

RAPIDASE® FILTRATION

Using Rapidase® Filtration to optimise the ageing of wines on lees.

Wine yeast, *Saccharomyces cerevisiae*, belongs to the ascomycetes family, a group of fungi whose cell walls are made up of β -(1 \rightarrow 3)-D-glucans and chitin (Figure 1). Yeast cell walls are also characterised by the abundant presence of mannoproteins: proteoglycans comprising 5 to 20 % peptides and 80 to 95 % D-mannose-containing chains. Chitin and β -(1 \rightarrow 3)-D-glucans are non-soluble components of the walls whereas mannoproteins are partially soluble and are released continuously during yeast growth and alcoholic fermentation.

As soon as alcoholic fermentation is complete, the viability of the yeast decreases dramatically and autolysis is initiated. Yeast autolysis is a very complex and slow process which involves hydrolytic enzymes such as proteases, nucleases, lipases and glycanases. Under the action of the endogenous hydrolases, yeast cytoplasm (peptides, amino acids, nucleotides, fatty acids,...) and wall (mannoprotein) components are released into the wine (Figure 2). This change in composition is associated with an organoleptic improvement in the wine which is manifest by better mouth feel, greater depth and more complex aroma. Wines aged on lees also present improved technological characteristics since their stability for both protein haze and tartrate crystals formation is improved by specific mannoprotein fractions.

The improvement in the wine due to spontaneous autolysis occurs very slowly during ageing on lees, which is traditionally carried out in oak barrels with weekly stirring of the lees. The marker for spontaneous autolysis during ageing is the release of mannoproteins. In the traditional process, less than 200 mg/l of mannoproteins become soluble within a 10-month period (Figure 3). The slow and limited nature of autolysis has been confirmed by electron microscopy scanning of 10-month-old lees where apparently intact ghost cells have been observed. These unaltered cell walls form a barrier against other yeast components being released into the wine and they dramatically limit the benefits of ageing. Recent studies have shown too, that the kinetics of mannoprotein release is quite independent of the yeast strain.

Fundamental research has demonstrated that mannoproteins become soluble when other cell wall components are hydrolysed through the action of endogenous yeast β -(1 \rightarrow 3)- and β -(1 \rightarrow 6)-D-glucanases and chitinases (Figure 4).

DSM has developed a modern solution to this issue which optimises the ageing process by allowing the autolytic process to be completed in a much shorter time period.

This solution is now available to winemakers as Rapidase® Filtration, a new enzyme formulation based on a blend of pectinase from *Aspergillus niger* and β -(1 \rightarrow 3)-D-glucanases from *Trichoderma harzianum*.

When Rapidase® Filtration is added to the wine at the beginning of ageing on lees, the release of mannoproteins is not only accelerated but also increased (Figure 5).

The total quantity of mannoproteins released is five to eight times greater than in untreated wine and this result can be reached in just two to three months instead of the period of the eight to ten months that is usually required. As previously mentioned, the fact that mannoproteins become soluble is a marker in a far more complex autolytic process. Wine treated with Rapidase® Filtration during ageing on lees is much preferred for its qualities of roundness and depth and for the improved "fusion" of the wine with the oak-flavour components.



In practice: with Rapidase® Filtration

Ageing on lees, a practice much used in white wine making, is being increasingly used in red wine vinification.

This type of ageing is currently carried out in barrels but there is a trend to using it for ageing in tanks. Nevertheless, the method imposes a number of constraints: the period of immobilisation, its use on small volumes (in barrels), a potential flavour of reduction. The use of Rapidase® Filtration enables such disadvantages to be mitigated.

In fact, its action means that ageing on lees can be optimised through autolysis of the yeast walls thus enabling mannoproteins to be released.

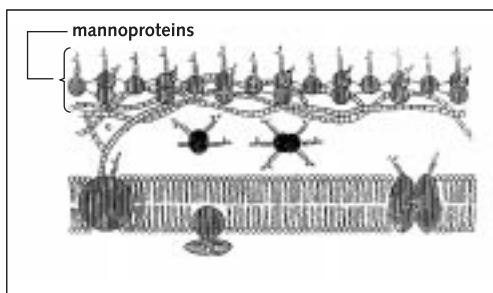
During ageing on lees, a dose of 2-3 g per hectolitre of Rapidase® Filtration is required for white wine and 3-5 g per hectolitre of Rapidase® Filtration for red wine.

The enzyme is incorporated at the end of alcohol fermentation whilst a minimum quantity of lees is retained. The duration of contact must be at least 4 weeks with the lees being stirred into suspension every week. This procedure increases the previously described autolysis effect, by releasing both nitrogenous elements and parietal glycoproteins. Weekly tasting will enable the organoleptic development of the wine to be monitored.

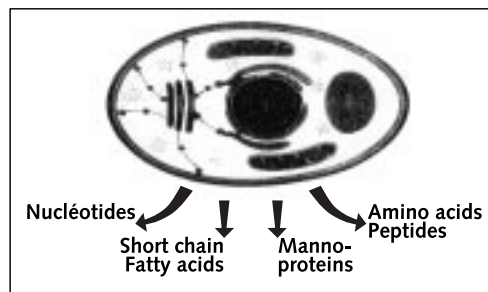
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Notes to Figures:

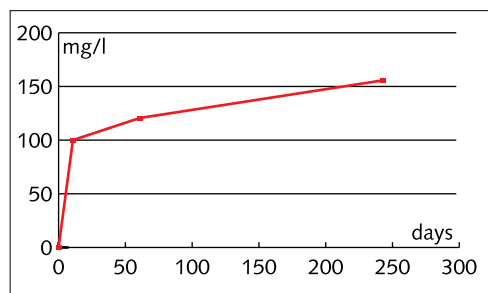
■ **Figure 1:** Schematic representation of the cell wall of a wine yeast: β -D-glucans are synthesised from the cytoplasm membrane and form a cross-linked network with chitin; mannoproteins are exposed at the external surface of the cell.



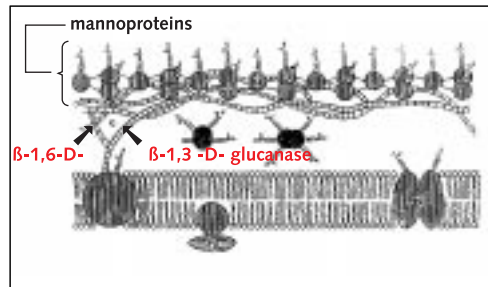
■ **Figure 2:** Release of yeast cytoplasm and wall components into the wine by autolysis.



■ **Figure 3:** Kinetics of the release of yeast mannoproteins into the wine during alcoholic fermentation and ageing on lees.



■ **Figure 4:** Action of β -(1→3)- and β -(1→6)-D-glucanases on a yeast cell wall: the hydrolysis of β -D-glucans allows the release of mannoproteins into the wine.



■ **Figure 5:** Release of mannoproteins into the wine during ageing on lees: the untreated control and the sample after the addition of 3 g/hl of Rapidase® Filtration

