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Research Note

Effects of pectolytic and glycosidase enzymes on the wine polysaccharide content of grapes and yeasts

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K e y w o r d s : polysaccharides, pectolytic enzymes, glycosidase enzymes, yeasts, grapes, wine.

Introduction: The two main sources of polysaccharides in wine are grapes and microorganisms. Wine colloids originating from yeasts are mainly mannoproteins and glucans, while pectins and the so-called neutral polysaccharides, consisting mainly of arabinose and galactose (arabans, galactans, arabinogalactans), are major components from grapes. Musts from grapes infected by *Botrytis cinerea* show a remarkable increase of glucans.

Not all polysaccharides show the same behaviour with respect to wines; glucans, for instance, are thought to be mainly responsible for filter colmatation (VILLETTAZ 1988) and slower membrane filtration (VERNHET *et al*. 1999); mannoproteins, on the other hand, seem to enhance growth of lactic bacteria (GUILLOUX-BENATIER *et al*. 1995), while at the same time influencing tartrate and protein stabilization of wine (MOINE and DUBOURDIEU 1995). Type II rhamnogalacturonans from grapes were shown to be involved in tartrate stabilization (GERBAUD *et al*. 1997).

Since the influence of the various types of polysaccharides on wine is different, enological treatments such as enzyme addition, aimed to selectively modify their content, are of major importance.

In the present study the influence of pectolytic enzymes, used in fermentation, on both the composition and the final content of colloids in Barbera wines was investigated. Fermentation tests were also carried out on synthetic media to evaluate whether pectolytic and glycosidase enzymes affect the content of mannoproteins from yeasts during vinification.

The results obtained support the idea that the use of pectolytic and glycosidase enzymes during must fermentation contribute to extract mannans from the cell wall of yeasts.

Material and Methods: A Barbera wine, vintage 1998, obtained from the experimental winery of the Istituto Sperimentale per l'Enologia (Asti) was used. To analyse glucidic colloids the colloidal fraction was isolated from 40 ml of wine, following the method of USSEGLIO-TOMASSET (1976). Polysaccharides were hydrolised with the two-step method of Saeman as reported by WILL and DIETRICH (1990). Monosaccharides were determined by gas chromatography via alditol acetates, following the method proposed by HARRIS *et al.* (1988): Fisons Instruments, Model HRGC MEGA 2 series equipped with flame ionization detector used in on-column mode. Column: OV 225 30 m, 0.32 mm, 0.25 µm (MEGA, Milano, Italy).

F e r m e n t a t i o n t r i a l s w i t h t w o m e d i a : (1) The synthetic polysaccharide free nutritive medium (SNM-pf) contained (a) the vitamins ($\mu g \cdot l^{-1}$), pyridoxine hydrochloride (400), thiamine hydrochloride (400), inositol (2000), biotin (20), calcium pantothenate (400), nicotinamide (400), and *p*-aminobenzoate; (b) the microelements ($\mu g \cdot l^{-1}$), $Na_2MoO_4 \cdot 2\ H_2O$ (200), $ZnSO_4 \cdot 7\ H_2O$ (400), $CuSO_4 \cdot 5\ H_2O$ (40), H_3BO_3 (500); KI (100); $FeCl_3 \cdot 6\ H_2O$ (400), and $MnSO_4 \cdot H_2O$ (400); and (c) the macroelements ($g \cdot l^{-1}$) $CaCl_2$ (0.1), NaCl (0.1), KH_2PO_4 (1.0), $MgSO_4 \cdot 7H_2O$ (0.5), $(NH_4)_2SO_4$ (0.944), $(NH_4)_2HPO_4$ (0.943), tartrate (3.0), KOH (to obtain pH 3.0) and sucrose (200). (2) The same medium as described above, but with malt extract (20 $g \cdot l^{-1}$) and yeast extract (10 $g \cdot l^{-1}$) added (SNM-polys.).

Fermentation was carried out in 300 ml flasks containing 200 ml of nutrient medium. A strain of *Saccharomyces cerevisiae* (10^6 cells$\cdot ml^{-1}$), previously grown on the same medium, was used. The incubation temperature was 20 °C, and every fermentation test was repeated twice. The fermentation time was 14 d. At the end of fermentation yeast was removed by centrifugation and the liquid was filtered through a 0.45 µm filter before the analysis of colloids was started.

The pectolytic enzyme VINOZIM FCE G (Novo Nordisk) with both, cellulase and hemicellulase secondary activities (0.5 $g \cdot l^{-1}$) and the glycosidase enzyme CITOLASE M-102 (Gist-Brocade) (2 $ml \cdot l^{-1}$) were used.

Results and Discussion: Statistically the study is based on 5 consecutive determinations of 5 separate colloidal fractions isolated from the same wine. The coefficients of variation were 1.7 % for galactose, 5.74 % for arabinose, 8.14 % for glucose and 8.29 % for mannose.

Tab. 1 shows the results of polysaccharide analysis of two Barbera wines made from the same grapes with the same vinification technique, but to one of them pectolytic enzymes were added. The latter wine had higher amounts of total colloids. Due to its specific action on the cell wall of skins, this enzyme seems to enhance the release of grape polysaccharides. Since mannose is a component of colloids produced by yeasts, a very similar concentration would be

T a b l e 1

Monosaccharides ($mg \cdot l^{-1}$) of the glucidic fractions of two wines, cv. Barbera, obtained from musts prepared in the absence (A) and presence (B) of pectolytic enzymes. tr: traces

Monosaccharides	A	B	Monosaccharides	A	B
Rhamnose	7.5	13.2	Xylose	0.8	1.8
Fucose	0.8	tr	Mannose	29.3	48.5
Ribose	tr	tr	Galactose	20.4	31.2
Arabinose	15.8	20.5	Glucose	7.7	5.6

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Table 2

Monosaccharides ($mg \cdot l^{-1}$) of the glucidic colloidal fractions at the end of the alcoholic fermentation in (1) a polysaccharide-free, synthetic nutrient medium (SNM-pf) with and without pectolytic and glycosidase enzymes and (2) a synthetic nutrient medium containing yeast and malt extracts (SNM-polys.) with and without pectolytic and glycosidic enzymes. tr: traces

Monosaccharides	SNM-pf	SNM-pf + pect. enzyme	SNM-pf + glycos. enzyme	SNM-polys.	SNM-polys. + pect. enzyme	SNM-polys. + glycos. enzyme
Rhamnose	0.3	1.5	tr	0.9	-	-
Fucose	tr	0.4	0.7	1.7	-	-
Ribose	-	-	tr	1.6	-	-
Arabinose	-	-	-	4.5	-	
Xylose	0.3	0.4	1.4	6.2	-	-
Mannose[(a)]	34.7	50.1	81.5	211.2	270.0	303.0
Galactose	-	0.8	1.3	7.8	-	9.7
Glucose	3.3	2.2	2.4	1096.0	11.2	23.4
Total	38.6	55.4	87.3	1329.9	281.2	336.1

[(a)]Anova $F_{(\text{enzyme addition})} = 52.9^{**}$

expected in both wines. The concentration of mannose, however, was higher in the wine treated with pectolytic enzymes, supporting the hypothesis that pectolytic enzymes work on both, cell walls of grapes and cell walls of yeasts.

To confirm this hypothesis fermentation tests were carried out in a synthetic medium free of polysaccharides. In the first test a pectolytic enzyme was added and in the second test glycosidase enzymes were added. As control, wine was fermented without enzymes.

Tab. 2 shows the polysaccharide content after fermentation. The data seem to confirm those obtained from Barbera wine. It may be argued that the enzymatic activity featured by the pectolytic enzymes, which helps to release mannans from the cell walls of yeasts, is probably a glycosidase activity occurring in the commercial preparation as a side activity.

Since mannans are intimately bound to glucans, it is possible that enzymes, such as glycosidases, hydrolyze glucan chains, thereby indirectly favouring the release of mannans from yeast cell walls.

In Tab. 2 the results of three fermentation tests, carried out with a synthetic nutrient medium containing polysaccharides from yeast and malt extracts, are reported. One of them was taken as a reference sample to evaluate yeast activity. Pectolytic and glycosidase enzymes were added to the other two trials, respectively. The data seem to confirm that the use of pectolytic and glycosidase enzymes, during must fermentation, supports the extraction of mannans from the cell walls of yeasts. The variance analysis (Tukey Test) calculated for the results shows a significant increase ($p = 0.01$) of mannose compared to the control and the two tests. The increase of mannose in the test with pectolytic enzymes and in the test with glycosidase enzymes was also significant ($p = 0.01$).

The hydrolysis of glucans, coming from the extracts added to the medium, by yeast was very poor. This supports the idea that the (1-3)-β-glucanase activity of yeasts, reported by HIEN and FLEET (1984) and FEUILLAT *et al*. (1989), targets only the glucans which form the yeast cell walls, while neglecting those occurring in the medium. According to HIEN and FLEET (1984) such an endoglucanase should therefore be strongly bound to the cell wall. In the presence of enzymes, glucans were almost completely hydrolyzed.

The cell mortality after the use of glycosidase enzymes, was not investigated. On the other hand, the ethanol production which was similar in all tests (11.8-12.2 %), regardless of enzyme addition, shows that the amount of enzymes added does not affect the cell wall stability.

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