound and its olfactory perception threshold. Aromatic compounds with OAV>1 are considered to have a contribution to the wine aroma (*2,5,9*), which can be evaluated with different sensory analyses as well. Sensory analysis involving ranking tests is one of the fast and easily feasible methods that do not require additional long-term trainings of the assessors. This fact, together with its simplicity, is a great advantage of the method (*20*).

The aim of the present work is to study the influence of a hybrid of *S. cerevisiae* hybrid and *S. paradoxus*, and a combination of *S. cerevisiae* and *T. delbrueckii* strains in comparison with reference *S. cerevisiae* strain on the chemical composition and sensory quality of Gewürztraminer wine.

Materials and Methods

Must handling

Grapes of Gewürztraminer (*Vitis vinifera* L.) vintage 2010 were destemmed and pressed with a pneumatic press immediately after crushing. The grape must was settled with SO_2 in the concentration of 50 mg/L and racked after 12 h. The must was divided into nine 5-litre carboys. The properties of the must were as follows: reducing sugars 205.5 g/L, pH=3.66, total acidity 5.40 g/L and volatile acidity 0.13 g/L.

Yeast strains and fermentation conditions

Four commercial wine yeast strains were supplied by the producers and used for three alcoholic fermentations (A–C). Fermentation A was carried out by *S. cerevisiae* Uvaferm 228 (Lallemand, Montreal, Canada), which was chosen as a reference strain with known β-glucosidase activity (*21*). Fermentation B was carried out by a hybrid of commercial strain VIN13 (*S. cerevisiae* hybrid) and a natural isolate of *S. paradoxus* strain RO88 (*22*). The strain was hybridized at Stellenbosch University in South Africa (*18*). Fermentation C was carried out by a starter culture consisting of *T. delbrueckii* strain 291 and *S. cerevisiae* strain 734 (*23*). Yeast strains used in fermentations A and B were inoculated once only, whereas the strains used in fermentation C were sequentially inoculated, first with *T. delbrueckii* and again after five days with *S. cerevisiae* strain. The dosage for each strain employed was 0.2 g/L. The fermentations were carried out in triplicate at a controlled room temperature (15 °C). They were monitored by weighing the carboys to estimate the amount of CO_2 released. After 19 days of alcoholic fermentation, the young wines were settled with SO_2 in the concentration of 50 mg/L and racked. The concentrations of reducing sugars and alcohol, pH, total and volatile acidity, and free volatile monoterpene alcohols were measured after one month of wine maturation.

Microbiological analysis

At the first sampling time, microbiological analyses were done only in fermentation C, where non-*Saccharomyces* yeast strains were used. This sampling was performed four days after the inoculation with *T. delbrueckii* strain. The second sampling was performed in all fermentations on the seventh day of alcoholic fermentation. Sampling was done aseptically. For each sample, a dilution series was prepared and cultured on a Wallerstein Laboratory (WL) Nutrient Agar (Merck KGaA, Darmstadt, Germany). After 3–5 days of cultivation at 26–28 °C, the concentration of the viable yeast cells was determined in the samples. The presence of *Torulaspora* and *Saccharomyces* yeasts was determined only in fermentation C, using morphological and microscopic inspection according to the plating of single yeast strain from a commercial yeast starter culture. The colonies of each yeast strain were morphologically very different when grown on WL agar, which enabled us to assume the dominance of a yeast strain with a great certainty.

Determining the chemical parameters

For determining the reducing sugars, total and volatile acidity and pH, the recommended EU methods were used (*24*). The concentration of alcohol in the wines was measured using the Wine Alcolyzer (Anton Paar GmbH, Graz, Austria).

Determining the free volatile monoterpene alcohols

Determining the free volatile monoterpene alcohols (α-terpineol, citronellol, geraniol, linalool and nerol) was performed using previously described methods (*3,19*), slightly modified by Bavčar *et al.* (*2*). The wine was diluted (1:4) with water (Milli-Q, Millipore, Billerica, MA, USA) to achieve a 1:3 ratio between the liquid and the headspace in a 20-mL SPME vial; 1.7 g of NaCl was added to adjust the ionic strength. The vial with the sample was heated at 40 °C for 60 min and monoterpene alcohols were absorbed on PDMS/DVB fibre (Supelco, Bellefonte, PA, USA). Monoterpene alcohols were identified and quantified with a gas chromatograph (Agilent 7890A; Agilent Technologies, Palo Alto, CA, USA) equipped with the MPS 2 automatic sampler (Gerstel, Mülheim an der Ruhr, Germany) and coupled with mass spectrometric detector (Agilent 5975C; Agilent Technologies). The incubation time in the automatic agitator was 5 min, the agitator speed was 250 rpm and the desorption time was 300 s. The chromatograph was equipped with a capillary column (INNOWax, 30 m×0.25 mm; film thickness 0.25 μm; Agilent Technologies), precolumn (FS deactivated 2 m×0.25 mm; Agilent Technologies) and liner. Helium gas (6.0) with a constant flow of 1.2 mL/min was used as a carrier. The injector temperature was set to 250 °C with the oven temperature gradient of 50 °C for 5 min, then

from 50 to 110 °C at 5 °C/min, from 110 to 150 °C at 2 °C/min, from 150 to 190 °C at 1 °C/min, from 190 to 250 °C at 20 °C/min and then at 250 °C for 15 min. The ion source temperature was 230 °C, the auxiliary temperature was 260 °C and the quadrupole temperature was 150 °C. One-point calibration was performed using a mixture of standards of all the analysed monoterpene alcohols. The calibration curve for this method was linear in the range of 0.1 to 50 μg/L; R^2 ranged from 0.9830 (nerol) to 0.9960 (linalool and α-terpineol). The limit of detection (LOD) was from 0.4 (linalool) to 3.0 μg/L (nerol), and the limit of quantification (LOQ) was from 1.5 (linalool and α-terpineol) to 10.1 μg/L (nerol). All the chemicals were of analytical grade and obtained from Sigma-Aldrich (St. Louis, MO, USA).

Sensory descriptive analysis

After three months of wine maturation at 12 °C, the sensory evaluation was performed in our sensory room by a group of experts (12 assessors). The wines were served at 12 °C. A simple ranking test was used for the following three attributes: tropical fruits and floral note flavours, and overall quality of the wine. This method is used when the objective of the test is to compare several samples according to a single attribute. Ranking is the simplest way to perform such comparisons and is less time-consuming than other methods. The subject receives the set of *t* samples in balanced random order, and the task is to rearrange them in rank order. The data obtained are merely ordinal, and no measure of the degree of difference is obtained from each respondent (*20*). Before ranking, standard solutions for aroma attributes were prepared in distilled water and presented to the assessors (in μg/L): citronellol 180, ethyl butyrate 200 and isoamyl acetate 750 for tropical flavour, and 2-phenylethyl acetate 2500 and linalool 300 for floral flavour. The panellists received the set of samples in randomized order. The panel ranked the wines obtained with the three fermentations (A, B and C) from the best (grade 3) to inferior (grade 1) separately for each attribute, using new samples coded differently. After that the rank sum was calculated for each attribute (*20*).

Statistical analysis

The analytical data were analysed for statistical significance using a one-way analysis of variance (ANOVA). The means were compared by a Fisher's least significant difference (LSD) procedure using a statistical software package (*25*). The results were considered significant if the associated p-values were below or equal 0.05. The results of the sensory evaluation were statistically evaluated using Friedman analysis (*20*).

Results

Fermentation kinetics

After five days of wine fermentation, when the yeasts were already in exponential growth phase, significant differences were found in the amount of CO_2 exhausted (Fig. 1). The kinetics was the fastest in fermentation B (3.6 g of CO_2 per 100 mL), followed by fermentation A (2.9 g of CO_2 per 100 mL). Statistically weakest kinetics was found in fermentation C (0.7 g of CO_2 per 100 mL). In all subsequent samplings, fermentation C had significantly weaker kinetics. However, at the end of the alcoholic fermentation, which lasted for 19 days, there were no significant differences in the exhausted CO_2 per 100 mL of must among all the tested strains and the amounts ranged between 10.1 (fermentation C) and 10.4 g (fermentation A).

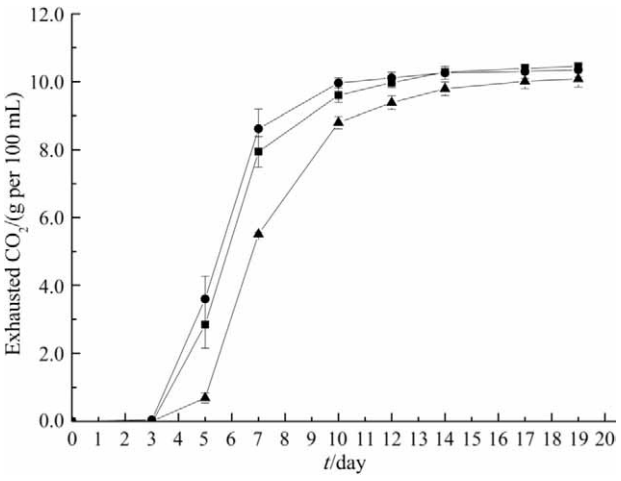

Fig. 1. The evolution of CO_2 during the three alcoholic fermentations of Gewürztraminer by selected yeast strains: A (-■-), B (-●-) and C (-▲-). The mean values of the triplicates and the standard errors are shown. Significant differences were determined at all sampling times from fifth day onward, except at the last sampling time

Monitoring of microbial population during alcoholic fermentation

Must fermented with *T. delbrueckii* strain (fermentation C) was sampled (fourth day of fermentation) before it was co-inoculated with the *S. cerevisiae* strain. The results of plate counting showed that the average number of colony forming units per mL (CFU/mL) was $1.2 \cdot 10^7$. Morphological and microscopic inspection, when compared to the strains of the commercial yeast starter cultures, showed that the dominant colonies belonged to *T. delbrueckii* strain. Two days after co-inoculation with the *S. cerevisiae* strain of the starter culture C (seventh day of fermentation), samples were taken from all fermentations. The results of plate counting showed a similar number of CFU per mL ($2.3 \cdot 10^7$) in the samples. Dominant colonies in co-inoculated fermentation C belonged to the *S. cerevisiae* strain.

Chemical parameters of wines

There were statistically significant differences in the concentrations of reducing sugars and alcohol, total and volatile acidities, and pH of wines after alcoholic fermentation (Table 1). The concentrations of reducing sugars in wines varied between 1.05 (fermentation B) and 2.75 g/L (fermentation C), and the volume fractions of alcohol between 12.40 (fermentation C) and 12.55 % (fermentation

Table 1. Chemical parameters and free volatile monoterpene alcohols in the wines produced using different yeast strains (fermentations A–C)

	Wines		
	A	B	C
Chemical parameters			
γ(reducing sugars)/(g/L)	(1.6±0.2)^{a}	(1.1±0.3)^{a}	(2.8±0.6)^{b}
φ(alcohol)/%	(12.55±0.01)^{b}	(12.52±0.03)^{b}	(12.40±0.07)^{a}
γ(total acidity as tartaric acid)/(g/L)	(6.62±0.02)^{b}	(6.3±0.2)^{a}	(6.3±0.2)^{a}
pH	(3.75±0.01)^{a}	(3.74±0.01)^{a}	(3.76±0.01)^{a}
γ(volatile acidity as acetic acid)/(g/L)	(0.46±0.05)^{a}	(0.6±0.1)^{ab}	(0.60±0.02)^{b}
Free volatile monoterpene alcohols/(μg/L)			
α-terpineol	(57±8)^{a}	(80±13)^{b}	(109±3)^{c}
citronellol†	(54±2)^{a}	(78±8)^{b}	(57±1)^{a}
geraniol†	(726±14)^{c}	(433±43)^{a}	(488±13)^{b}
linalool	(41±4)^{a}	(38±2)^{a}	(49±2)^{b}
nerol	(72±2)^{b}	(42±3)^{a}	(40±1)^{a}
total	(949±25)^{c}	(671±29)^{a}	(742±17)^{b}

The results are expressed as mean values±standard deviations (N=3). The different letters indicate significant differences at $p \leq 0.05$ obtained using the LSD test
†monoterpene alcohols detected above the olfactory perception thresholds

A). The wines had a total acidity ranging from 6.30 (fermentations B and C) to statistically higher value of 6.62 g/L for wine in fermentation A. The pH was comparable between the wines (3.74–3.76). Statistically higher volatile acidity was determined in the wine obtained in fermentation C (0.60 g/L) in comparison with the wine obtained in fermentation A (0.46 g/L).

Concentration of free volatile monoterpene alcohols

The results of the determination of the concentrations of free volatile monoterpene alcohols are shown in Table 1. Those for citronellol and geraniol were detected well above their thresholds (18 and 130 μg/L, respectively (4)). The significantly highest concentration of geraniol, which was the most abundant monoterpene alcohol, was detected in the wine in fermentation A (726 μg/L) and lower concentrations were found in the wine in fermentations C (488 μg/L) and B (433 μg/L). The highest concentration of citronellol was measured in the wine in fermentation B (78 μg/L) and significantly lower in the wine in fermentations C (57 μg/L) and A (54 μg/L). Linalool in wine samples was detected just below its threshold (50 μg/L; (4)). However, significantly higher concentration was determined in the wine in fermentation C (49 μg/L), followed by wine in fermentations A and B (41 and 38 μg/L). The concentrations of α-terpineol were lower than its threshold (400 μg/L; (4)) in all samples. However, significantly higher amounts were detected in wine in fermentation C (109 μg/L), followed by samples in fermentations B (80 μg/L) and A (57 μg/L). Although the concentrations of nerol in the wines were also lower than its threshold (400 μg/L; (4)), a rather significant difference exists between the samples with the highest (wine in fermentation A with 72 μg/L) and the lowest concentrations (wine in fermentation C with 40 μg/L). Significantly higher total concentration of free volatile monoterpene alcohols was detected in the wine in fermentation A (949 μg/L), followed by wine in fermentation C (742 μg/L) and wine in fermentation B (671 μg/L).

To determine the contribution of each free monoterpene alcohol to Gewürztraminer wine aroma, the odour activity values (OAV) were calculated, dividing the concentration of each free monoterpene alcohol by its olfactory perception threshold. Aroma compounds with OAV>1 are considered to have an important impact on the wine aroma, which was in our study shown for citronellol and geraniol (Table 2). The highest impact on wine aroma was found by geraniol, with significantly highest OAV in the wine in fermentation A (5.58), followed by the wine in fermentation C (3.75), and significantly lowest in the wine in fermentation B (3.33). The OAV of citronellol was significantly higher in the wine in fermentation B (4.31) compared to the wine in fermentations A (2.98) and C (3.17). Other three monoterpene alcohols were considered less important for wine aroma since their OAVs were less than one. The OAV of linalool was just below one, but was significantly higher in the wine in fermentation C (0.97) in comparison with the wines in fermentations A (0.82) and B (0.77). A much lower OAVs were determined for nerol (0.10–0.18) and α-terpineol (0.14–0.27), but with significant differences among wines as well. When we sum up the OAVs of compounds contributing to floral aroma (α-terpineol, geraniol, linalool and nerol), the wines are classified in the same order as for geraniol: fermentation A (6.72)>fermentation C (5.09) >fermentation B (4.40).

Sensory quality of the wines

The results of the sensory evaluation are reported in Table 3. Statistical differences among the wines were

Table 2. Olfactory perception thresholds of free volatile monoterpene alcohols and their odour activity values (OAVs) in the wines produced using different yeast strains (fermentations A–C)

Compound	Olfactory perception threshold/(μg/L)	Wine		
		A	B	C
α-terpineol	400	(0.14±0.02)[a]	(0.20±0.03)[b]	(0.27±0.01)[c]
citronellol	18	(2.98±0.10)[a]	(4.31±0.43)[b]	(3.17±0.04)[a]
geraniol	130	(5.58±0.10)[c]	(3.33±0.33)[a]	(3.75±0.10)[b]
linalool	50	(0.82±0.08)[a]	(0.77±0.03)[a]	(0.97±0.03)[b]
nerol	400	(0.18±0.00)[b]	(0.10±0.01)[a]	(0.10±0.00)[a]

The results are expressed as mean value±standard deviation (*N=3*). The different letters indicate significant differences at $p \leq 0.05$ obtained using the LSD test

Table 3. Rank sums for the three sensory attributes of wines produced by selected yeast strains (fermentations A–C)

Sensory attribute	Wines (*N*=12)			Significant differences among the wines*
	A	B	C	
	Rank sums			
flavour – tropical fruits	24	30	18	B and C
flavour – floral notes	24	24	24	n.s.
overall wine quality	20	21	31	A and C, B and C

*The critical value (LSD_{rank}) of the multiple comparisons was 9.60. Any two samples whose sums differ by 10 points or more were significantly different at the 5 % level; n.s.=not significant

found for the sensory attributes of the flavour of tropical fruits and overall wine quality. For the sensory attribute of the flavour of tropical fruits, the highest ranked wine was wine in fermentation B. The wine in fermentation C was ranked significantly lower. There were no significant differences among the samples for the sensory attribute of the flavour of floral notes. Wine in fermentation C was ranked significantly higher than in fermentations A and B for overall wine quality.

Discussion

It is well known that the population of *Saccharomyces* and especially non-*Saccharomyces* yeast strains influences the kinetics of wine fermentation (*14,26*). In our study, the *T. delbrueckii* strain employed in fermentation C confirmed the already observed behaviour, since it showed a weaker fermentation rate than fermentations A and B inoculated with reference *S. cerevisiae* strain, or hybrid of *S. cerevisiae* hybrid and *S. paradoxus*. However, after inoculation of starter culture C with *Saccharomyces* yeasts, the fermentation kinetics enhanced and the final amount of exhausted CO_2 was comparable among the fermentations. Additionally, statistically higher volatile acidity was determined in the wine in fermentation C, which is not completely in agreement with the fact that *T. delbrueckii* is often described as a low acetic acid producer under standard conditions (*15*). However, the same authors also showed that inoculating *Saccharomyces cerevisiae* after five days' fermentation with *T. delbrueckii* had lower effect on volatile acidity in comparison with co-inoculation.

Nevertheless, our study focused on the influence of selected yeast strains on the concentration of free volatile monoterpene alcohols in relation to the sensory quality of the wine. Monoterpene alcohols are one of the principal components, contributing to the floral, fruity and citrus aroma of many aromatic wine cultivars (*1–3, 5*), such as Gewürztraminer (*8*), used in our study. We showed that the concentrations of free volatile monoterpene alcohols are significantly dependent on the yeast strain used in wine fermentation, since they may have a different β-glucosidase activity and a different ability to biotransform monoterpene alcohols or even synthesize them (*10–12*). The most abundant monoterpene alcohol in the produced wines was geraniol, which, like linalool, contributes to flowery, particularly rose-like aromas. On the other hand, citronellol contributes to the aroma of tropical fruits. The concentrations of α-terpineol (lily of the valley-like aroma) and nerol (rose-like aromas) in the produced wines were well below their olfactory perception thresholds. The *Saccharomyces* strain and the hybrid of *S. cerevisiae* hybrid and *S. paradoxus* in fermentations A and B produced either more geraniol (fermentation A) or citronellol (fermentation B) than the co-inoculated yeast strains in fermentation C, when considering only monoterpene alcohols with exceeded odour thresholds. Mixed yeast starter culture of *T. delbrueckii* and *S. cerevisiae* in fermentation C, on the other hand, produced the highest amounts of α-terpineol and linalool, the latter just below the OAV. Total concentration of free volatile monoterpene alcohols produced by *T. delbrueckii* and *S. cerevisiae* combination or by the hybrid of *S. cerevisiae* hybrid and *S. paradoxus* did not reach the concentration produced by the reference strain.

If compared to the reference *S. cerevisiae* strain, there were no significant differences among the samples regarding the sensory attribute of the flavour of floral notes, although significant differences in the concentrations of α-terpineol, geraniol, linalool and nerol were found. However, for the sensory attribute of the flavour of tropical fruits, the highest ranked wine was wine in fermentation B, which is consistent with the highest amount of citronellol and consequently the highest OAVs for tropical fruits aroma in this sample. The fruity characteristics and intense aroma of wines, produced by the hybrid of *S. cerevisiae* hybrid and *S. paradoxus* and its parental strain *S. paradoxus* RO88, were also confirmed by other authors (*18*,*27*), as well as the fast fermentation ability and moderate aroma production potential of the parental strain *S. cerevisiae* hybrid (VIN13) (*12*,*18*). On the other hand, regardless of lower total concentration of free volatile monoterpene alcohols than in the wine produced with the reference strain, wine in fermentation C was rated as the best in overall wine quality. This could possibly be attributed to the more balanced concentrations of monoterpene alcohols than in the other two wines, and slightly higher concentration of reducing sugars as well. The good quality of the wine resulting from the use of *T. delbrueckii* in combination with *Saccharomyces* strain in wine fermentation was achieved by other authors as well (*15*,*28*).

Conclusion

In conclusion, the selection of yeast strains for the alcoholic fermentation of Gewürztraminer significantly influenced the concentration of free volatile monoterpene alcohols and the sensory quality of wine. We also confirmed that with the selected hybrid of *S. cerevisiae* hybrid and *S. paradoxus*, or with a combination of *Saccharomyces* and *T. delbrueckii* strains, either a better flavour or overall wine quality than with the reference strain can be achieved. It was also confirmed that the sensory attributes of wine are not necessarily correlated, as we showed for flavour of tropical fruits and overall wine quality of the wines in fermentations B and C. Finally, the interactions between aroma precursors in grape must and the prevailing yeast strain in the alcoholic fermentations play a crucial role in modulating the aroma and overall quality of the wine.

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