# RELEASE OF FUNCTIONAL POLYSACCHARIDES BY WINE YEAST AND THEIR INTERACTION WITH WINE POLYPHENOLS

**S. ESCOT[(1)], M. FEUILLAT[(1)], A.JULIEN[(2)], et C.CHARPENTIER[(1)]**

[(1)] Laboratoire d'Œnologie, Institut Universitaire de la Vigne et du Vin, BP 27877 Campus Universitaire, 21078 Dijon Cedex, France

[(2)] Lallemand S.A, BP 4412, 31405 Toulouse Cedex France

e-mail: sandraescot@yahoo.fr

Polysaccharides represent one of the main macromolecular groups of wine. Some of these macromolecules, such as pectic compounds and neutral polysaccharides originate from the berry. Others are of fungal origin, the most well-known amongst these polysaccharides being a glucan of 1000 kDa produced by *Botrytis cinerea* upon infestation of the grape berry. Finally, there is an important group of polysaccharides that are produced or released by yeast of the genus *Saccharomyces*, namely glucans and mannoproteins. Mannoproteins are released by *S. cerevisiae* during alcoholic fermentation (Llaubères 1988) or during autolysis (Feuillat et al 1989).

The effect of macromolecules, namely “protective colloids” on wine stability is known since 1933 (Ribéreau-Gayon). Traditionally, however, these colloids were removed by fining or filtration (Feuillat et al 1987) because of their limiting membrane filtration performance.

Mannoproteins are described as having multiple effects in enology: They act as stabilizing agents regarding tartrate (Lubbers et al 1994) and protein precipitations (Moine-Ledoux et al 1992), as aroma support (Lubbers et al 1994) as well as being stabilizers for phenolic compounds (Saucier et al 1997).

Phenolic compounds and particularly anthocyanins and tannins contribute extensively to the organoleptic qualities of red wines, since they are responsible for color and texture, respectively.

The first observations made on the interactions between yeast cell walls and phenolic compounds go back to work from Augustin (1986) who showed that the addition of yeast hulls led to increased color intensity and hue. The contribution of free anthocyanins to red color was reduced while the contribution of $SO_2$-decolorizable tannin-anthocyanin complexes increased. Additionally, the tannins reacted less with the protein fractions of gelatin. These results have been confirmed by Llaubères in 1988, who showed that the addition of active dry yeasts (ADY) or mannoproteins extracted from these yeasts led to a reduction of the gelatin index which corresponds to a joint fining including tannin inactivation.

Saucier et al (2000) have shown that certain polysaccharides could control or prevent colloidal instability by “wrapping up” tannins. This phenomena is associated with the concept of “good” tannins and the organoleptic sensation of body and roundness.

Trials carried out during the last years on red wine ageing on fine lees have led to several observations on the evolution of polyphenol quality. Accordingly, astringency can not be explained simply by the chemical structure of the tannins but also by the incidence of their combination with non-phenols, such as mannoproteins.

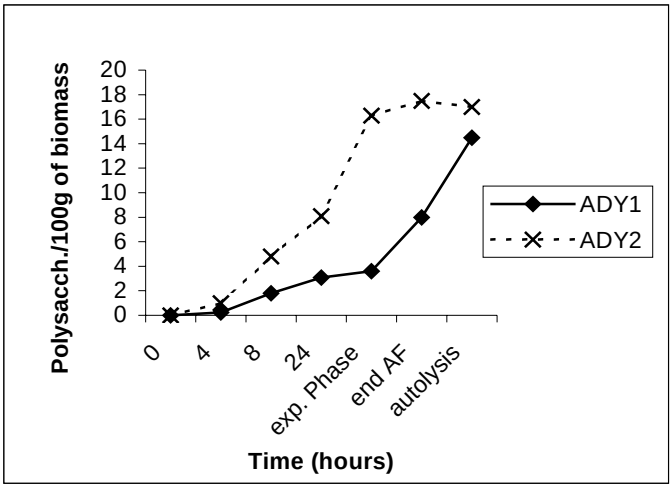
The understanding of the influence of mannoproteins on the stabilization of phenolic compounds requires a better knowledge of their exact composition as well as the mechanisms of their release and reactions involved. Hence, these observations have been completed by a more refined laboratory study and applied validation studies.

## 1-Release and composition of mannoproteins, and influence of the yeast strains

A study including 27 wine yeasts (Rosi et al 1998) showed that the release of polysaccharides by these yeasts during fermentation in the same must was strain dependent. Under the experimental conditions described, the quantity of released mannoproteins ranged between 15 and 144 mg/l. 48% of the yeasts studied released 51 to 80 mg/l and only 4% liberated more than 121 mg/l. Following this observation, studies were carried out in order to understand the mechanisms leading to the "yeast effect" dependant release of polysaccharides during alcoholic fermentation. For this, fermentations were carried out in a model medium simulating grape must initially depleted of colloids. Two yeast strains were used, one releasing low quantities of mannoproteins (ADY1) and a strong mannoprotein liberating yeast (ADY2). At the end of alcoholic fermentation, the yeasts were left in the medium for 10 days to simulate the beginning of yeast lees ageing.

The mannoproteins were harvested by ethanol precipitation at different fermentation stages: exponential growth phase, end of alcoholic fermentation and after 10 days of yeast autolysis. The amount of released mannoproteins was determined with a High Performance Liquid Chromatography (HPLC) system by peak area integration and comparison with commercial and purified mannoprotein standards. The results are given in mg/l.

*Figure 1 : Release of mannoproteins by 2 strains of selected commercial yeasts, ADY1 and ADY2. Determination by HPLC.*




It can be noticed that mannoproteins were accumulated in the medium during the fermentation and this accumulation continued during the conservation of the medium on the yeast lees (Figure 1). This accumulation was already considerable at the start of alcoholic fermentation for the strain ADY2, while being delayed for strain ADY2. In fact, after 8 hours of fermentation, strain ADY2 released 76% more mannoproteins as strain ADY1. After 2 weeks of autolysis, the difference remained only at 15%, but still in favor of ADY2.

The composition of these macromolecules was determined. They contained 80-90% polysaccharides and 10-20% proteins, which confirmed their cell wall origin. The principal sugars of the polysaccharide fraction were mannose and glucose.

The glycoproteins derived from strain ADY1 were composed of 80% mannose and 20% glucose, independently from the fermentation stage, while the cell wall glycoproteins derived from strain ADY2 had a mannose glucose ratio close to 1.

The mannoproteins are linked in the cell wall either to ß 1-3 or ß 1-6 glucan, or to chitin through ß 1-3 glucan. The cell wall composition of both strains was studied, as well as their sensitivity towards Quantazyme, in order to verify if the liberation phenomena were related to cell wall modifications.

Quantazyme is a very pure β 1-3 glucanase from Quantum Biotechnologie and its application on yeast cell walls allows to characterize structural changes thereof.

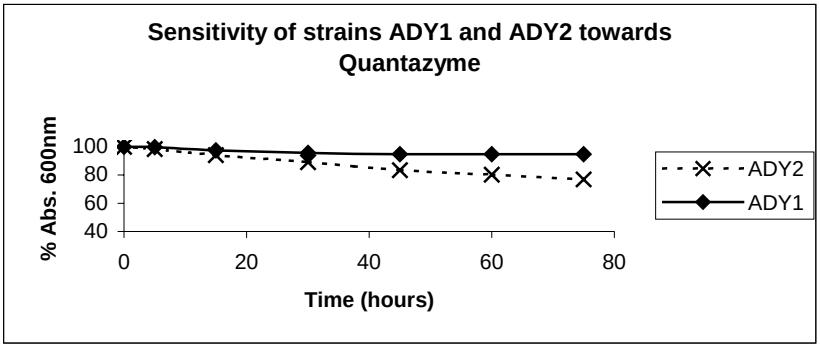
The experiments were carried out in duplicate with yeast in exponential growth phase.

Only strain ADY2 was sensitive towards the enzyme (Figure 2). At this stage of the fermentation, this yeast had already released many macromolecules (16.3 g / 100 g of dry biomass versus 3.6 g for strain ADY1). This means that the protein cover of the cell wall was less important, which would certainly make it more easily accessible for the enzyme.

The results of the ADY2 cell wall protein fractionation at this fermentation stage showed a content in laminarinase (a β 1-3 glucanase having a β 1-6 glucanase activity) releasable proteins that was 2-fold lower than for the cell walls of strain ADY1. This may indicate that the macromolecules released were mannoproteins linked to ß 1-6 glucan in the cell wall.

Finally, it is important to note that the content in cell wall chitin of the ADY2 strain was 2-fold higher than for strain ADY1.

*Figure 2 : Sensitivity of strains ADY1 and ADY2 towards Quantazyme during exponential growth phase.*




## 2- Interaction between mannoproteins and phenolic compounds:

The quantitative and qualitative differences among the mannoproteins released, led us to study their interactions with certain wine constituents, notably polyphenols, since strain ADY2 was known for making round wines with stable colors.

Tannins play an essential role for the organoleptic and visual qualities of red wines, as well as for their ageing capacity. They intensify the color by associations with anthocyanins (Ribereau-Gayon 1973). Tannins can also interact with macromolecules like mannoproteins and influence astringency, and the chemical and colloidal stability of wine. During wine ageing, numerous bindings between tannins and polysaccharides apparently increase (Glories, 1978) thus preventing the reaction of tannins with saliva proteins. Saucier (1997)

showed that after reaching a certain concentration, a colloidal stabilization occurred by adsorption of polysaccharides around the colloidal particles of certain tannins (procyanidins).

Initially, the study of polyphenol-polysaccharide interactions was carried out in the laboratory by adding purified mannoproteins derived from the two yeast strains to a young Pinot Noir wine to give a concentration of 100 mg/l. After 10 days of contact, different indices yielding information about the structure of tannins and the degree of linkage of anthocyanins were measured (Table 1).

*Table 1: Influence of mannoproteins released by two yeast strains on the properties of phenolic compounds*

| | **Yeast strain** | **Gelatin Index (%)** | **PVPP Index (%)** | **Ethanol Index (%)** |
|---|---|---|---|---|
| Control | | 68 | 33 | 6 |
| + 100 mg/l mannoproteins obtained from alcoholic fermentation | ADY1<br>ADY2 | 58<br>22 | 38<br>57 | 8,8<br>15 |
| + 200 mg/l mannoproteins obtained from alcoholic fermentation | ADY1<br>ADY2 | 58<br>22 | 37<br>57 | 6<br>13 |
| + 100 mg/l mannoproteins obtained from autolysis | ADY1<br>ADY2 | 29,5<br>29,5 | 34<br>36 | 7,1<br>8,5 |
| + 200 mg/l mannoproteins obtained from autolysis | ADY1<br>ADY2 | 27,5<br>23 | 36<br>34 | 7,2<br>10 |

The results obtained prove a positive influence of certain purified mannoproteins on phenolic compounds.

The mannoproteins obtained during alcoholic fermentation of strain ADY2 interacted strongly with phenolic compounds. A 30% decrease of the gelatin index (representing the astringency of tannins) can be noticed, as well as an increase of mannoprotein / tannin complexes (ethanol index) and combined anthocyanins (PVPP index). This phenomenon was not observed for the mannoproteins obtained from strain ADY1.

The mannoproteins obtained from autolysis led to a less marked difference regarding the interaction of the mannoproteins with tannins but they had no effect on the combinations formed with tannins and the polymerization degree of anthocyanins.

## 3- Field studies :

In order to support previous results and validate the effect of the yeast strains ADY1 and ADY2 on color intensity and mouthfeel of wines, different studies were realized during the harvest of 2000.

The studies were run by National Diploma in Enology (DNO) students in different areas: Burgundy, Beaujolais and Madiran. In all areas, the tanks were filled homogenously, the course of fermentation followed, and the yeast implementation verified. Then, various parameters (wine color, evolution of phenolic compounds) were measured at different stages of the fermentation and ageing.

Wherever the wines were produced, the same analytical and sensory profiles were observed. The results favored strain ADY2 from filling and up to 6 months of ageing with rounder tannins and a more stable color.

Here, the example of Madiran is shown, where the wines are more tannic and the difference between the various indices was more marked.

The analyses were carried out at the end of alcoholic fermentation, after malolactic fermentation and after 6, 10 and 15 months of ageing.

As can be seen in Figures 3 and 4, from the filling and up to 6 months of ageing, the results for the different indices favored ADY2, with more stable color (color intensity and PVPP indices higher) and rounder tannins (ethanol and ionization indices higher, lower tannin power index).

However, it can be seen that over time certain differences were reduced. This is in accordance with the laboratory studies involving the two strains: strain ADY1 released certain mannoproteins later, during autolysis. Nevertheless, and also in agreement with the laboratory studies, it is noticeable that the interaction of these mannoproteins (released during autolysis) with phenolic compounds was less important.

*Figure 3 : Course of ethanol index during ageing of 2 wines made with strains ADY1 and ADY2*

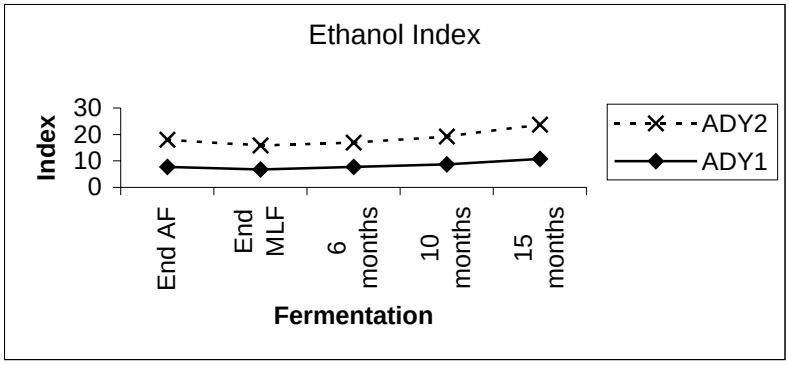
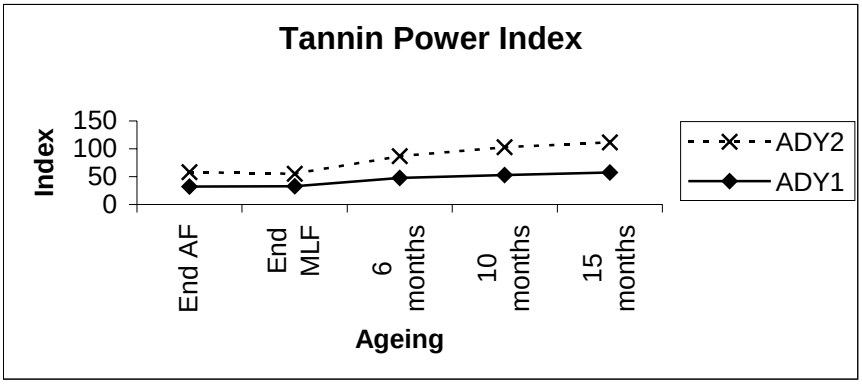


*Figure 4 : Course of tannin power during ageing of 2 wines made with strains ADY1 and ADY2*




The results of the sensory analysis followed the same trend: 8 tasters out of 12 found the wines made by strain ADY2 smoother (significant at 5%) than those made by strain ADY1. 9 tasters out of 12 also found that the wines produced by strain ADY2 had more volume and were less tannic (11/12) than the wines produced by strain ADY1.

## Conclusion

In view of these experiments it seems that the yeasts are capable of liberating variable amounts of mannoproteins during fermentation and autolysis with the final composition of these mannoproteins differing in dependence on the yeast strain used and the moment of liberation.

This work also reveals a positive effect of certain mannoproteins on the organoleptic qualities of red wines. In fact, certain mannoproteins interact positively with phenolic compounds. Two families of mannoproteins can be distinguished: those released during the fermentation and those released during autolysis.

Certain yeasts release mannoproteins that interact with phenolic compounds during alcoholic fermentation and this property has an influence on wine astringency and color stability.

The fractions of various mannoproteins released during fermentation and autolysis are currently being studied in order to explain the positive role of some amongst them on the astringency of red wines and the stabilization of their color.

The field experiments carried out for validation revealed modifications of wine composition that were certainly attributable to the effect of mannoproteins beneficial for the organoleptic equilibrium of the wines (roundness, mouthfeel). Strain ADY2 produced wines with better integrated tannins whatever the grape variety and vinification method was. This strain therefore reveals itself as being well adapted for the production of young wines or wines with short ageing.

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