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USE OF ENZYMES WITH GLUCOSIDASE ACTIVITY IN MUSTS AND WINES MAINLY FROM NORTH ITALIAN VARIETIES, A FEW SAMPLES

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INTRODUCTION

Several trials employing enzymes with glucosidase activity in both white and red musts and wines have been carried out since 1990 at the Agricultural Institute at San Michele all'Adige. All those experiences were performed on a semi-industrial scale, using experimental as well as commercial enzymes at their maximum dosage, as shown in the information sheets. We worked on one hand under usual winemaking conditions, and we also tried to evidence possible alterations by the use of higher dosages. Musts and wines - of mono-varietal or mono-clonal origin - were vinified at the experimental winery of the Institute.

MATERIAL AND METHODS

We would like to review here those experiences - a large part of which was already published [Nicolini et al., 1993a; Nicolini et al., 1993b; Nicolini et al., 1994a; Nicolini et al., 1994b; Nicolini et al., 1996] - focusing on both positive features and problems observed. Some employment advice is provided. Some considerations are also included regarding the possibility of verifying the varietal origin of wines produced with glucosidase. Material and methods are reported in the quoted previous works.

DISCUSSION

1. Effects on aroma compounds.

1a. Enzymes with β-glucosidase activity applied in musts.

In a supervised ring-control, two glycosidases (P and L), applied in white and red musts in comparison with untreated controls (T), showed a similar activity (Tab. 1), the higher being for L. As reported by Shoseyov et al., [1987], Cordonnier et al. [1989] and Gunata et al. [1990], this activity proved lower in the case of wines with high residual sugars (e.g. *Rose Muscat*, sugar: 40-50 g/L). A precise balance between increased free forms and corresponding decreased bound forms is difficult to be performed, because of chemical reactions and of the metabolic activity of yeasts on monoterpenols - e.g. the transformation of geraniol into citronellol and other non volatile compounds [Gramatica et al., 1982; Versini et al., 1990a] -, however, bound geraniol showed to be the preferential target of the glycosidases, particularly for *Rose Muscat*, *Traminer* and *Nosiola*. This late variety is usually considered as neutral.

The ho-diendiol (I) contents and the unreported furanic and pyranic oxides seem not to be affected by the enzymic treatments.

The increase of free benzyl alcohol, which seems not to be justifiable only on the

basis of the hydrolysis of the bound form, is likely due to a higher hydrolysis efficiency of the used glycosidases compared to that of enzymes employed for chemical analyses of bound forms. Similar increases were observed also by Gunata [1992] and confirmed in other our trials in which increasing contents of glycosidase were used in wines (Tab. 2).

Also a partial hydrolysis of norisoprenoids glycosides (see the case of 3-oxo-α-ionol) is verified. This fact can give the possibility for increasing the "tobacco-spicy" scent in some wines, like in *Rhine Riesling*. In the case of high bound benzyl alcohol content - as recorded sometimes in *Prosecco* [Nicolini et al. 1994c] - and in connection with even partly botrytised grapes [Goetghebeur et al., 1991], benzaldehyde could theoretically pass the sensory threshold. Anyway we do not know about actual cases.

Increasing contents of 4-vinyl-guaiacol (4VG) in P and L wines are noticed, confirming previous data about the production of volatile phenols in relation to the use of several commercial enzymes with pectinase and cellulase activities reported by Dugelay et al., [1992]. This volatile phenol can play a positive sensory role with its spicy-like note in the Trentino *Gewürztraminer* typicality [Versini, 1985] or contribute to the aroma complexity in others [Versini et al., 1990b], but - at high concentrations - it is generally considered an off-flavour [Rapp et Versini, 1996]. Yeast strains with Pof(+) or Pof(-) activity [Grando et al., 1992] can be used in directing the volatile phenols production.

The wines of Tab. 1, submitted to the tasters judgement (tab. 3), showed a trend in enhancing the perceived colour intensity, particularly in the case where enzyme L is used. The floral and rose-like notes increase in statistically significant way at the Duncan's test in all the varieties as a consequence of the enzymic treatments. An interesting oregano/spicy-like note arises in the two treated *Rhine Riesling* without bringing about an increased typicality; a phenolic scent appears in *Chardonnay* L, but a clear analytical explanation has not been found for it.

The typicality judgement is positively affected by the enzymic treatments for those varieties in which the rose-like note plays a considerable part (*Gewürztraminer*, *Rose Muscat*), or it is not modified in a statistically significant way in the other varietal wines. The enjoyableness judgement is particularly interesting also for *Nosiola*, in relation to the technological opportunity of "managing" the aroma.

Highly significant and positive results in increasing the rose-like note had already been obtained in 1990 by a glucosidase employed in a *Traminer* wine; in that wine, as a consequence of the halving of bound geraniol, free geraniol increased from 10 to 150 μg/L.

Sometimes the P - but particularly L - wines (Tab. 3) seemed to taste as if longer aged; this fact can be considered as positive only for *Nosiola*, in which it contributes to the disappearance of an evident green-unripe tone.

A mushroom-like scent was observed in some P - but also L - white wines at the end of fermentation. It was not clearly noticed any more after bottling three months later, so that the tasters were not requested to perform any sensory analysis about this scent; nevertheless some undefinable and unpleasant note connected with it could explain the overall impression for the *Traminer* P, which presents about the same free geraniol and linalool contents as the L treatment.

1b. Enzymes with β-glucosidase activity applied in wines.

- Figure 1, including the sensory effects produced by increasing enzyme doses added to a *Traminer* wine from a different vintage, seems to confirm the above. At the highest dosage (5 g/hL), the so-called "mushroom" note was significantly perceived, negatively affecting the overall enjoyableness evaluation, even if the highest "floral-rose" note was present. However, with ageing (2 - 3 months) the already noticed tendency of this off-flavour to fade seems to be confirmed. It cannot be excluded that the experimental enzymes available in the two vintage years may have had an effect either on non determinable glucosides or by fostering the formation of artifacts [Sefton and Williams, 1991], which were perceptible but unstable in time.

The sensory data quite agree with the results of the gas chromatographic (Tab. 2) and the spectrophotometric analyses, the latter being shown below.

No peculiar olfactory sensation appeared in a *Rhine Riesling* wine added with the same dosages as the *Traminer*; in this variety (Fig. 2) along with a rising general "floral" note, the "tea" and "spicy/oregano" were enhanced, in particular with the highest dosages employed. The enjoyableness evaluation generally rewarded the higher olfactory intensity of the floral and spicy notes in the 3 g/hL treatment, probably because the tasting panel felt the intensity of those or the "tea" note in the 5 g/hL treatment a bit exaggerate. The sensory variations perceived here can be only partly explained with the analytical data of free terpenes (Tab. 2); however there is much shared knowledge about the role that other substances, i.e. hydrolysable norisoprenoids [Schreier e Drawert, 1974; Straüss et al., 1987; Winterhalter et al., 1990; Gunata et al., 1992], may play in the sensory profile and in the floral and tea notes in *Riesling* and in other varieties [Williams et al., 1991].

Also three Sauvignon blanc wines of different origin underwent an enzymatic treatment (Fig. 3). In this case too the general floral note of wines was significantly stronger than in the control treatments. That did not always involve a better enjoyableness judgement, anyhow the wines treated with enzyme were not negatively affected as regards enjoyableness or typicality. The rise of the floral note accords with the results by Williams [Williams et al., 1992] who - by stressing the sensory-chemical differences between chemical and enzymatic hydrolyses - describes *Sauvignon blanc* as an "intermediate" variety of *Vitis vinifera* between the "floral" (*Muscat-* or *Riesling*-type) and the "non-floral" (i.e. *Chardonnay* and *Semillon)* cultivars. The fact that terpenes could play a role in the floral aroma of *Sauvignon blanc* had previously been stressed also by Versini et al. [1988].

Table 4 regards trials performed on 4 *Garganega* wines. The sensory analysis results show clearly that the enzyme always brought about wines that were significantly different from the relevant controls and characterized by a higher olfactory intensity. In only one case this implied a significant preference evaluation, probably as a consequence of a certain deviation from the typicality olfactory characteristics of wines. It seems unlikely to attribute only to the terpene component the sensory differences perceived; in fact, even after the treatment with enzymes, the free terpenes (citronellol + linalool + geraniol) did not rise over 10 μg/L. Some role may have been played also by the methyl salicilate whose free form is used in perfume-making as modifier of floral fragrances [Bauer et al., 1990] and whose olfactory threshold in white wines of good aroma complexity lies, according to our experience, around 30-50 μg/L with sweetish and medicine hints. This compound is present in bound form in several varieties, at levels similar to those mentioned.

In another experience (Fig. 4), eight Müller-Thurgau wines (1 year aged) were treated with a glycosidase and their sensory analysis was performed on the basis of 2 broad keywords: nose intensity and typicality. An increased and significant global nose intensity was perceived for four wines but a higher typicality was observed only in one. Since today we know the bound terpene profile of Müller-Thurgau (with only linalool and a bit of geraniol of technological interest) [Nicolini et al., 1995; Versini et al., 1995], we can understand why the enzymic treatment did not particularly affect typicality. By the enologists, however, the use of glycosidase on a slightly aged Müller-Thurgau table wine was considered an technological opportunity which can be sometimes effective in improving the marketability of the product.

A lot of monovarietal Italian red and white wines were treated with glycosidase and sensorially evaluated in black glasses by different panels of consumers. Some of the results are shown in Tab. 5. The treated wines proved frequently different and with a higher nose intensity than the control ones, but there was no significant result at the preference test, except for Nero d'Avola of Sicily. In that treated wine, an anomalous and disagreeable scent was clearly noticed. No other anomalous situation was recorded in about 20 red and white other Italian wines (among which many Sangiovese wines) treated with glycosidase.

2. Effect on colour and side-activities.

The use of glycosidase - beside the sensory effects, that anyway prove interesting - also showed some problems, apart from the possible formation of artifacts even having sensory importance:

- a tendentially higher colour intensity in white wines was recorded, although usually within a technological level of acceptability (Fig. 5).
- An esterase side-activity was noticed on cinnamyl derivatives (Figg. 6a-6b) - with a higher possible production of volatile phenols from the yeasts - but also on some acetates and esters important for the fruity scent (Fig. 7).
- A degradative activity was observed on anthocyanins when enzymes were used in red wines (Tab. 8) with decrease in colour intensity and increase of hue as for longer-aged wines. This effect was not evident when enzymes were applied in red musts (Tab. 7). An esterase activity on p-coumarate anthocyanins causing the formation of free p-coumaric acid was demonstrated (Tab. 8) by performing the reaction on a pure fraction of anthocyanins in a model wine solution. This esterase activity was causing the complete hydrolysis of anthocyanins, leading to the formation of corresponding amount of p-coumaric acid. It was shown to operate not only on all free coumaric esters of anthocyanins, but also on the red pigments. The amount of p-coumaric acid hydrolyzed is approximately one third of the amount of total p-coumarate anthocyanins, and can therefore be of many mg/L in varieties such as Cabernet sauvignon, Cabernet franc, Merlot, Teroldego, Marzemino, Lagrein, Carignan. A new colourless flavonoid compound, whose structure is not yet ascertained, appeared as a consequence of the anthocyanins degradation. (Fig. 8).

CONCLUSIONS

Trials - performed with rather high glycosidases doses and evaluating the results on thoroughly treated wines, not on blends between treated and not treated - gave generally interesting results. The collection of performed trials allowed us to provide the users of this biotechnological product with suggestions that should help optimizing the results and minimizing the problems recorded.

In case of white varieties - except for the cases in which time is essential - we deem to be safer and more easily manageable the use of glycosidase on young wine rather than on must, because of no glucose inhibition and the possibility to perform preliminary tests which can allow to manage the aroma blending from wines processed with or without enzymes.

In case of red aromatic varieties, it seems preferable to use glycosidase in musts, in particular for varieties having a low natural colour intensity.

In all cases where an excessive production of volatile phenols could occur (which can be linked to the variety, to the winemaking process, to the refermentation, etc.) it is safer to use a Pof (-) yeast jointly with the enzyme.

Glycosidase can best work on the typicality of those varieties in which free geraniol particularly contributes to the aroma and that are characterized by a reasonable amount of bound geraniol (*Gewürztraminer, Rose Muscat, Nosiola,* in some cases *Prosecco*). The increase of free norisoprenoids, although interesting for many varieties (e.g. *Riesling, Syrah*) is probably less easily manageable by winemakers because of the less complete knowledge available on these compounds.

The use of glycosidase with the quoted side activities can be detected by the analysis of free anthocyanins and/or of hydroxycinnamic acids, since it modifies the typical distribution pattern - linked to the variety (Fig.9) [Mattivi et al., 1995] - of these compounds.

The availability of more precise data about the complete characterization of the aroma composition of the different part of the berry of each variety would be of great help in designing winemaking process, including the use of glycosidase. Even where such data are notavailable, a simple preliminary test - with the organoleptic evaluation of the samples treated with enzyme - can be used in everyday pratice for an estimate of the potential benefits (such as actual increase in aroma, standardization of the results through different vintage years, etc.).

LEGENDA:

Varieties: CH= Chardonnay, TRAM= Gewürztraminer, NOS= Nosiola, RIES= Rhine Riesling, GARG= Garganega, S.B.= Sauvignon blanc, PROS= Prosecco, M.R.= Rose Muscat (red variety), TER=Teroldego (red variety), LFF= Lambrusco a foglia frastagliata (red variety);

Treatments: T= untreated control wine; P, L, P92, P93, C, R, G, N, H commercial or experimental pectolitic-glycosidase enzymes of different producers.

Phenolics (mg/L): ANT.LIB.= free anthocyanins, malv.; ANT.TOT.= total anthocyanins, malv.; ANT.ACIL.= anthocyanins, sum of acetic esters, malv.; ANT.PCUM.= anthocyanins, sum of p-coumaric esters), malv.

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FREE VOLATILE COMPOUNDS (μg/L)	CHARDONNAY			GEWÜRZTRAMINER			NOSIOLA		
	T	P	L	T	P	L	T	P	L
LINALOOL	4.6	6.1	13.0	23.0	96.6	101	4.4	8.5	16.6
α-TERPINEOL	<0.5	0.5	<0.5	6.5	18.4	27.0	1.1	1.2	2.1
CITRONELLOL	9.2	13.6	6.3	20.4	425	472	3.1	52.0	87.7
GERANIOL	3.8	4.7	5.5	83.6	880	826	8.9	56.5	53.8
HO-DIENDIOL (I)	15.4	18.5	16.8	108	110	101	13.6	6.9	16.7
BENZYL ALCOHOL	96.0	270	358	70.0	422	469	142	323	327
4-VINYLGUAIACOL	1.9	6.5	3.4	154	232	171	<0.5	15.9	13.5

BOUND VOLATILE COMPOUNDS μg/L	CHARDONNAY			GEWÜRZTRAMINER			NOSIOLA		
	T	P	L	T	P	L	T	P	L
LINALOOL	19.1	14.0	11.0	15.1	5.7	4.9	3.0	0.5	0.3
NEROL	3.9	3.3	1.5	395	98.3	38.6	6.8	0.8	0.3
GERANIOL	8.9	2.5	2.6	1956	156	97.3	145	6.8	5.0
trans GERANIC ACID	5.3	1.8	1.4	327	156	44.8	11.6	2.7	0.6
HO-DIENDIOL (I)	<1.0	2.4	<1.0	12.4	7.6	8.8	2.1	2.2	2.0
trans 8-OH-LINALOOL	23.0	7.8	6.5	39.5	7.7	2.3	22.4	1.3	<1.0
cis 8-OH-LINALOOL	68.3	21.3	16.0	521	117	40.8	93.9	13.0	10.0
3-OXO-α-IONOL	106	74.0	58.0	147	90.0	51.2	127	27.4	30.4
BENZYL ALCOHOL	96.3	49.1	19.0	155	40.2	16.1	66.2	5.7	10.8
2-PHENYLETHANOL	101	62.3	22.9	184	29.0	32.4	111	26.7	35.7

FREE VOLATILE COMPOUNDS μg/L	ROSE MUSCAT			RHINE RIESLING		
	T	P	L	T	P	L
LINALOOL	495	852	777	198	265	236
α-TERPINEOL	291	464	473	157	225	220
CITRONELLOL	205	607	621	4.3	18.8	19.5
GERANIOL	104	540	379	38.2	45.3	54.5
HO-DIENDIOL (I)	60.5	66.4	46.6	463	536	602
BENZYL ALCOHOL	228	740	705	65.0	268	407
4-VINYLGUAIACOL	1.7	4.2	7.4	49.0	128	104

BOUND VOLATILE COMPOUNDS μg/L	ROSE MUSCAT			RHINE RIESLING		
	T	P	L	T	P	L
LINALOOL	102	130	107	25.0	17.0	7.9
NEROL	655	455	390	6.5	2.3	0.9
GERANIOL	2212	934	827	51.5	9.2	2.5
trans GERANIC ACID	347	226	189	21.5	3.5	0.9
HO-DIENDIOL (I)	1.6	0.5	0.5	22.5	30.0	36.4
trans 8-OH-LINALOOL	90.0	55.7	51.0	41.0	13.5	7.6
cis 8-OH-LINALOOL	184	92.1	77.0	296	89.5	44.1
3-OXO-α-IONOL	60.5	25.3	29.0	90.0	49.0	43.0
BENZYL ALCOHOL	175	130	103	140	30.6	14.8
2 PHENIL ETHANOL	363	1100	542	290	135.0	134.0

Tab.1: Free and bound aroma compounds in wines; use of glucosidases on the musts.

VOLATILE COMPOUNDS µg/L	GEWUERZTRAMINER 1992				RHINE RIESLING 1992			
	untreated	1 g/hL	3 g/hL	5 g/hL	untreated	1 g/hL	3 g/hL	5 g/hL
LINALOOL	19.5	29	35	68	67	67	79.5	73.5
GERANIOL	32.5	61.5	138	262.5	7.1	9.5	29.5	49.5
BENZYL ALCOHOL	40	100	218	498	37.5	114.5	407.5	493

Tab. 2: Variations of the content of some free volatile compounds as resulting from the treatment of wines with increasing doses of an experimental enzymic product having glycosidase activity.

descriptive	CHARDONNAY			RHINE RIESLING			GEWUERZTRAMINER			NOSIOLA			ROSE MUSCAT		
terms	T	P	L	T	P	L	T	P	L	T	P	L	T	P	L
COLOR INTENSITY	-0.62 b	-0.42 b	1.03 a	-1.01 c	0.29 b	0.72 a	-1.01 c	0.2 b	0.81 a	-0.51 b	-0.59 b	1.1 a	-0.91 c	0.26 b	0.65 a
HUE													0.53 a	-0.11 b	-0.42 b
FLORAL	-0.35 b	0.34 a	-0.01 ab	-0.61 b	0.26 a	0.34 a				-0.47 b	0.33 a	0.14 ab			
ROSE							-0.72 c	-0.07 b	0.79 a				-0.94 b	0.63 a	0.31 a
TEA	-0.1 a	-0.13 a	0.23 a	0.13 a	-0.06 a	-0.07 a									
SPICY -4VG							-0.31 a	0.01 a	0.3 a						
SPICY OREGANO				-0.67 b	0.09 a	0.58 a									
PHENOLIC	-0.5 b	-0.23 b	0.73 a												
GREEN UNRIPE										0.54 a	-0.25 b	-0.29 b			
MORE AGED	-0.09 a	-0.15 a	0.24 a	-0.73 c	0.02 b	0.71 a	-0.72 b	0.32 a	0.40 a						
TYPICALITY	0.06 a	0.26 a	-0.32 a	-0.24 a	0.4 a	-0.16 a	-0.48 b	-0.34 b	0.82 a	0.17 a	0.16 a	-0.33 a	-0.73 b	0.35 a	0.41 a
ENJOYABLENESS							-0.56 b	-0.31 b	0.87 a	-0.53 b	0.40 a	0.13 ab	-0.75 b	0.37 a	0.38 a

Tab. 3: Results of the sensory analysis performed on the wines of Tab.1. (T= control ; P-L= different glycosidases applied in musts)

WINE	DUO-TRIO TEST		PAIRED-DIFFERENCE TEST (higher nose intensity)			PAIRED-PREFERENCE TEST		
	correct results	significance	control wine	treated wine	significance	control wine	treated wine	significance
GARGANEGA n° 1	18/20	0.001	1	14	0.001	3	12	0.04
GARGANEGA n° 2	15/20	0.03	3	12	0.02	5	10	n.s.
GARGANEGA n° 3	17/20	0.005	2	13	0.005	4	11	n.s.
GARGANEGA n° 4	16/20	0.01	2	13	0.005	6	9	n.s.

Tab. 4: Difference and preference test performed on 4 pairs of Garganega wines (control wine vs. the same wine treated with a commercial glycosidase)

WINE	DUO-TRIO TEST significance	PAIRED TEST (higher nose intensity)	PREFERENCE TEST
Pinot gris	*	n.s.	n.s.
Pinot gris	*	n.s.	n.s.
Pinot blanc	*	n.s.	n.s.
Pinot blanc	*	enzyme *	n.s.
Verdicchio	**	enzyme *	n.s.
Verdicchio	***	enzyme *	n.s.
Cortese	**	n.s.	n.s.
Tocai	**	enzyme *	n.s.
Vernaccia	**	enzyme *	n.s.
Trebbiano	**	n.s.	n.s.
Inzolia	*	n.s.	n.s.
Lambrusco Salamino	*	enzyme *	n.s.
Corvina	n.s.	n.s.	n.s.
Rondinella	*	n.s.	n.s.
Corvinone	n.s.	n.s.	n.s.
Marzemino	n.s.	n.s.	n.s.
Frappato	***	enzyme **	n.s.
Nero d'Avola	***	enzyme ***	control ***

Tab. 5: Difference and preference tests performed on untreated control wines vs. wines treated with glycosidase (5 g/hL). (n.s.= non significant; *=p<0.05; **=p<0.01; ***=p<0.001).

wine	ANT.LIB.	ANT.TOT.
TER.-T	226	483
TER.-L	32	231
LFF.-T	189	333
LFF.-L	50	202
LFF.-T	41	140
LFF.-P	23	61
LFF.-L	23	61
LFF.-R	14	43
TER.-T	543	958
TER.-G (*)	237	633
TER.-H (*)	392	825
TER.-T	715	1277
TER.-G (*)	259	609
TER.-H (*)	427	849
LFF.-T	203	504
LFF.-G (*)	78	346
LFF.-H (*)	137	432.

Tab. 6: Anthocyanins content of different wines treated with different glycosidases at the highest doses suggested by the producers in their technical brochures or at double dose (*).

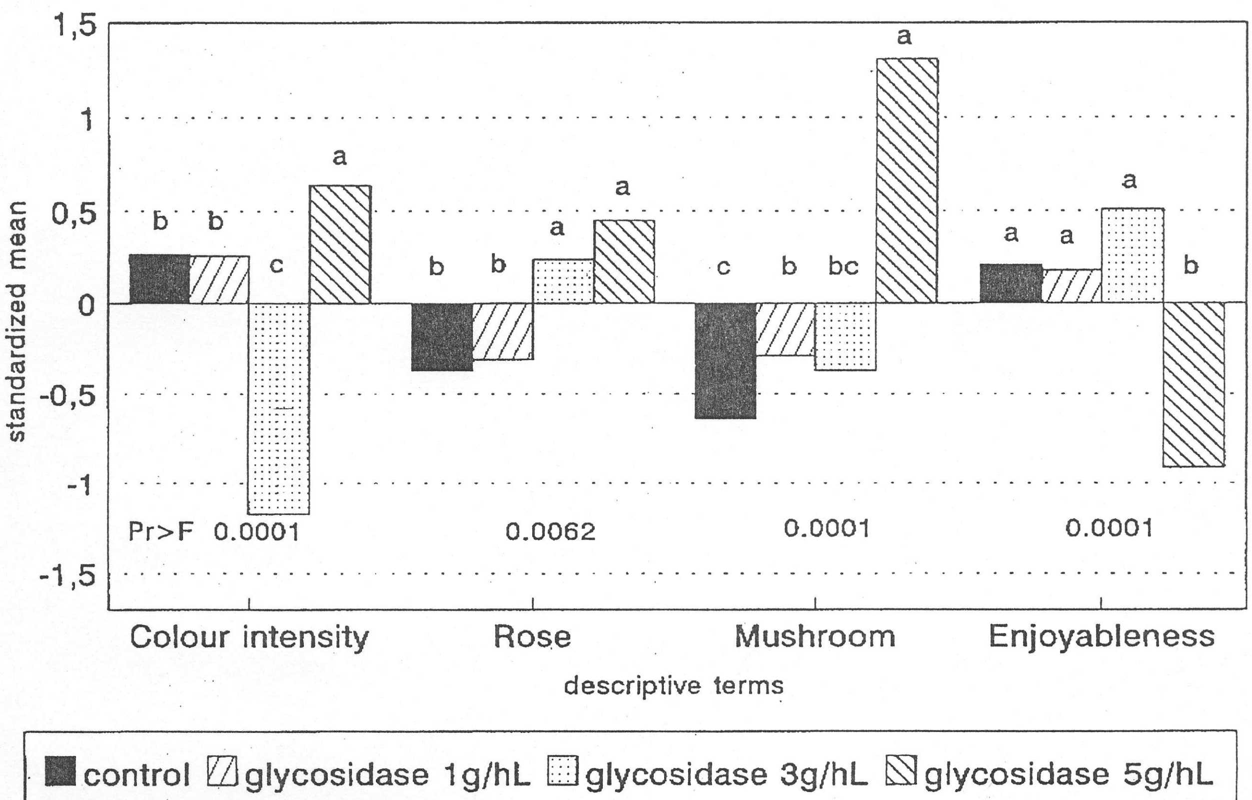
WINE-TREATMENT	TOTAL POLY-PHENOLS (mg/L +cat.)	PRO-ANTHOCYANIDINS (vanillin) (mg/L +cat.)	TOTAL ANTHOCYANINS (mg/L malv.)	FREE ANTHOCYANINS (mg/L malv.)	COLOUR INTENSITY ***	HUE (o.d. 420/520)
M.R.-T	1370	760	246	121	5.19	0.732
M.R.-P	1711	1086	279	131	7.00	0.703
M.R.-L	1725	1185	300	127	7.35	0.689
TER.-T	1296	574	846	452	9.90	0.589
TER.-P	1361	672	745	442	9.34	0.613
TER.-L	1473	709	907	498	11.68	0.597

Tab. 7: Phenolics and colour characteristics in red wines from musts treated with different glycosidases. (***=O.D.420+520+620nm, 1cm)

VINI wines	FLAV. NON ANTOC.	ANT. LIB.	ANT. ACIL.	ANT. PCUM.	ACIDO CAFFEICO caffeic acid		ACIDO p-CUMARICO p-coumaric acid		ACIDO FERULICO ferulic acid	
					LEGATO Σ bound	LIBERO free	LEGATO Σ bound	LIBERO free	LEGATO bound	LIBERO free
LFF test	1191	59.23	3.72	1.82	40.47	4.00	12.00	4.07	2.13	1.14
LFF 5g/hL	1236	22.12	0.92	0.00	11.36	18.57	5.20	11.89	1.66	0.74
LFF 10g/hL	1265	11.05	0.51	0.00	3.78	27.03	5.27	13.40	1.74	1.11
LFF test	1232	41.08	2.76	1.44	42.48	3.39	11.93	2.46	2.05	1.05
LFF 5g/hL	1269	22.67	1.33	0.00	16.63	16.70	4.94	11.71	1.87	0.66
LFF 10g/hL	1290	13.12	0.63	0.00	6.01	24.23	4.53	13.31	1.89	0.86
TER test	865	137.89	53.40	14.66	26.75	2.88	16.53	5.15	1.63	1.23
TER 5g/hL	955	58.12	18.34	0.00	1.56	23.54	3.03	29.65	0.00	0.60
TER 10g/hL	993	30.72	7.18	0.00	1.33	25.11	2.63	30.95	0.00	1.06
TER test	1149	221.18	113.18	19.78	33.08	2.01	21.11	3.97	1.47	1.32
TER 5g/hL	1236	50.64	20.86	0.00	3.41	25.73	4.94	33.86	0.00	1.01
TER 10g/hL	1236	51.14	21.90	0.00	3.06	26.75	4.91	33.98	0.00	0.43
SOL.SINT. test	n.d.	60.83	54.24	30.58	0.00	0.00	0.00	0.00	0.00	0.00
SOL.SINT. 10g/hL	n.d.	26.94	7.04	0.00	0.00	0.00	0.00	12.61	0.00	0.00

Tab. 8: Polyphenolic content (mg/L) in different red wines treated with different doses of the same glycosidase (SOL.SINT.= like-wine synthetic solution).

GEWURZTRAMINER 1992
18 tasters - test F - Duncan's test



ig.1: Results of sensory analysis performed on wine treated with different doses of glycosidase

RHINE RIESLING 1992
16 tasters - test F - Duncan's test

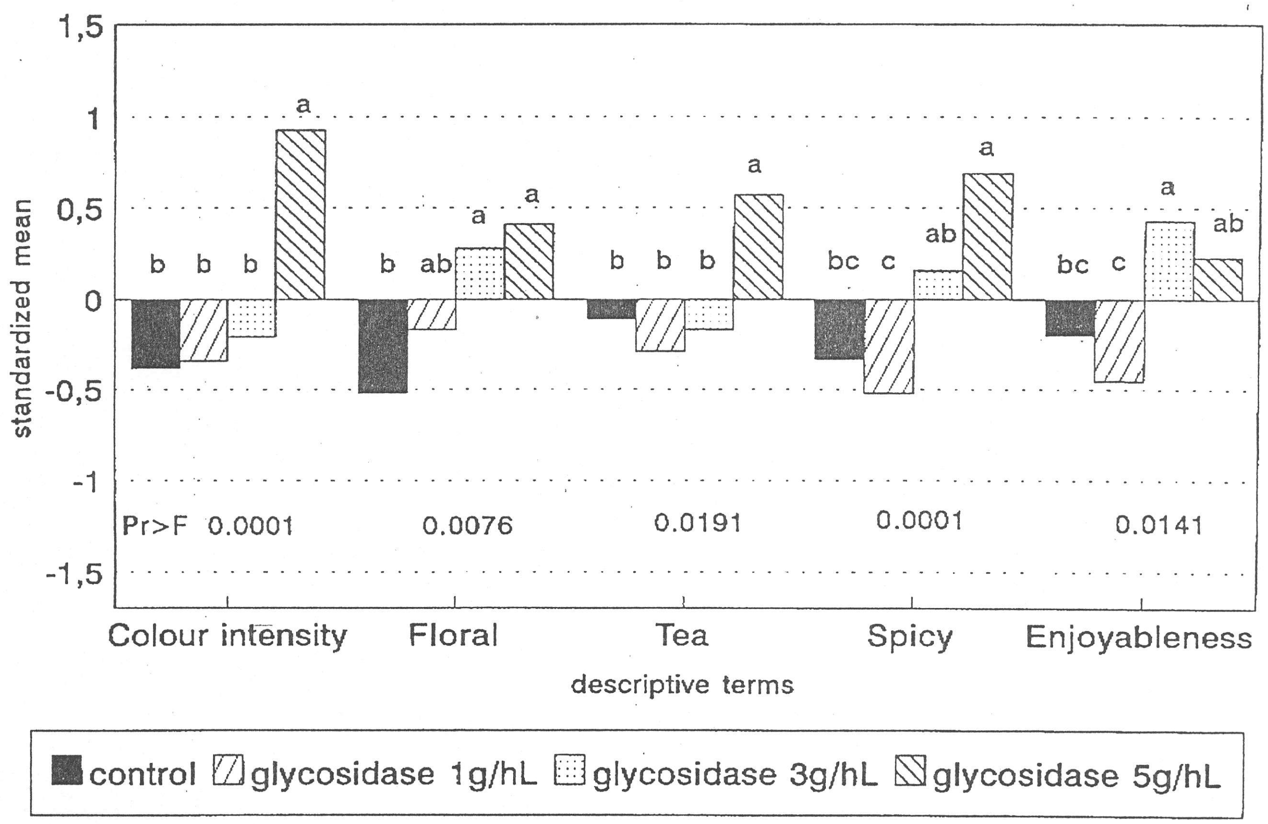


Fig.2: Results of sensory analysis performed on wine treated with different doses of glycosidase.

SAUVIGNON BLANC
7 tasters - test F - Duncan's test

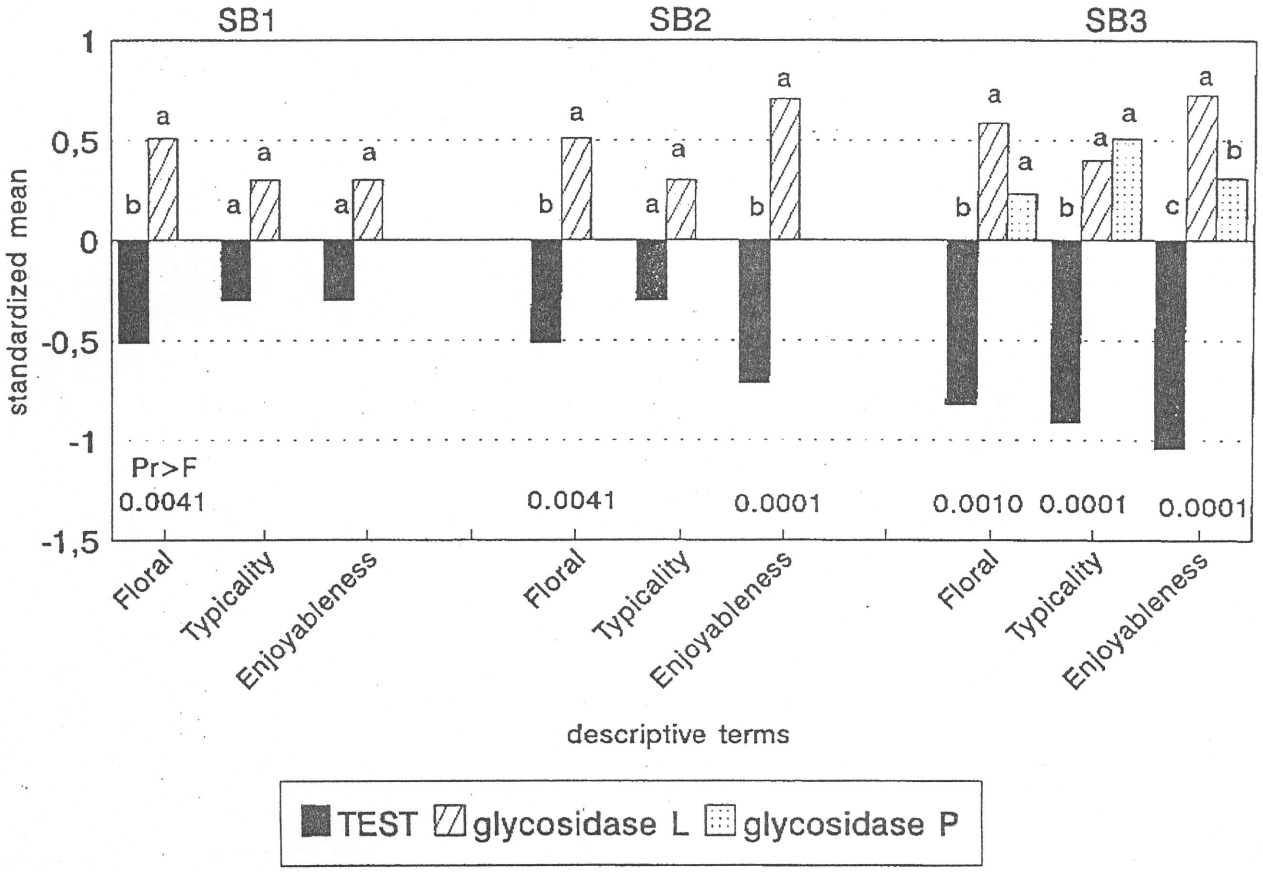


Fig.3: Sensory analysis performed on 3 different Sauvignon blanc wines treated with 2 glycosidases

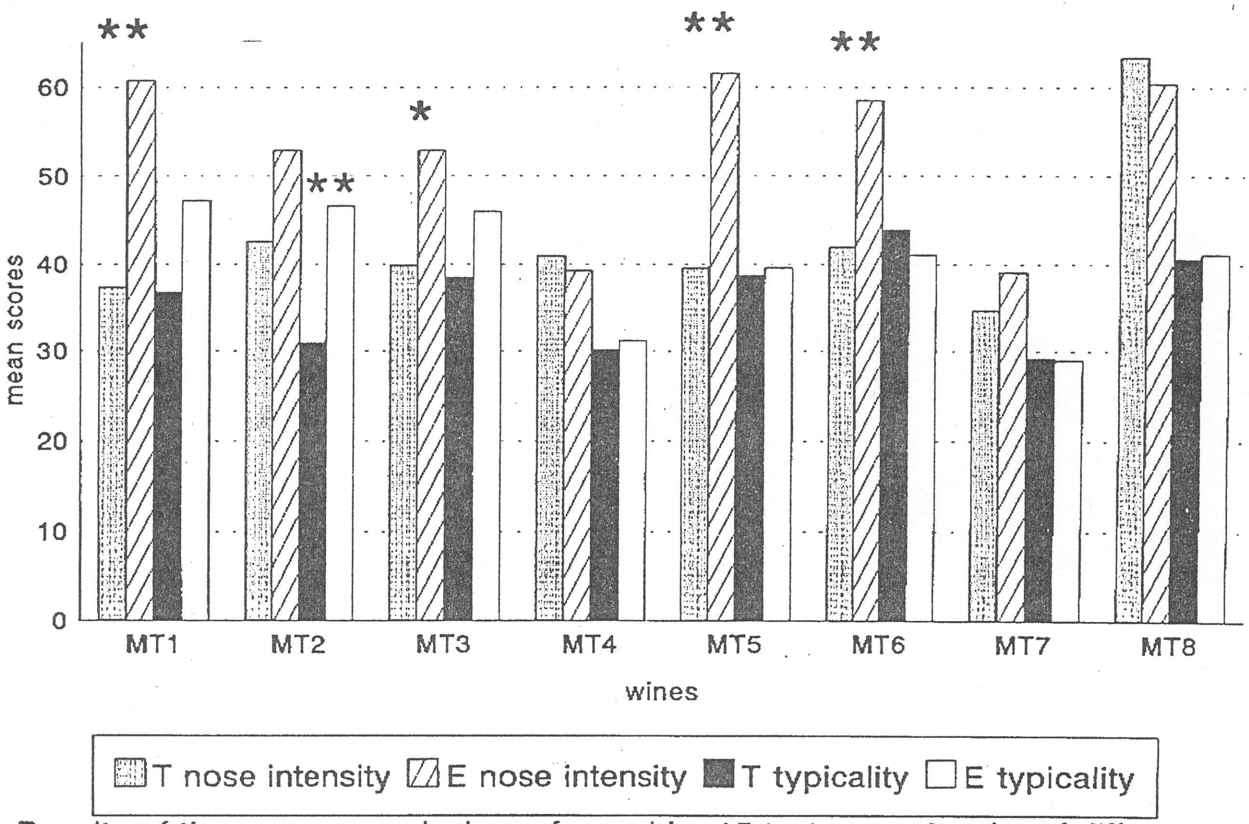
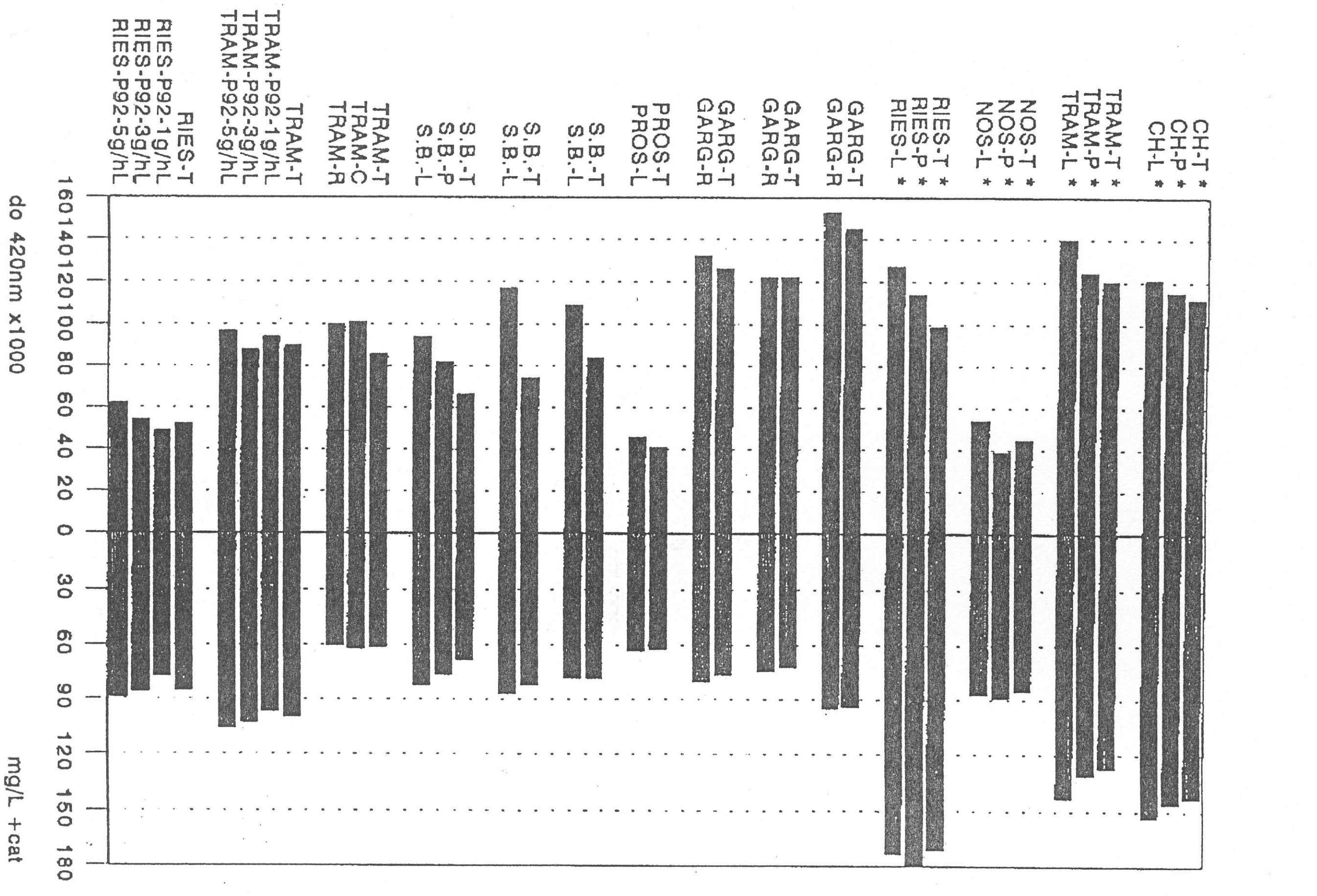


Fig.4: Results of the sensory analysis performed by 15 tasters on 8 pairs of different wines; control (T) vs. treated wine (E: glycosidase 5 g/hL) (*=p<0.05, **=p<0.01, ***=p<0.001)



ig.5: Total polyphenols and adsorbances at 420 nm (10mm) in white wines; glycosidases used in musts (*) or in wines.

Fig. 6a: Content of free cinnamic acids (mg/L) in wines; glycosidases used in musts

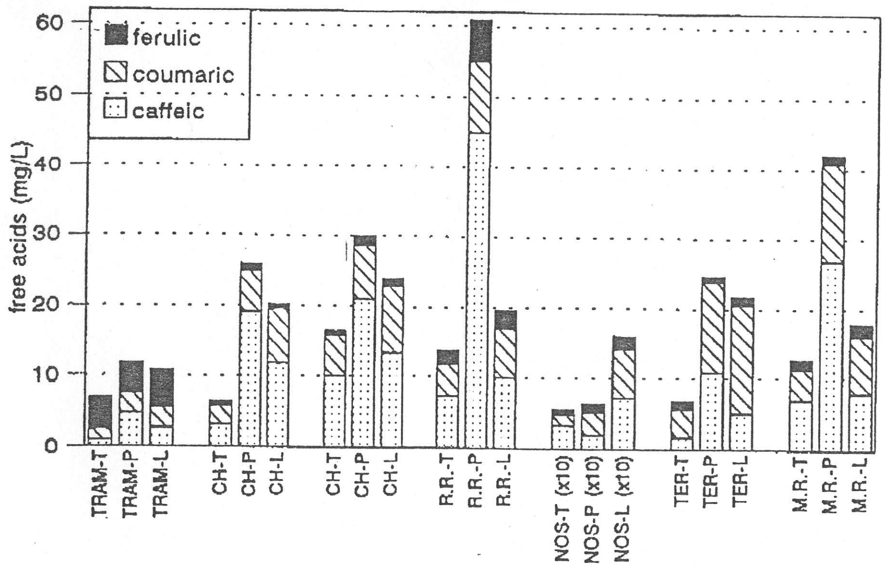


Fig. 6b: Percentage of ester forms and of free acids on the total cinnamic derivatives (as mg/L C.A.E.) and content of the single free acids (mg/L) in wines; glycosidases used in wines.

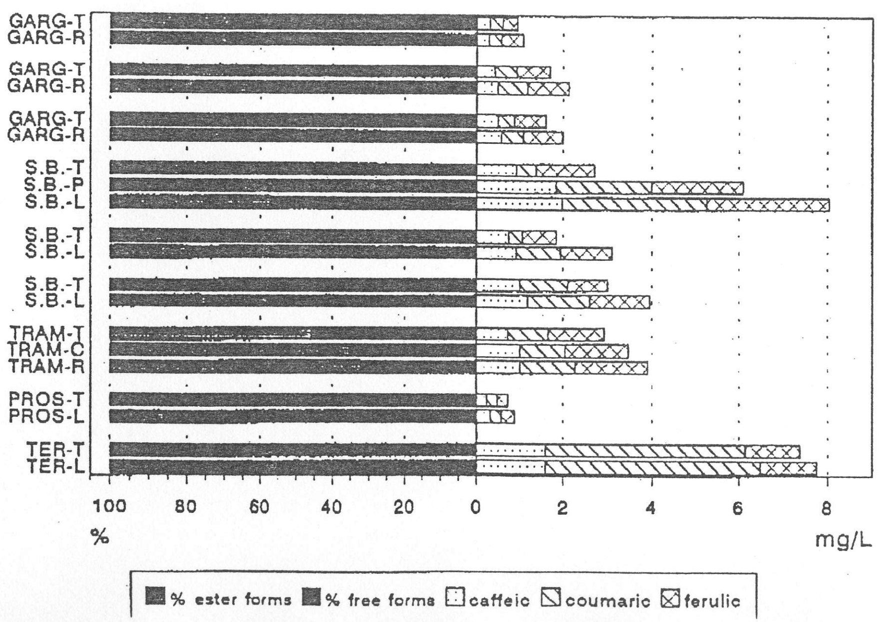
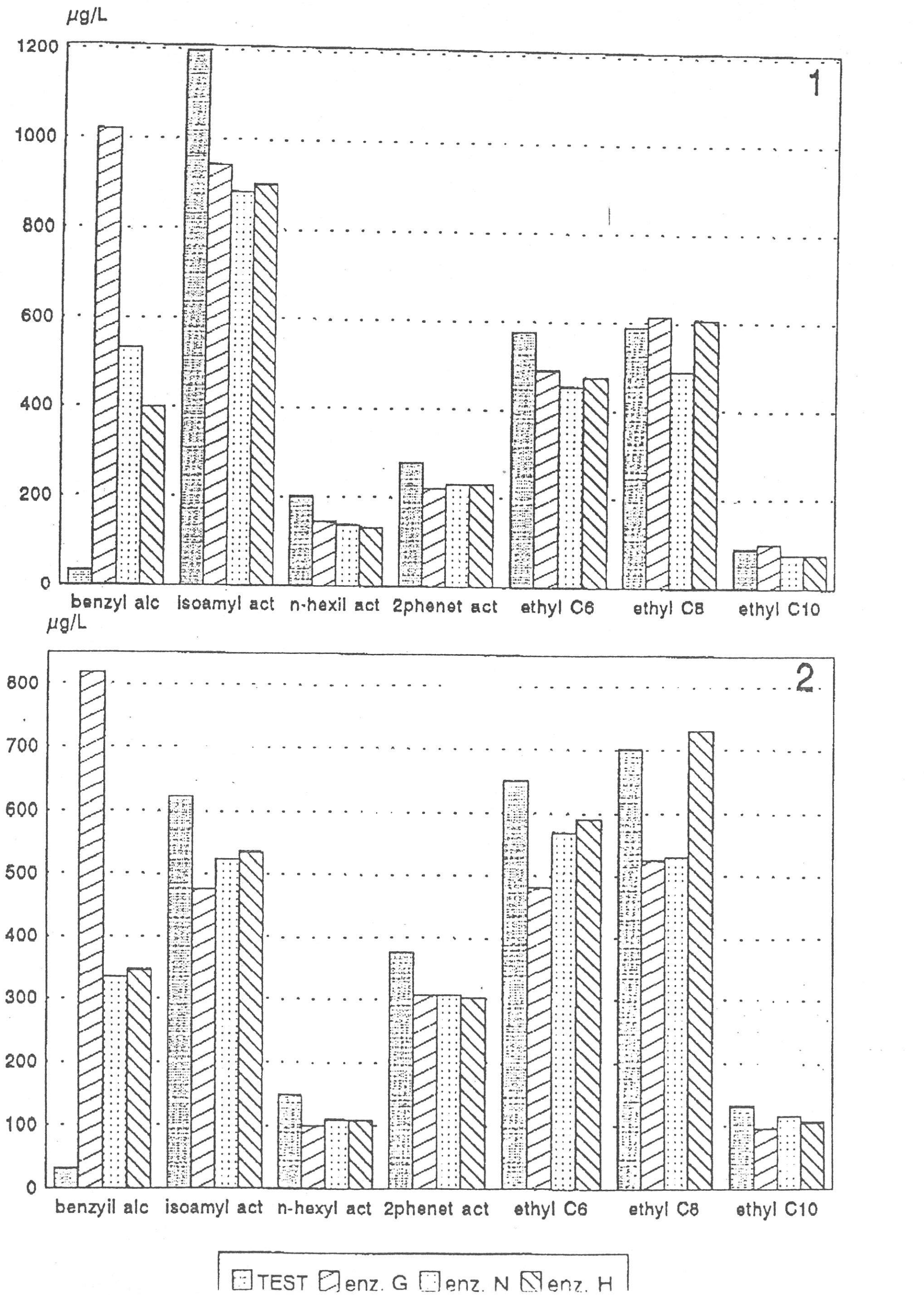


Fig. 7: Volatile compounds in different Müller-Thurgau wines (1 and 2) treated with 3 glycosidases



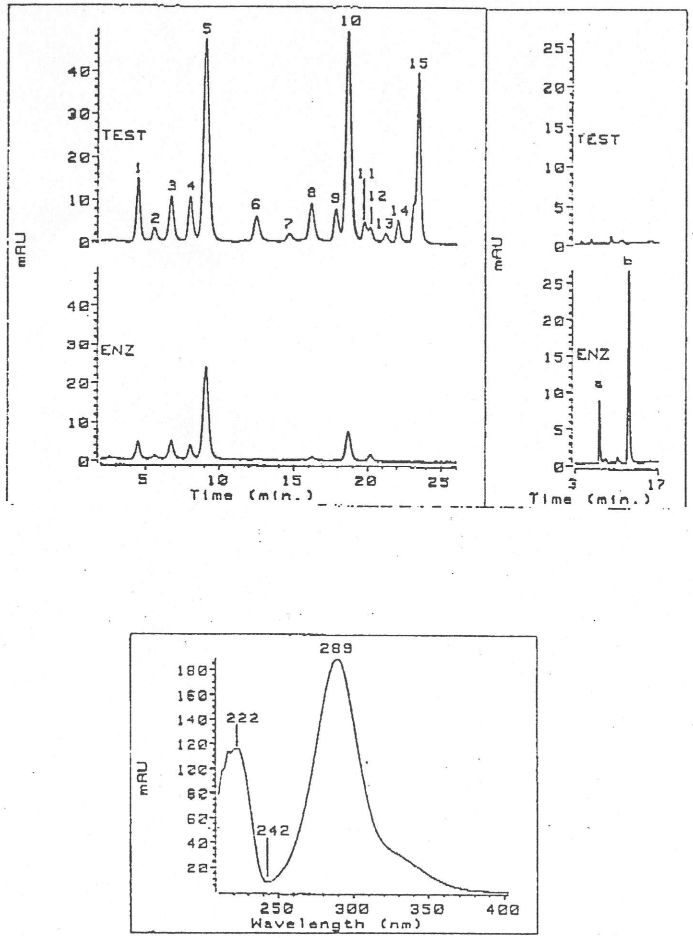


Fig. 8: Chromatographic separations (above) of free anthocyanins (left) and hydroxycinnamic acids (right) of a sinthetic solution before (test) and after (enz) treatment with glycosidase P93. UV-spectrum (down) of the unknown compound "a". [Nicolini et al., 1994b]

(Legenda: 1=delphinidin-3-monoglucoside; 2=cyanidin-3-monoglucoside; 3=petunidin-3-monoglucoside; 4=peonidin-3-monoglucoside; 5=malvidin-3-monoglucoside; 6,7,8,9,10=1,2,3,4,5-acetic ester; 11,13,14,15 = 1,2,3,4+5-p-coumaric ester; 12= unknown anthocyanin; a= reaction product, unknown; b= p-coumaric acid).

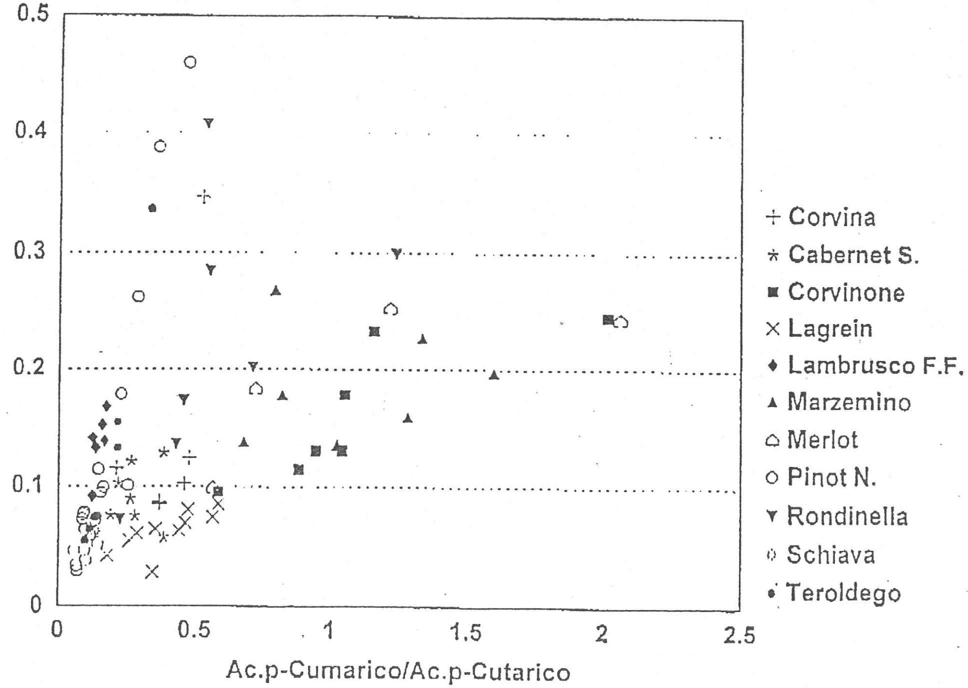


Fig. 9: Ratio of hydrolysis of caffeoyl- and p-coumaryl-tartaric esters in monovarietal red wines. [Mattivi et al., 1995]