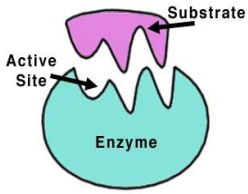
Enzymes and winemaking

Enzymes are nature's tools. They are needed in nowadays winemaking processes for mastering the production steps, creating value and most important protecting the quality of the wine and the environment. The grapes themselves have many enzymes, acting during the fruit maturation and along the winemaking process.

What is an enzyme?

An enzyme is a protein with a very strong catalytic power. An enzyme makes reactions happen fast and in a specific way: the enzyme acts as an accelerator. The transformations are known as being key-lock systems: only one enzyme catalyses one type of reaction. This is very important, as we are sure – ought to this characteristic - that in winemaking we can keep the process under control.



key-lock model

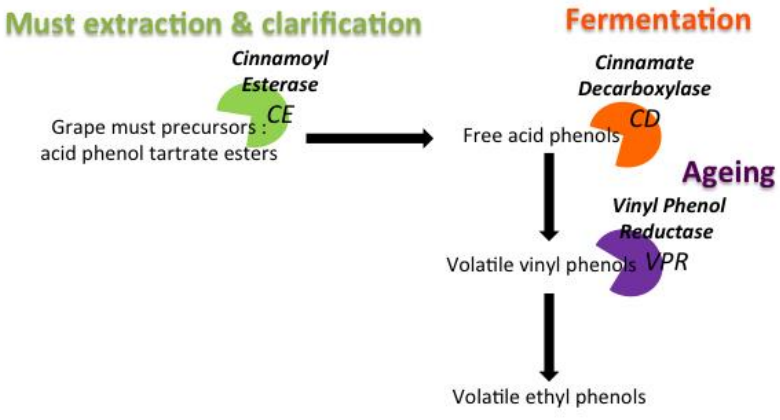
Thanks to their specificity, enzymes catalyse only one type of reaction. For example, polygalacturonase is splitting the main pectin chain between two molecules of galacturonic acid, thus reducing the size of the chain into smaller units.

Which enzymes in winemaking?

Most of the enzymes are hydrolases, meaning they hydrolyse substrates and liberate products. Enzymes need to be purified as the microorganisms used in fermentation – commonly Aspergillus *sp* - and the fermentation conditions favour the production of negative enzyme activities such as the cinnamoyl esterase (CE) activity.

The FCE concept: enzyme purification

FCE stands for Free of Cinnamoyl Esterase activity. To remove this negative enzyme activity, an extra purification step is needed at enzyme production level. The liquid pectinase formulation is maintained for several hours at very low pH to inactivate the CE enzyme. This enzyme, first described in 1976 by Burkhardt as a « depsidase » is responsible for the loss of freshness and fruitiness of fine white wines, altering their aroma profile. The work done by Barbe in 1992 has allowed Novozymes to purify their pectinases from this enzyme activity and to use the term FCE on their labels with a limit on activity, almost equal to zero.



Source of CE: pectinases A.niger, Botrytis
Source of CD: yeasts S. cerevisiae, Brettanomyces
Source of VPR: yeast Brettanomyces

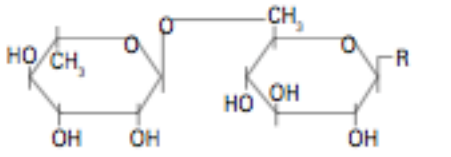
The Cinnamoyl-Esterase or CE starts the reaction by liberating higher concentration of free acid phenols. These molecules are then decarboxylated into volatile phenols (vinyl 4 phenol and vinyl 4 gaiacol) by the Cinnamate Decarboxylase – CD - of the yeast. The Vinyl Phenol Reductase – VPR - of *Brettanomyces* is further reducing the volatile phenols into ethyl phenols (source: Revue des Œnologues n°148, July 2013). Therefore using FCE pectinases is securing the winemakers to keep the initial composition of their must during the extraction and clarification processes.

Pectinases for extraction and clarification

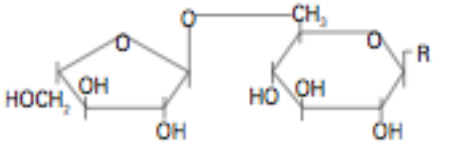
Under the term pectinases, many enzyme activities are present. In fact to hydrolyse pectins various enzymes are needed such as polygalacturonase, pectin esterase, pectin lyase, arabinanase, galactanase, rhamnogalacturonase, … Commercial enzymatic preparations contain a mixture of these enzymes needed in the application. The enzymes are obtained by fermentation of the fungi *Aspergillus sp*. either in a fermenter (submerged fermentation) or on plates (surface fermentation). The **enzyme concentration** is a key factor in the applications; therefore the dose and the contact time are detrimental to get the best results. These pectinases are preferably FCE - Free of Cinnamoyl Esterase activity - for use in winemaking applications.

Glycosidases for aroma liberation

These enzymes are also produced by *Aspergillus sp*. Under the term glycosidases, many enzyme activities are present like the glucosidase, arabinosidase, rhamnosidase, apiosidase, … However to liberate the aroma molecules (R) present in the glycosylated aroma precursors mostly under the form of glycosylated terpens, glucosidase is the main activity liberating the aroma in the free form (source: Revue des Œnologues n°149, November 2013) and aroma can be smelt only if they are in their free form.



6-O-α-rhamnopyranosyl-ß-D-glucopyranoside



6-O-α-arabinofuranosyl-ß-D-glucopyranoside

***R**: aroma molecule. The glucosidase activity is hydrolysing between the glucose and the aroma molecule, liberating the aroma compound as an odorous molecule.*

Glucanases for ageing on lees

In winemaking applications, the glucanases involved in the hydrolysis of glucans - originating either from the yeast or from *Botrytis cinerea* (mouldy grapes) - are composed of two major enzyme activities: the beta 1.3 glucanase and the beta 1.6 glucanase. These enzymes favour the release of mannoproteins by hydrolysing the cell wall yeast's glucan. Acid proteases might also be present in the preparation liberating small peptides. These glucanases differ from the glucanases needed to hydrolyse cellulose. It is important to note that the main activity involved in the hydrolysis of the cellulose is the beta 1,4 glucanase. These enzymes are produced by strains of *Trichoderma sp*. The strain *Trichoderma harzianum* has been especially selected to hydrolyse wine glucans thanks to the Ph. D research work of Pr. Dubourdieu in 1982. The preparation also

contains an acid protease very similar to the yeast protease, thus explaining the release of savoury peptides during the ageing on lees.

Pectinases/glucanases blend for filtration
In this application, pectinases are needed to hydrolyze pectins, in particular the ones released from the pomace in red winemaking in the press wines and glucanases to hydrolyze the glucan produced by the yeast during the alcoholic fermentation. Little is known about the impact of yeast strains on the release of glucan and its effect on wine filterability. Preliminary work conducted by Dr. Llaubères in 1988 has shown differences in the glucan concentration according to the yeast strain used for the fermentation.

Lysozyme or muramidase for microbial control
Lysozyme is a food preservative. This enzyme degrades the cell walls of gram-positive bacteria such as lactic bacteria in wine: *Oenococcus*, *Pediococcus* and *Lactobacillus*. Its use can greatly reduce the need for SO2, which poses a health hazard to individuals allergic to sulphites.

All enzyme activities applying in winemaking are listed by the OIV: Office International de la Vigne et du Vin. The documents can be consulted on line at www.oiv.int (oeno 11-18, 2004). Analytical methods for measuring the enzyme activities have been developed and are available on the OIV website under resolutions oenology 2007 and 2008. The list and the methods are regularly updated.

Mastering the processes
How to get the best out of your grapes using enzymes?

Enzymes are present in the grapes, in the yeasts and in the bacteria. The whole winemaking process is depending on enzymatic transformations (endogenous enzymes).

The major applications concern the use of pectinases either on grapes or on the juice (exogenous enzymes). On **grapes**, pectinases are used for direct pressing, skin contact and red maceration. On **juice**, enzymes are used for clarification in various processes: static clarification, flotation, centrifuge and filtration (Rotary Vacuum Filter for example).

The parameters affecting enzyme activity are the pH, the temperature and the time. In order to get good results, the **enzyme dose** is very important because the other parameters are given and cannot be changed easily. The dose is linked to the enzyme concentration in the product. Among the oenological parameters, sulphites (SO2) are not affecting the enzyme activity up to 500 ppm. Enzymes should not be used in presence of bentonite, the major enzyme inhibitor. In all applications, it is advisable to use the enzymes prior bentonite addition. If bentonite has been used, a racking should be done before adding any enzymes.

Treatments	Impact on enzymes
SO2 addition	bearable up to 500 ppm
bentonite	non bearable, enzyme activity lost even at low dosage
gelatine	bearable, more effective if used after enzyme
silice gel	bearable, more effective if used after enzyme addition

Enzymes are available both in solid and liquid forms. Solid enzymes should be diluted 1 to 10 (100 g for 1000 ml of juice or water). The enzyme solution can be prepared for one day of operation in the winery, as it remains stable. Typical enzyme doses vary from 3-4 g/100 kg grapes in extraction (dose can be higher with thick and slippery grape cultivars up to 5-6 g/100 kg) and 1-3 g/hl in clarification. The dose depends on the enzyme concentration. Large producers favour the enzymes in liquid form for convenience of use. Specific liquid purified pectinases exits also for wine like the newly introduced **Vinozym Ultra FCE**.

Extraction and clarification

The major effect of pectinases is on the **viscosity reduction** of the mash or the juice by hydrolysing the larger polysaccharide molecules into smaller fragments. As a result, the juice is extracted easily, at lower pressure, the juice yield is increased and the process runs smoothly. In red extraction, pumping overs are facilitated as more juice is available from the mash, colour extracted quicker and stabilized earlier with tannins. Hue is bluer. Settling, in respect to clarification, is speeded up because when pectins are hydrolysed, agglomeration and sedimentation starts rapidly. The enzyme should be added as soon as possible in the process: at the crusher for white and rosé pressing, when filling the maceration tank in red production, at the bottom of the vat when filing it for clarification. The addition can be done with a dosing pump, a drop-by-drop system, or using a watering can.

Results on extraction: example on direct pressing and red maceration.

The use of pectinases on grapes, for white or rosé wine production, at the crusher or during the filling of the maceration tank will help decreasing the viscosity of the mash. Enzymes remain active as long as pectins are present. The dose is the key parameter and need to be monitored according to the grape variety and its ripeness. Grapes varieties with thick and slippery berries need addition of higher enzyme dose. Unripe grapes will also need higher dose. Typical dosage varies from 3 to 5 g/100 kg grapes. For hard to press or extract varieties like Muscat or unripe grapes, the dose needs to be set at 5-7 g/100 kg grapes for getting the best results.

Measured parameters	with enzymes*	without enzymes
Total yield increase	6%	
Increase of free run juice	12%	
Effect on color : OD 420 nm	0,3	1,7
Effect on polyphenol : OD280 nm	10	17,6
Effect on turbidity reduction: NTU	87	508
Reduction of lees volume	50%	

** Vinozym Ultra FCE at 3 ml/100 kg*

Thanks to viscosity reduction lower oxidized colour (OD420) and reduced phenolic compounds (OD280) are released in the juice.

In red winemaking, the use of enzymes on grapes has the same effect: quicker and higher juice release as shown on the picture and therefore milder and more efficient extraction through mechanical actions using either pumping over, rack and return or punching down.

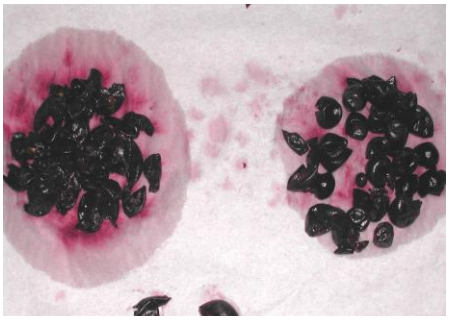
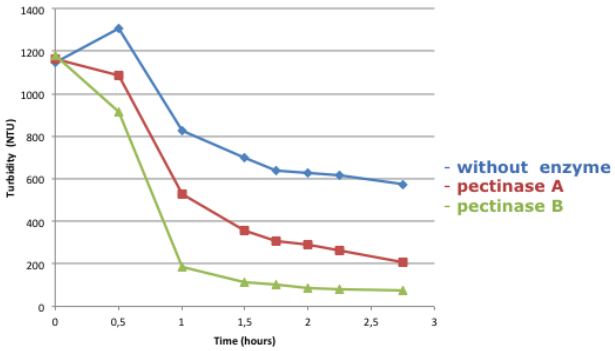


Photo: Wine Experimental Cellar, Novozymes-Lamothe-Abiet

The day after filling the tank, the enzyme treated Merlot grapes on the left (Vinozym Vintage FCE: 3,5 g/100 kg, addition on grapes when filling the tank) show more juice coming out from the mash and also more colour. The mash without enzymes on the right is showing more intact berries and less juice and colour release.

Results on clarification

The use of pectinases on the juice allows to rapidly starting the agglomeration and the sedimentation phases as soon as the first step – the flocculation - has been taking place.



To allow flocculation, pectins need to be hydrolysed. The speed of this first step in the clarification process is given by the enzyme concentration used and therefore **the enzyme dose** to get rapidly a negative pectin test. As soon as a negative pectin test is achieved, agglomeration and sedimentation can start. Then the total time for settling will depend on tank geometry and temperature. A negative pectin test is very important in flotation to achieve a quick and performing clarification. If not achieved winemakers can increase the enzyme dose to get it. If this depectinisation phase is well mastered, reduced dose of flocculent can applied.

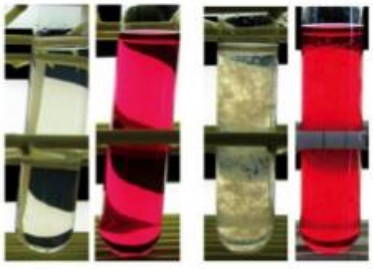
How to control enzyme efficiency?

Trials can be run at the start of harvest in order to define the adequate enzyme dose rate. Unripe grapes, thick grape skins and slippery grape varieties like Muscat or Semillon among other cultivars need a higher enzyme dose. Clarification trials can help define the dose by measuring the turbidity reduction over time and performing the pectin test every 30 minutes. A negative pectin test (tubes on the left) indicates that pectin is hydrolysed and agglomeration can start. The time needed for sedimentation will then depend on the temperature and the geometry of the tank.

The **pectin test** – or alcohol test – is a simple tool to monitor enzyme performance. The presence of pectin is visualized by adding acidified alcohol (ethanol + 1% pure HCl at 37%) into a grape juice or a wine sample. The sample taken from the tank should stand for 5 minutes prior to the test. To run the test, add in a tube:

- 5 ml of must
- 10 ml of the acidified alcohol
- Mix gently and let stand before reading

Wait 5 minutes to read the results. The pectin agglomerates in presence of alcohol.



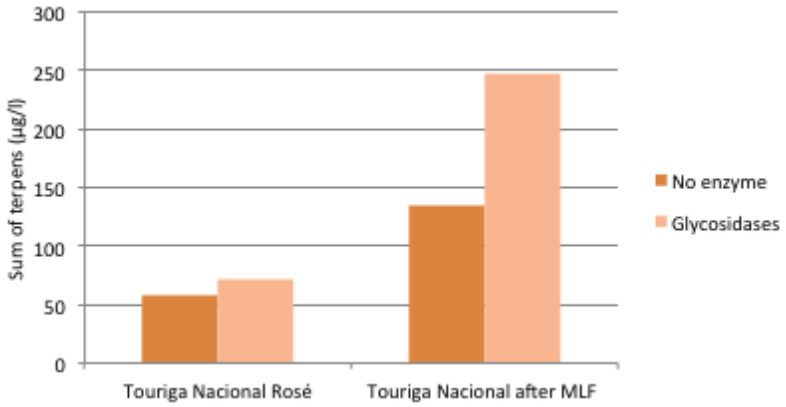
Left tubes: negative pectin test - Right tubes: positive pectin test

This test is used in flotation to control if enzyme addition has been efficient enough to obtain a negative pectin test. This is mandatory to be able to produce the flocculation. Addition of flotation agent can be used to help in the process but might be reduced in case a good depectinisation is achieved.

Aroma liberation

Glycosidases are added into the young wine right after the alcoholic fermentation. They are reversibly inhibited by glucose meaning that when glucose is consumed the activity of the enzyme is back. Fructose has no inhibitory effect on glycosidases. Trials conducted at various addition points: beginning, middle and end of alcoholic fermentation have shown that this enzyme gives best sensory results when added into the young wine. The presence of high level of CE in the glycosidase product available into the market can be one of the reasons. At the end of alcoholic fermentation, glycosidases can benefit from higher temperature and activation by the ethanol. The contact time is around 2 to 3 weeks. Results can be monitored by sensory evaluation. The level of free SO_2 has to be checked during the treatment. The enzyme activity can be stopped by bentonite addition (20 g/hl).

Trials conducted on red varieties like Touriga nacional have shown that it is possible to modulate the aroma profile by using glycosidases.



The graph shows the total terpenol levels in 2010 wines following 6 months bottle aging as obtained by Sygmington et al. (2011)*

** Symington C., Ferreira A. and Rogerson F., 2011. Industrial trials modulating Touriga Nacional aroma typicity. OIV XXXIV World Congress of Vine and Wine "The Wine Construction" 20-27th June 2011, Porto – Portugal.*

Glycosidases help release the terpene aroma molecules on Muscat and related cultivars. They can be used to treat a portion of wine and produce aromatic base wines for blending purposes.

Ageing on lees

In this application, enzyme blends contain beta (1,3-1,6) glucanases to help release the mannoproteins linked to the yeasts cell-wall glucan, often associated with pectinases to hydrolyse the pectins released during pressing in red winemaking. Glucanases from *Trichoderma harzianum* are specific for the hydrolysis of beta (1,3-1,6) yeast and *Botrytis* glucan. This high molecular weight polysaccharide is responsible for clarification and filtration problems. The only way to get rid of this polysaccharide is to hydrolyse it with enzymes. As for pectinases, it is advisable to use the glucanase or the pectinase/glucanase blends at the end of the alcoholic fermentation. In red winemaking, these enzymes are added under the cap towards the end of alcoholic fermentation

or just right after devatting in tanks or barrels. In white winemaking, they are preferably added after racking at the end of alcoholic fermentation in tanks or barrels for ageing on fine or gross lees. As the optimum temperature for glucanases is higher than for pectinases, the enzyme will benefit from higher temperature occurring during the malolactic fermentation. The presence of acid protease in the enzyme preparations will favour the release of small peptides.

Filtration

Enzyme preparations used in the pre fermentation steps – extraction and clarification processes – will have an impact on filterability improvement of the young wines. Also the use of pectinases/glucanases blend at the end of alcoholic fermentation will help to clarify, stabilize and filter the young wine. Any fining procedure will be more efficient if done after hydrolysing the long chain polysaccharides. In case no enzymes have been used in none of the processing steps, it is advisable to add enzyme as soon as possible in the young wine in order to hydrolyse the large chains of pectin particularly in red wines and the glucan released by the yeast. The performance can be check by running a filterability test using the CFLA (Critère de Filtration Lamothe-Abiet) measurement. This test is equivalent to the filterability test used prior bottling using membranes of higher porosity (5 and 1,2 micron).

Creating value

How to master your production costs using enzymes?

The enzyme treatment cost can easily be compensated by the yield increase in the extraction and clarification processes thanks to quicker sediment compaction in the latter, the reduction of fining agent use and wine losses during fining and filtration steps thanks to size reduction of the larger polysaccharide molecules. Also time can be reduced, performances of equipment like presses, flotation, centrifuges and rotary vacuum filter can be improved. In large wineries operation time is valuable as large quantities of grapes are coming in every day. All the savings can be documented by running dedicated trials at the start of the harvest. The yield increase averaged from 4-6% in direct pressing in white and rosés production and 3-4% in red wines with very short or short maceration time.

Protecting and revealing quality

How to maximize the quality with well-mastered processes?

Thanks to milder extraction with the use of enzymes, juice can be released at lower pressure avoiding extracting harsh tannins in particular if the grapes are not enough ripe. In red grape varieties, colour is easily released and stabilized. Clarification of both must and young wine is better performing allowing clarifying quicker and ensuring better microbial stability. Also less classical chemicals are needed and the use of SO2 can be better rationalized. Thanks to enzymes, well-mastered processes have an impact of final quality in all steps of winemaking.

Enzymes are very valuable tools for the winemakers. These biocatalysts help in mastering the production processes. Today time is an important parameter for large production operations and thanks to their properties enzymes have a role to play. They are convenient to use and they are biodegradable. Mastering their use – in particular the dose and the addition point – is important to get the best results in the applications.

Reference:
Enzymes and wine quality, Rose-Marie Canal-Llaubères in Managing wine quality, vol. 2: oenology and wine quality, ed. By Andrew G; Reynolds, Woodhead Publishing, 2010.