RAPIDASE

HIGH SPEED ENZYMES SINCE 1922

Rapidase® Filtration

Liquid enzyme for reducing wine clogging power

Finding effective, innovative wine filtration enzymes

During filtration, wine moves due to the action of a pressure gradient and separates from the dispersed solid particle content, because the latter is held back by a porous filter device through which the liquid flows.
The smaller the size of the transit pores and the higher the resistance posed by the solid particles (which accumulate on the surface of the filter during filtration and form a deposit that increases as filtration proceeds), the higher the resistance posed by the filtering layer. This deposit progressively reduces filter permeability, so it eventually clogs and stops the filtration.

The cloudiness seen in wine is caused by two different particles:

1. colloidal dispersion of gums, mucilages, pectins, tannins, dyes and proteins; these are macromolecules or even molecular aggregates.
2. suspended, flocculated colloids, bacteria, yeast and mould.

At Oenobrands, the mission was to perfect an enzymatic preparation that would significantly reduce filtration interference caused by polysaccharide compounds in colloidal dispersion.
There are two objectives:

1. ensure that wines with obvious difficulties, but due to unknown causes, can be filtered
2. prevent filtration issues in wines that may be prone to these issues.

Numerous formulations were tested on various wines under laboratory conditions to perfect the new filtration enzyme. The two best formulations were then compared directly in the cellar, first on small-scale, then on large volumes (200–500 hl). One of the formulations proved to be particularly effective in most of the situations in which it was tested, and was therefore selected and subjected to further investigation regarding the impact on filterability and on the variation it induces in the polysaccharide composition of the wine. Tests have been conducted on different types of wines in many countries: Italy, France, Spain, Chile, Germany, Portugal and South Africa. Many tests are still in progress, and their aim is to expand the knowledge regarding the enzyme's impact in the most diverse conditions.

Rapidase Filtration: the ideal enzyme for any filtration

Rapidase Filtration is a unique, complex enzyme that applies various actions to facilitate wine filtration, regardless of the filter system used (filtering layers, streaming, membrane, crossflow).
This enzymatic preparation for oenological use contains high polygalacturonase and α-N-arabinofuranosidase activity, obtained from selected strains of *Aspergillus niger*, and endo-1,3 (4) - β-glucanase obtained from a selected strain of *Talaromyces emersionii*, as well as secondary activities derived from normal microorganism metabolism and retained by the finished product.
Aspergillus activities allow the degradation of both simple and complex pectic polysaccharide chains, while glucanase activity leads to the degradation of any glucans mainly from *Botrytis* contaminated grapes.
The enzyme's liquid formulation allows it to be easily added and mixed with wine at any time during the winemaking process.

Thanks to its specificity, **Rapidase Filtration** acts only on target molecules and does not in any way affect the quality standards of the wine. In addition, unwanted activities have been tested and kept at naturally low levels, with no significance from a winemaking or quality perspective.

The use of the enzyme leads to direct advantages for wine filtration and indirect benefits for cleaning and longevity of the filters.
The use on wines rich in lees and hence unfilterable, has led to the recovery of products that would otherwise go to waste or have to undergo treatments affecting quality and shelf life.

The enzyme has proven to be very flexible in application, adapting to working at very low pH (tested on white and red vinegar) and very high sulphite levels (laboratory tests on preserved must).
It has also been tested on fermenting must and on secondary fermentation, with remarkable advantages for processing time.
Similar to other enzymes, the minimum technological temperature limit is about 10 °C. As temperature increases, treatment times can be considerably reduced. Generally speaking, at least one week at a minimum temperature of 15 °C, or about two weeks at temperatures below 15 °C (but not lower than 10 °C), are recommended. A longer contact time does not create any kind of problem: once the substrate has been converted, the enzyme ceases to function. In the case of evident and abundant glucans, a treatment of at least two weeks at a temperature of 15 °C or higher is recommended for optimal results.
To clearly illustrate the enzyme process and efficacy, two successful filtration case studies are shown below: one on Porto wine lees and the other on a red wine from central Italy.

Red Port wine lees

The lees was that of the previous harvest, filtered with a Padovan ceramic membrane cross-flow filter in order to recover as much wine as possible.
There was 30% solids in a total volume of 9,000 litres.

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After just one hour of filtration, without use of the enzyme, the product was already showing heavy flow reduction, and after about four hours in total, the flow was obstructed. The flow was set at 300 litres per hour, with the minimum decided at 100 litres.
A dose of 5 g/hl of enzyme was added at a temperature of about 25 °C, with enzyme contact time of 48 hours.
Figure 1 shows filtration values:

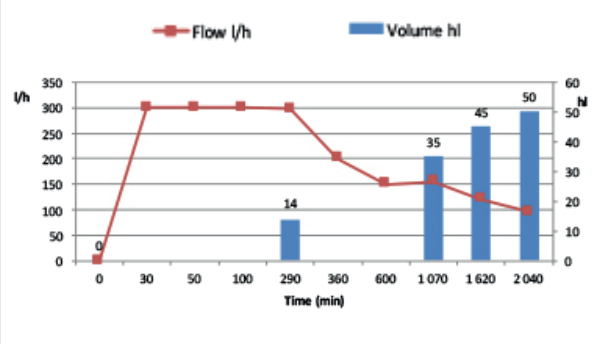

Figure 1. Parameters for the filtration of year-old Porto red wine lees, with 30% of solids. Padovan ceramic membrane cross-flow filter. Filters were effective for 290 minutes maximum, working with a sample with no added enzymes.

For each sample, the polysaccharides present in the different wine fractions collected during the test were quantified; **Figure 2** shows the quantities for the different classes of the most significant polysaccharides for testing purposes.

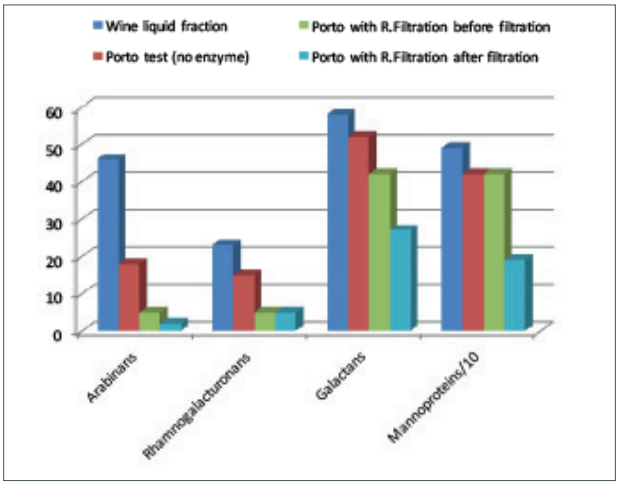

Figure 2. Polysaccharide analysis (for basic sugars in wine polysaccharides and galacturonic acid, using liquid chromatography after precipitation, lyophilization and hydrolysis) for the liquid fraction (without lees) and in Porto wine (liquid fraction and lees) with and without the enzyme, and with the enzyme before and after filtration.

Detail of the sugars analysed: galacturonic acid – above all homogalacturonans; arabinose from arabinan and arabinogalactan pectin side chains; xylose from pectin xylans and arabinoxylans and from xyloglucans; rhamnose from RG-II; galactose from pectin galactans and arabinogalactans; mannose from mannoprotein mannans; glucose from fungal glucans and xyloglucans.

As can be seen:
- homogalacturonans are present in reduced amounts and do not undergo significant alterations;
- arabinan side chains are hydrolysed to 90% and then removed by filtration;
- rhamnogalacturonans are hydrolysed by the enzyme; in this way they cannot clog filters and reduce filtration flow;
- galactans are partly hydrolysed by the enzyme and are then removed in part by filtration;
- mannoproteins are not hydrolysed by the enzyme as it does not contain measurable quantities of α-mannanase activity. They are present in high concentrations, given the high content of yeast lees and are removed in part by filtration (hence regardless of enzyme treatment) ;
- glucans are partly hydrolysed and removed by filtration (about 40%).

Test conducted on red wine

The wine used for the test was a Merlot IGT produced in central Italy by a medium-sized cellar.
The wine was divided into two 100-hl tanks; 5 ml/hl of the enzyme was added to one of the two tanks. The contact time was about 14 days and the temperature was around 15 °C. Subsequently, the wine from both tanks was subjected to separate filtration using a Juclas hollow fibre crossflow.

For the enzymated wine, the following differences were noted:
- when processing was complete, the filter was much cleaner;
- work continued seamlessly to the end, while in the case of the control sample wine, filtration had to be interrupted to carry out a wash cycle;
- flow for both samples were 17 hl/h, constant throughout the cycle; for non-enzymated wine, the flow progressively decreased and a wash cycle was required, while filtration of the enzymated wine continued without interruption to the end.

For this test, polysaccharides in the various wine fractions were again quantified; **Figure 3** shows the quantities for different classes of polysaccharides (reference molecules are indicated).

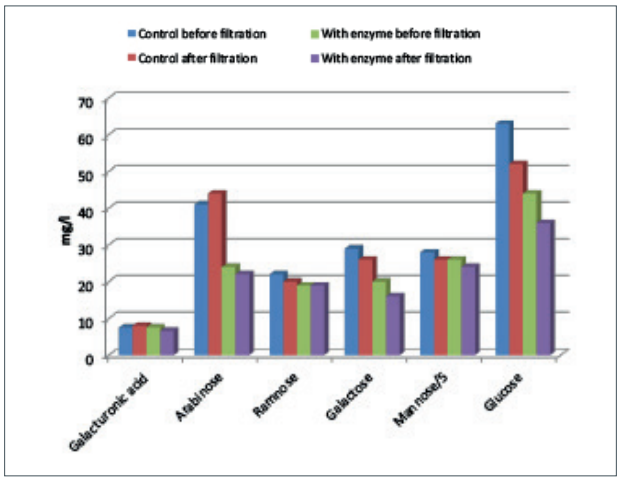

Figure 3. Polysaccharide analysis (method analysing the basic sugars of polysaccharides in wine and galacturonic acid, using liquid chromatography after precipitation, lyophilization and hydrolysis) in the control sample wine before and after filtration, and in the enzymated wine before and after filtration.

The analysis highlighted the following facts:

- only small amounts of linear pectic chains (homogalacturonans) are present in this wine and are therefore barely affected by the presence of the enzyme;
- pectin arabinan side chains are hydrolysed by 50%;
- RG-II was not really affected by the enzyme but for practical purposes, it showed no clogging effect on the filters;
- galactans were partially hydrolysed by the enzyme and about 50% was then removed by filtration;
- mannoproteins are not hydrolysed by the enzyme; nor did filtration affect the mannoprotein fraction;
- glucans were significantly hydrolysed by the enzyme, with a reduction of 30% before filtration and values almost halved after filtration. The action was definitely one which influenced filtration performance the most;
- insignificant amounts of xyloglucans and xylans are present in the wine (data not shown on the chart).

Conclusion

From all the tests conducted so far, the use of **Rapidase Filtration** brought significant advantages, as follow:

1. enhanced filtration flow, more evident if there are any clogging problems attributable mainly to glucans or complex pectic polysaccharides. In this case, it was possible to filter larger wine volumes without having to wash filters;
2. better filterability index, especially when the control sample wine shows high indices. In situations of trouble-free filtration, benefits can be seen during analysis;
3. in many cases filters are found to be cleaner, with long term benefits for filter life and processing time;
4. an option for filtration of wines that quickly clog filters, as in the case of wines made from unhealthy grapes or wines rich in polysaccharides;
5. the enzyme can be used in a wide variety of conditions (pH, sulphites, type of wine, temperature, alcohol content);
6. no organoleptic impact, even when adding larger amounts of enzyme;
7. enzyme can also be used in first and secondary fermentation, without affecting fermentation kinetics.

OENOBRANDS SAS
Parc Agropolis II - Bât 5
2196 Boulevard de la Lironde
CS 34603 - 34397 Montpellier Cedex 5
RCS Montpellier - SIREN 521 285 304
info@oenobrands.com
www.oenobrands.com

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