

**SYMPOSIUM LALLEMAND**

**"SENSORY CONTRIBUTION  
OF YEASTS TO WINE"**

**WEISSENKIRCHEN/WACHAU, AUSTRIA**

**MAY, 21 - 24 1992**

# USE OF *S. CEREVISIAE* STRAINS PRODUCING DIFFERENT VOLATILE PHENOLS CONTENT IN GEWÜRZTRAMINER WINE

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## INTRODUCTION

Research dedicated to the aroma of Gewürztraminer wine has identified in 4-vinyl guaiacol an important compound that gives rise to its characteristic spicy-like flavour. This volatile phenol is perceived, in fact, as a cloves-like sensation, at the olfactory threshold of 200  $\mu\text{g/L}$ . Together with the rose-like note of monoterpenols, in particular geraniol, it contributes to the typical cultivar character and quality of Gewürztraminer.

It is also been observed that the concentration of volatile phenols is higher in Traminer wines produced from grapes slightly over-ripened, as found for 4-vinyl phenol in Chardonnay wine (Versini *et al.*, 1989). The same effect has been obtained from juices subjected to skin-contact. The fermentative origin of 4-vinyl guaiacol has been confirmed from following its formation-kinetics in wine (Versini, 1985; Aurich *et al.*, 1987; Marais and Rapp, 1988).

In fact, the ability of *Saccharomyces cerevisiae* strains to decarboxylate 4-hydroxycinnamic acid (*p*-cumaric) and 3-methoxy-4-hydroxycinnamic acid (ferulic) is known and the presence in the wine of 4-vinyl phenol and 4-vinyl guaiacol is attributed to the yeast metabolism (Albagnac, 1975).

It has not been established, however, if yeasts can affect the transformation of the likely precursors such as hydroxycinnamic acid-tartaric acid esters or the liberation of glycosidically-bound volatile phenols (Gunata *et al.*, 1990)

A molecular genetic analysis conducted on *S. cerevisiae* brewery strains has cloned and identified a nuclear gene called *POF 1* (phenolic off-flavour) that confers to the yeast the ability to carry out this decarboxylation reaction (Meaden and Taylor, 1991).

While in the brewing industry the use of *Pof<sup>-</sup> Saccharomyces cerevisiae* is preferred, except in the case of wheat beer (Schieberle, 1991), data concerning the distribution of *Pof* phenotype among wild *S. cerevisiae* strains in grape musts and strains selected for their wine-making properties, are scarce (Thornton and Bunker, 1989; Dubourdieu *et al.*, 1989).

In order to evaluate the specific contribution and potential of wine yeasts for wine organoleptic characteristics, which are associated with the concentration of volatile phenols, Traminer wines obtained from juices fermented with *S. cerevisiae* strains of phenotype *Pof<sup>+</sup>* and *Pof<sup>-</sup>*, were compared.

## MATERIALS AND METHODS

### Yeast strains and determination of Pof phenotype

The capacity to decarboxylate the phenolic acids was determined for 115 *Saccharomyces cerevisiae* strains obtained from the Yeasts Collection of the Istituto Agrario - San Michele all'Adige. The majority of these strains were isolated from Trentinian grape musts. Among these, 86 were selected for their acceptable or good fermentative abilities (Cavazza and Romano, 1987), the other 26 strains were not characterized (wild strains).

The Pof phenotype was also determined for 24 strain cultures isolated from ADY commercial preparations for oenological purposes.

Each strain was inoculated in culture medium containing 0.5 mM of *p*-cumaric acid or ferulic acid. The production of volatile phenols in the media was determined, qualitatively, after a period of 48 hours at an incubation temperature of 30 °C, by olfactory analysis (ferulic-medium) and by spectrophotometric analysis (*p*-cumaric-medium) (Albagnac, 1975).

Gas chromatographic analysis, as described below for the wines, was used to establish the presence of volatile phenols in culture media fermented from four Pof<sup>-</sup> strains and from one Pof<sup>+</sup> strain (two of these strains were subsequently used in wine-making trials) and in the same culture medium non-inoculated.

### Fermentation trials

All samples of Gewürztraminer grapes (1991 vintage) from the Trentino region, were harvested at the same ripening stage.

On a laboratory-scale (2 L) fermentations of Traminer juice (I) were prepared to compare the hydroxycinnamic acid decarboxylase ability (HCD) of the *S. cerevisiae* strains: VL1 (Intec), CH101 SMA and R1 SMA.

Four strains: Fermivin CRYO (Gist-brocades), CH101 SMA, RM1515 SMA and R1 SMA were used for experimental cellar-scale (30 L) fermentations of a Traminer juice (II).

In cellar conditions CH101 SMA and RM1515 SMA strains were used for fermentations of another Traminer juice (III).

In the latter two cases the grapes were subjected to skin-contact for 12 hours at 18-20 °C. The grape musts were inoculated after having been cooled and allowed to settle and also 50 mg/L SO<sub>2</sub> had been added. For the experimental cellar-scale fermentations, fresh culture of each strain in sterile grape must was added at 1.5% v/v, except in the case of Fermivin CRYO strain which, as for ADY, had been previously rehydrated.

In the case of the cellar trials, fermentations were run in a routine manner: *pie de cuve* was added, which was maintained by the multiplication of each strain in clarified must.

### Microbiological control

During all the fermentation trials, the yeast species present were closely monitored. In particular, electrophoretic karyotyping analysis was performed to distinguish *S. cerevisiae* strains and to determine the population composition, according to a described procedure (Grando and Cavazza, 1992), thus evaluating the effectiveness of inoculation and the stability of the Pof phenotype.

### Gas-chromatographic analysis

Presence of 4-vinyl guaiacol and 4-vinyl phenol in wines was assessed by gas chromatographic analysis, following the published methods (Versini *et al.*, 1988), but altering the pH of the wine to achieve 7 before adsorbment on Amberlite XAD-2. This procedure was employed in order to avoid fixing of phenolic acids and therefore excluding the possibility of neo-formation of volatile phenols during the actual analysis.

### Sensory evaluation

The four wines derived from Traminer juice (II) and the two cellar products were sensorially evaluated on the basis of the spicy-like/phenolic olfactory descriptor, on a non structured scale. The standard descriptor was the synthetic medium supplemented with 0.5 mM ferulic acid, after fermentation with RM1515 SMA strain.

The data from these trials were subjected to analysis of variance (ANOVA) statistical testing.

## RESULTS AND DISCUSSION

Results obtained from the olfactory and spectrophotometric analysis, with regard to the ability of *S. cerevisiae* strains to decarboxylate respectively ferulic acid and *p*-cumaric acid, showed a high degree of concordance.

Therefore the Pof<sup>+</sup> phenotype was attributed to 89 % of *S. cerevisiae* strains isolated from Trentinian grape musts. An important frequency difference was not observed between the group of selected strains (90 % Pof<sup>+</sup>) and the group of wild strains (86 % Pof<sup>+</sup>). The isolated cultures obtained from commercial ADY preparations also showed a high proportion of Pof<sup>+</sup> strains but with a lower frequency (75 % Pof<sup>+</sup>) (Tab. 1).

The gas chromatographic analysis has allowed the confirmation of the observed phenotype. In the synthetic media supplemented with ferulic acid, after fermentation with the four Pof<sup>-</sup> strains, the concentration of 4-vinyl guaiacol was found to be weak when compared with 48 mg/L produced from R1 SMA (Pof<sup>+</sup>) strain. Moreover, no 4-vinyl guaiacol was detected in the non-inoculated sample, after the same time period.

High differences in the volatile phenol concentrations were also found in the laboratory- scale wines (Tab. 2).

The results from gas chromatographic (Tab. 2) and sensory analysis (Tab. 3) indicate that wines obtained from the four experimental cellar-scale fermentations can largely be differentiated on the basis of 4-vinyl guaiacol and 4-vinyl phenol levels.

The behaviour of yeast strains, with regard to their capacity to produce volatile phenols, was in fact as anticipated, according to the phenotype assigned. Even if in Traminer wine obtained from using a wild strain (R1 SMA) highest concentrations of phenols were observed, from sensorial analysis the spicy-like note was perceived to be significantly more intense in the wine produced from the selected strain RM1515 SMA.

The effective dominance of each strain inoculated has been confirmed by microbiological control. This is demonstrated by the same profiles obtained from electrophoretic karyotyping analysis of *S. cerevisiae* yeast cells having been isolated after 2 and 4 days from starting of fermentation.

The cellar fermentations, instead, yielded two Traminer wines with the same 4-vinyl guaiacol and 4-vinyl phenol concentrations (Tab.2) and, by using sensory evaluation, were found to be not significantly different from each other.

The lowest concentrations of volatile phenols detected in these cases could be attributed to the must composition. However, some *S. cerevisiae* strains, different from those inoculated, were isolated from all the samples taken for microbiological control. The flora present during fermentations was shown, in fact, to be very heterogenous and to have been enriched by the skin-contact process. Before the addition of *pied de cuve*, the must already contained more than  $2 \times 10^6$  *S. cerevisiae* cells/ml and about  $8 \times 10^6$  apiculated yeast cells/ml. The latter type continued to be observed until five days after the start of fermentation at a level of approximately  $10^7$  cells/ml.

The identification of *S. cerevisiae* strains by electrophoretic karyotyping (Fig. 1), associated with the fermentation test for control of HCD activity, has demonstrated that the inoculated strains, in both cases, were present with a frequency always of less than 30 % and the expression of the Pof character was maintained stable. In both fermentations, the foreign *S. cerevisiae* strains observed from before starting of the process showed both Pof<sup>+</sup> and Pof<sup>-</sup> phenotypes.

## CONCLUSIONS

As the majority of wine *S. cerevisiae* strains seem to possess the ability to decarboxylate ferulic and *p*-cumaric acids, it is thus important to assess the contribution of volatile phenols to the aroma of different wines. Our trials have allowed the verification of the character expressed by strains Pof<sup>+</sup> and Pof<sup>-</sup> in fermentation of Traminer grape musts.

The influence of the yeast strain Pof phenotype has been confirmed from the analytical results of 4-vinyl guaiacol and 4-vinyl phenol and from organoleptic evaluation. This indicates the real possibility to obtain products very different with respect to a factor that can affect the quality and typicality of wine.

The microbiological aspect has explained the lack of differences observed in the volatile phenols concentration of Traminer wine, obtained from the trials performed under normal cellar working conditions. It was not possible to differentiate the populations of *S. cerevisiae* actually present in the above described fermentations, with respect to the parameter Pof phenotype. This arose from the contemporary presence of inoculated strains and a high population of indigenous flora of the same species.

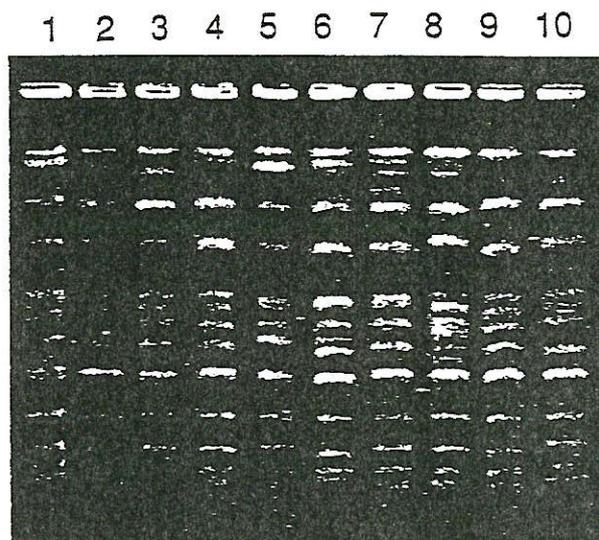
Strains Pof<sup>+</sup> for fermentation can therefore be used to exploit the aromatic potential of Traminer cultivar.

Given the diffusion of Pof<sup>+</sup> phenotype in wine yeasts, from our data one can deduct that, if the contribution of the volatile phenols is detrimental for a wine, the use of Pof<sup>-</sup> strains would have an effect only in the conditions that consent the yeast to dominate in fermentation.

## REFERENCES

- ALBAGNAC G., 1975 - *La décarboxylation des acides cinnamique substitués par les levures*. Ann. Technol. agric., 24, 133-141.
- AURICH M., VERSINI G. and DALLA SERRA A., 1987 - *Influenza dell'epoca di vendemmia e della macerazione sulle caratteristiche di tipicità dei vini Traminer Aromatico dell'Alto Adige*. In: Primo Simp. Int. Le sostanze aromatiche dell'uva e del vino. San Michele all'Adige, pp. 223-232, Manfrini Ed., Rovereto.
- CAVAZZA A. and ROMANO F., 1987 - *Valutazione tecnologica di ceppi di Saccharomyces cerevisiae isolati in diversi ambienti: influenza del fattore "velocità di fermentazione"*. Mic. Ital., 3, 199-210.
- DUBOURDIEU D., DARRIET P., CHATONNET P. and BOIDRON J.N., 1989 - *Intervention de systemes enzymatiques de Saccharomyces cerevisiae sur certain précurseurs d'aromes du raisin*. In: Actualité Oenologiques '89, pp.151- 159, Dunod Ed., Paris.
- GRANDO M.S. and CAVAZZA A., 1992 - *Il controllo della purezza fermentativa: riconoscimento dei ceppi della specie Saccharomyces cerevisiae*. Biologia Oggi, 1-2, 191-197.
- GUNATA Z., BITTEUR S., BAUMES R., SAPIJ J.C. and BAYONOVE C., 1990 - *Activité glycosidases en vinification. Perspectives d'exploitation des précurseurs d'arome du raisin, de nature glycosidique*. Rev. Fr. Oenol., 122, 37-41.
- MARAIS J. and RAPP A., 1988 - *Effect of skin-contact time and temperature on juice and wine composition and wine quality*. S. Afr. J. Enol. Vitic., 9, 22-30.
- MEADEN P.G. and TAYLOR N.R., 1991 - *Cloning of a yeast gene which causes phenolic off-flavours in beer*. J. Inst. Brew., 97, 353-357.
- SCHIEBERLE P., 1991 - *Primary odorants of pale lager beer*. Z. Lebensm. Unters. Forsch., 193, 558-565.
- THORNTON R.J. and BUNKER A., 1989 - *Characterization of wine yeasts for genetically modifiable properties*. J. Inst. Brew., 95, 181-184.
- VERSINI G., 1985 - *Sull'aroma del vino Traminer Aromatico o Gewürztraminer*. Vignevini, 1-2, 57-65.
- VERSINI G., DALLA SERRA A., DELL'EVA M., SCIENZA A. and RAPP A., 1988 - *Evidence of some glycosidically bound new monoterpenes and norisoprenoids in grapes*. In: Bioflavour '87, pp. 161-170, Schreier Ed., Berlin, New York.
- VERSINI G., SCIENZA A., DALLA SERRA A., DELL'EVA M. and MARTIN C., 1989 - *Rôle du clone et de l'époque de récolte sur l'arôme du Chardonnay: aspects analytiques et sensoriels*. In: Actualité Oenologiques '89, pp. 69-74, Dunod. Ed., Paris.

Fig. 1 Electrophoretic karyotypes of *Saccharomyces cerevisiae* strains.



Lane 7: RM1515 SMA, inoculated strain;  
 Lanes 1, 2, 3, 4=9, 5, 6, 8 : indigenous strains;  
 Lane 10: S 288 C, reference strain.

Tab. 1: Pof phenotype distribution among *Saccharomyces cerevisiae* wine strains tested.

n. YEAST STRAINS	SOURCE	Pof <sup>+</sup> strains		Pof <sup>-</sup> strains	
		n.	*	n.	*
86	SMA selected strains	77	RM 1515	9	CH 101
29	SMA wild strains	25	R 1	4	-
24	Commercial strains	18	-	6	VL 1 CRYO

SMA = Yeasts Collection Istituto Agrario - San Michele all'Adige  
 (\*) Yeast strains used for winemaking trials.

Tab. 2: Levels of 4-vinylguaiacol and 4-vinylphenol (mg/L) after fermentation with Pof<sup>+</sup> and Pof<sup>-</sup> wine strains.

YEAST STRAIN	POF	FERULIC ACID 0.5 mM medium	TRAMINER wines 1		TRAMINER wines 2		TRAMINER wines 3	
			4VG	4VP	4VG	4VP	4VG	4VP
BA 027	-	0.251						
TR 203	-	0.318						
PG 717	-	0.199						
CH 101	-	0.264	0.024	0.013	0.195	0.143	0.318	0.154
R1	+	47.967	0.424	0.797	1.210	0.474		
RM 1515	+				0.976	0.431	0.336	0.148
VL 1	-		0.024	0.012				
CRYO	-				0.154	0.146		

- 1 = laboratory-scale trials, juice I;  
 2 = experimental cellar-scale trials, juice II;  
 3 = cellar-scale trials, juice III.

Tab. 3: Analysis of Variance, Duncan's Multiple Range Test for variable "Spicy like/phenolic" (Traminer wines from juice II).

DESCRIPTOR	SOURCE	DF	MS	F	Pr>F	YEAST STRAIN Duncan MEAN			
						RM 1515	R 1	CRYO	CH 101
SPICY LIKE/ PHENOLIC	strain	3	6711	28.20	0.0001				
	panelist	7	256	1.07	0.4133	a	b	c	c
	error	21	238			75	57	18	18
	corr.tot	31							