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Selective use of wine yeast strains having different volatile phenols production

by

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Summary: Among *Saccharomyces cerevisiae* wine yeasts, we found a high frequency of strains having the ability to decarboxylate 4-hydroxycinnamic acid and 3-methoxy-4-hydroxycinnamic acid. From Gewürztraminer juices fermented by *S. cerevisiae* wine strains with and without such character, we obtained wines with considerably different levels of volatile phenols and some interesting evidences of the likely precursors of 4-vinylguaiacol and 4-vinylphenol. The identification of yeast strains by electrophoretic karyotyping gave us the possibility of evaluating the effective contribution of the yeast in the organoleptic characteristic of Traminer wines associated with the concentration of such volatile phenols.

Key words: wine yeast, volatile phenols, Gewürztraminer.

Introduction

Research dedicated to the aroma of Gewürztraminer wine has identified 4-vinylguaiacol (4VG) an important compound that gives rise to its characteristic spicy-like flavour (VERSINI 1985). This volatile phenol is perceived, in fact, as a cloves-like sensation, at the olfactory threshold of about 200 µg/l. Together with the rose-like note of monoterpenols, in particular geraniol and with a not yet attributed honey-lime note, it seems to contribute to the typical varietal character and quality of Gewürztraminer wines produced in Trentino-South Tyrol (Italy). It has also been observed that the concentration of volatile phenols is higher in Traminer wines produced from grapes slightly over-ripened (AURICH *et al.* 1987; MARAIS and RAPP 1988), as found for 4-vinylphenol (4VP) in Chardonnay wine (VERSINI *et al.* 1989). The same effect has been obtained from juices subjected to skin-contact.

The fermentative origin of 4VG has been confirmed from following its formation-kinetics in wine. In fact, the ability of *S. cerevisiae* strains to decarboxylate 4-hydroxycinnamic acid (*p*-coumaric) and 3-methoxy-4-hydroxycinnamic acid (ferulic) is known and the presence in the wine of 4VP and 4VG 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 (WINTERHALTER *et al.* 1990, 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⁻ *S. 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).

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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$^+$ and Pof$^-$, 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 properties (CAVAZZA and ROMANO 1987), the other 29 strains were not characterized (wild strains). The Pof phenotype was also determined for 24 strain cultures isolated from ADY commercial preparations for enological purposes. Each strain was inoculated in culture medium containing 0.5 mM of *p*-coumaric acid or ferulic acid. The production of volatile phenols in the media was determined, qualitatively, after a period of 48 h at an incubation temperature of 30 °C, by olfactory analysis (ferulic medium) and by spectrophotometric analysis (*p*-coumaric 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 4 Pof$^-$ strains and from one Pof$^+$ strain (2 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: Zymaflore 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 typical cellar conditions, CH101 SMA and RM1515 SMA strains were used for fermentation of another Traminer juice (III). In the latter two cases the grapes were subjected to skin-contact for 12 h at 18—20 °C. The grape musts were inoculated after having been cooled and allowed to settle and also 50 mg/l SO_2 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: *pied 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 4VG and 4VP 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 the coelution of phenolic acids in the organic fraction and therefore excluding the possibility of neoformation of volatile phenols during the actual analysis.

HPLC analysis: The hydroxycinnamic acid-tartaric acid esters and hydroxycinnamic acids contents in wine were assessed by HPLC, non-diluted samples being injected directly under the above described instrument conditions (NICOLINI *et al.* 1990). The calibration curve used in the assessment of free acids was derived from pure acids, whereas tartaric acid esters — for which no commercial standard is available — were measured as caffeic acid at 320 nm (caffeic acid equivalent, mg/l).

Sensory evaluation: The 4 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 *Saccharomyces cerevisiae* strains to decarboxylate respectively ferulic acid and *p*-coumaric acid, showed a high degree of concordance. Therefore the Pof$^+$ 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$^+$) and the group of wild strains (86 % Pof$^+$). The isolated cultures obtained from commercial ADY preparations also showed a high proportion of Pof$^+$ strains but with a lower frequency (75 % Pof$^+$) (Tab. 1).

Table 1

Pof phenotype distribution among *Saccharomyces cerevisiae* wine strains tested.

n. YEAST STRAINS	SOURCE	Pof$^+$ strains n.	*	Pof$^-$ strains 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.

The gas chromatographic analysis has allowed the confirmation of the observed phenotype. In the synthetic media supplemented with ferulic acid, after fermentation with the 4 Pof$^-$ strains, the concentration of 4VG was found to be weak when compared with 48 mg/l produced from R1 SMA (Pof$^+$) strain. Moreover, no 4VG 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 4 experimental cellar-scale fermentations can largely be differentiated on the basis of 4VG and 4VP levels. The behaviour of yeast

Table 2

Levels of 4-vinylguaiacol and 4-vinylphenol (mg/l) after fermentation with Pof+ and Pof- 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.

Table 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			
SPICY LIKE/ PHENOLIC	strain	3	6711	28.20	0.0001	RM 1515	R 1	CRYO	CH 101
	panelist	7	256	1.07	0.4133	a	b	c	c
	error	21	238			75	57	18	18
	corr.tot	31							

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 d from starting of fermentation. The cellar fermentation, instead, yielded two Traminer wines with the same 4VG and 4VP 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. cerevisae* 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 2×10^6 *S. cerevisiae* cells/ml and about 8×10^6 *Kloeckera apiculata* cells/ml. The latter type continued to be observed until 5 d 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 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+ and Pof- phenotypes.

HPLC analysis (Tab. 4) of Traminer wines obtained from the 4 experimental cellar scale fermentations allow some assumptions on the precursors of volatile phenols. The two wines fermented by Pof⁻ yeast strains still show a very high residual level of free cinnamic acids (ferulic and *p*-coumaric) in comparison to the relevant wines, fer-

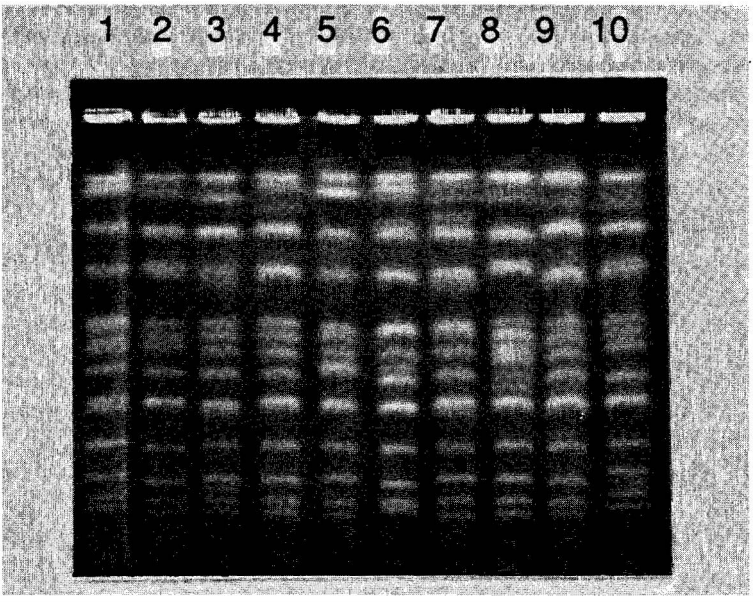

Fig. 1: Electrophoretic karyotypes of *Saccharomyces cerevisiae* strains. Ethidium bromide stained agarose gel on which the chromosomal DNA molecules of *S. cerevisiae* have been resolved. Each yeast strain possesses a unique chromosome pattern differentiating it from all other strains: lane 1, 2, 3, 4 = 9, 5, 7, 8 indigenous strains; lane 6, RM1515 SMA inoculated strain; lane 10, S 288 C reference strain. Through the analysis of some samples of the *S. cerevisiae* colonies isolated during fermentation, it is possible to identify the inoculated strain and to calculate its frequency. The subsequent determination of its hydroxycinnamic acid decarboxylase ability ascertains the stability of the phenotype Pof. — Pulsed field gel electrophoresis was performed through a 1 % agarose gel in 0.5 X TBE (Tris-Borate EDTA) buffer at 14 °C, for 23 h with a switching interval of 60 s for 15 h and 90 s for 8 h, in a CHEF-DR II cell (Bio-Rad Laboratories) apparatus.

Table 4

Cinnamic compounds content (mg/l) in Traminer wines.

	Yeast strains (Pof⁺)			Yeast strains (Pof⁻)		
	R1	RM 1515		CH 101		CRYO
cinnamic compounds	wine 2	wine 2	wine 3	wine 2	wine 3	wine 2
caftaric acid	1.80	1.71	1.13	1.33	1.54	2.19
coutaric acid-glucoside	1.35	1.36	0.64	1.17	0.76	1.49
p-coutaric acid	0.69	0.60	0.34	0.44	0.46	1.26
2-S-glutathionyl-caftaric acid	2.95	2.83	1.66	2.08	1.79	2.45
fertaric acid	6.06	6.38	2.64	6.02	3.15	6.24
caffeic acid	1.07	1.12	0.16	1.50	0.42	1.19
p-coumaric acid	0.88	0.69	0.02	1.81	0.14	1.33
ferulic acid	1.25	1.56	0.24	3.42	0.68	3.85

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

mented by Pof+ strains. Such differences can derive from the diverse usage by yeasts for the production of volatile phenols. The hydroxycinnamiltartaric acids content in the 4 wines (Fig. 2), on the other hand, does not seem to have varied in an analogous way. In particular, the fertaric acid content is actually not really different among the 4 wines. Such remarks give place to the assumption of an exclusive or anyway predominant origin of volatile phenols from free hydroxycinnamic acids, rather than from their tartaric esters. HPLC analysis of precursors in wines deriving from cellar-scale fermentation of Traminer juices indicate a content of both free and esterified hydroxycinnamic acid, which is far lower than in the wines made at experimental cellar-scale.

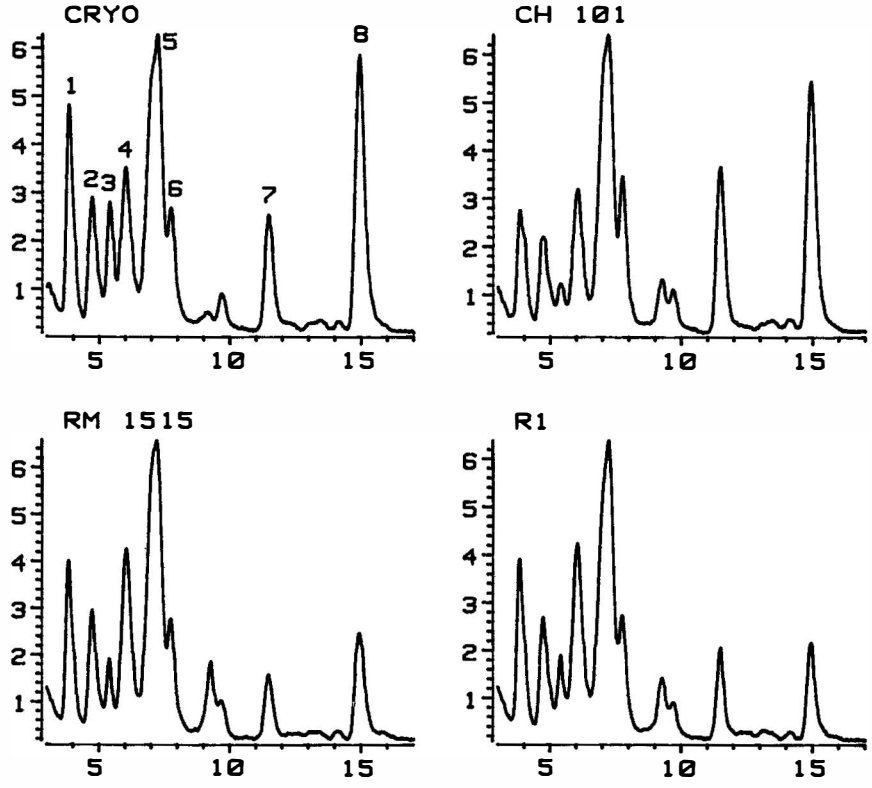

Fig. 2: HPLC analysis of cinnamic compounds in wines from Traminer juice II. 1 = caftaric acid; 2 = p-coutaric acid glucoside; 3 = p-coutaric acid; 4 = 2-S-glutathionyl caftaric acid; 5 = fertaric acid; 6 = caffeic acid; 7 = p-coumaric acid; 8 = ferulic acid.

Conclusions

As the majority of wine *S. cerevisiae* strains seem to possess the ability to decarboxylate ferulic and *p*-coumaric 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+ and Pof- in fermentation of Traminer

grape musts. The influence of the yeast strain Pof phenotype has been confirmed from the analytical results of 4VG and 4VP 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 typicity 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.

HPLC analysis of precursors allows the supposition that volatile phenols are originated from free *p*-coumaric and ferulic acids rather than from their tartaric esters. Strains Pof$^+$ for fermentation can therefore be used to exploit the aromatic potential of Traminer cultivar. Given the diffusion of Pof$^+$ 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$^-$ 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 cinnamiques substitués par les levures. Ann. Technol. Agric. **24**, 133—141.

Aurich, M.; Versini, G.; Dalla Serra, A.; 1987: Influenza dell'epoca di vendemmia e della macerazione sulle caratteristiche di tipicità dei vini Traminer Aromatico dell'Alto Adige. In: Scienza, A.; Versini, G.; (Eds.): Primo Simp. Intern. Le Sostanze Aromatiche dell'Uva e del Vino, San Michele all'Adige, Italy, 25—27 Giugno 1987, 223—232.

Cavazza, A.; Romano, F.; 1987: Valutazione tecnologica di ceppi di *Saccharomyces cerevisiae* isolati in diversi ambienti: influenza del fattore 'velocità di fermentazione'. Micol. Ital. **3**, 199—210.

Dubourdieu, D.; Darriet, P.; Chatonnet, P.; Boidron, J. N.; 1989: Intervention des systèmes enzymatiques de *Saccharomyces cerevisiae* sur certains précurseurs d'arômes du raisin. In: Actualités Oenologiques 89. C.R. 4. Symp. Intern. Oenol. Bordeaux, 15.—17. Juin 1989, 151—159. Dunod, Paris.

Grando, Maria Stella; 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.; Sapis, J. C.; Bayonove, C.; 1990: Activités glycosidases en vinification. Perspectives d'exploitation des précurseurs d'arôme du raisin, de nature glycosidique. Rev. Franç. Oenol. **30** (122), 37—41.

Marais, J.; Rapp, A.; 1988: Effect of skin-contact time and temperature on juice and wine composition and wine quality. S. Afr. J. Enol. Viticult. **9**, 22—30.

Meaden, P. G.; Taylor, N. R.; 1991: Cloning of a yeast gene which causes phenolic off-flavours in beer. J. Inst. Brew. **97**, 353—357.

Nicolini, G.; Mattivi, F.; Dalla Serra, A.; 1991: Iperossigenazione dei mosti: conseguenze analitiche e sensoriali su vini della vendemmia 1989. Riv. Viticolt. Enol. **42** (3), 45—56.

Schieberle, P.; 1991: Primary odorants of pale lager beer. Z. Lebensm.-Untersuch. -Forsch. **193**, 558—565.

Thornton, R. J.; 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 **12** (1—2), 57—65.

— —; Dalla Serra, A.; Dell'Eva, M.; Scienza, A.; Rapp, A.; 1988: Evidence of some glycosidically bound new monoterpenes and norisoprenoids in grapes. In: Schreier, P.; (Ed.): Bioflavour 87, 161—170. Walter de Gruyter, Berlin, New York.

— — ; SCIENZA, A.; DALLA SERRA, A.; DELL'EVA, M.; MARTIN, C.; 1989. Rôle du clone et de l'époque de récolte sur l'arôme du Chardonnay: aspects analytiques et sensoriels. In: Actualités Oenologiques 89. C. R. 4. Symp. Intern. Oenol. Bordeaux, 15.—17. Juin 1989, 69—74. Dunod, Paris.

WINTERHALTER, P.; SEFTON, M. A.; WILLIAMS, J.; 1990: Two-dimensional GC-DCCC analysis of the glycoconjugates of monoterpenes, norisoprenoid, and shikimate-derived metabolites from Riesling wine. J. Agricult. Food Chem. **38**, 1041—1048.

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