The A – Z of Wine Enzymes

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l'œnologie par nature

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LAFFORT is a fourth generation family owned business founded in 1895 by Jean LAFFORT who subsequently participated in all the major œnology developments following on from Pasteur's discoveries. **Laffort**'s extensive research and development budget has allowed the company to develop a range of œnological products combining ***research*** and **innovation**, with ***tradition*** and **expertise**.

The use of commercial enzymes is widely accepted as an integral part of winemaking today. With countless suppliers offering a vast array of enzymes under different brand names, the choice available to winemakers is staggering. Many view enzymes as commodity products, purchased on price alone. However, significant differences in quality exist between suppliers' offerings, and a good understanding of enzymes is necessary to make informed decisions. This booklet explains the basic concepts of wine enzymes.

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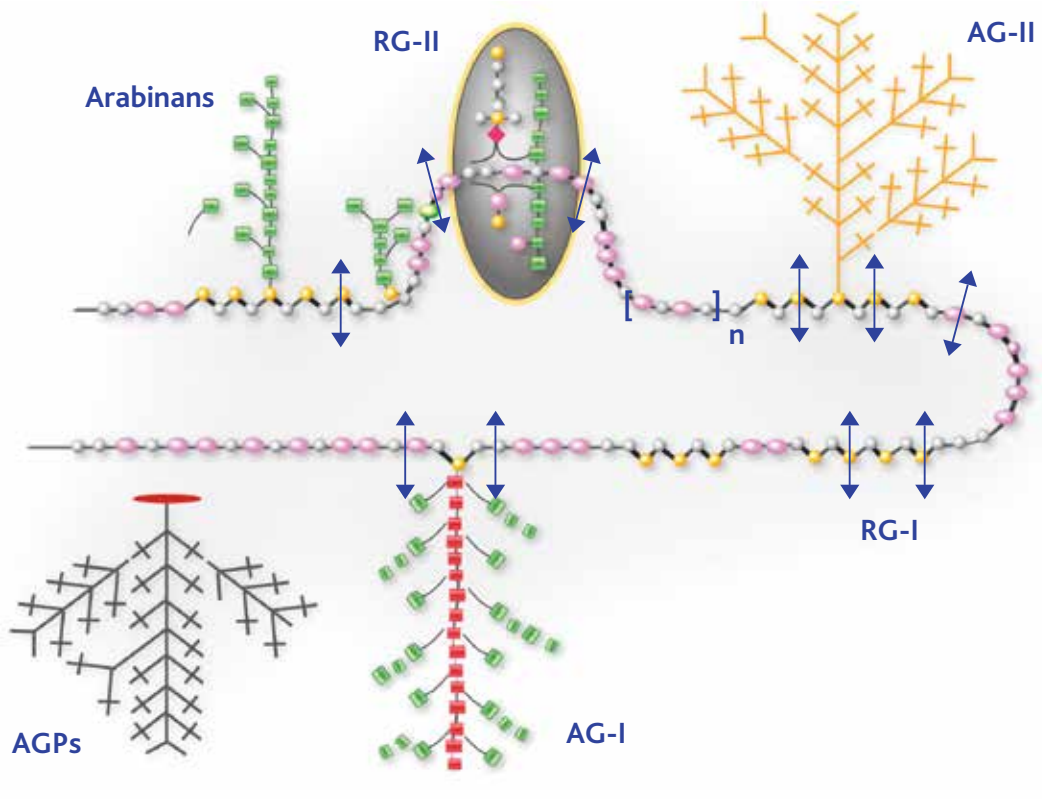


A graphical representation of the pectin molecule structure. The molecule consists of linear portions as well as highly branched portions of different sugar molecules. The sugar molecules can be linked with proteins. AG: arabino-galactan, AGP: arabino-galactan protein, RG: rhamno-galacturonan. Source: Doco et al. 1995 (4)

WHAT ARE ENZYMES?

Enzymes are proteins which act as biological catalysts. They can facilitate as well as accelerate reactions, without being changed through the process. Enzymes are highly specific, acting on one substrate, or a limited number of substrates under mild conditions. All living organisms contain enzymes. Wine is the product of the enzymatic transformation of grape juice. The most well-known wine enzymes are the pectinases.

WHAT IS PECTIN?

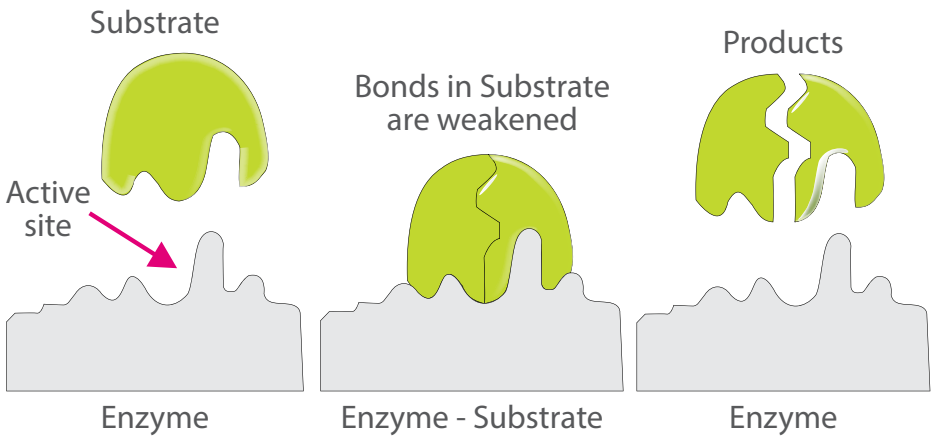
Pectin is a structural polysaccharide, a chain of various sugar molecules. It is found in the cell walls of plants, including grapes. It has various functions, the most important one being the integrity of the plant tissues. The structure of pectin is very complex. A very simplified explanation is that pectin consists of a main chain of specific sugar molecules, composed mostly of galacturonic acid units, with side chains of different sugar molecules such as arabinose, galactose and rhamnose. Together with other polysaccharides such as glucans, cellulose and hemicellulose, grape pectins play a role in the viscosity, clarity and filterability of wines. In general, the longer the pectin chain – the lower the filterability of a wine. Polysaccharides also play a role in the perception of tannin astringency (3).

WHAT IS GLUCAN?

A glucan molecule is a polysaccharide (sugar chain) consisting of D-glucose units only, which can be branched. There are many different types of glucan molecules existing in nature. The way in which the glucose molecules are bound together determines the type of glucan. Cellulose (ß 1.4 linked glucose units) is the most well-known and prevalent glucan in nature (making up approximately 33% of all plant matter on earth) and is found in grape cell walls.

When *Botrytis cinerea* infects grape berries, it secretes a specific glucan (ß 1.3-1.6 linked glucose units) into the grape juice. This polysaccharide is highly viscous. Its exact nature and structure has been determined by Dubourdieu (5). The same type of glucan produced by *Botrytis* is also found in wine yeast cell walls. It is released by wine yeasts during and post fermentation. The amount of glucan produced differs from strain to strain (7).

Spoilage lactic acid bacteria such as *Pediococcus* also produce glucan – ß 1.3-1.2 linked glucose units responsible for high viscosity (ropiness). As with pectins, the glucan chain length and structure determine its effect on wine filterability.



A schematic diagram explaining the concept of enzyme – substrate interaction: the key-lock model (2). Enzymatic catalysis.

WHAT IS HEMICELLULOSE?

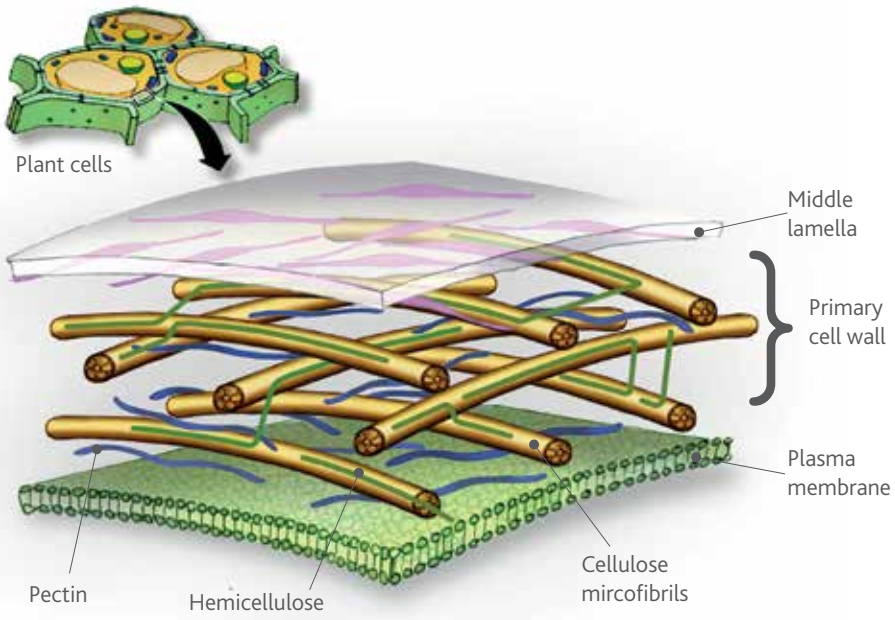
Hemicellulose is a generic term for polysaccharides related to cellulose. They make up approximately 20% of most plants. They differ from cellulose in that they are much shorter chains of not only glucose, but various other sugars too. These are predominantly xylose in addition to glucose (xylan, xyloglucan), as well as mannose, galactose, rhamnose and arabinose. Hemicellulose is branched, whereas cellulose is un-branched. Hemicellulose is found together with pectins and cellulose in grape cell walls. Together these three structural components of grape cell walls form a physical barrier around the grape cell, and therefore need to be hydrolyzed during the winemaking process in order to release the juice, aroma, color etc...

WHY USE ENZYMES FROM FUNGAL ORIGIN?

In addition to having polysaccharides in their cell walls, grapes also have some enzymes (mostly pectinases) to break these polysaccharides down. This is an important feature in grape berry (and fruit in general) ripening. Ripening is associated with fruit softening to the point where birds and insects can break through the skins and release the seeds. This ripening is due to the change in pectins resulting from the grape pectinases becoming active as the berry matures. In winemaking, however, these grape pectinases are inhibited by the low temperatures, SO_2 and alcohol.

Some wine yeasts can secrete polysaccharide degrading enzymes, but this activity is limited under winemaking conditions and doesn't occur during the pre-fermentation stages.

Fungi produce a much broader range of polysaccharide degrading enzymes than grapes. They are very active under winemaking conditions. For example, pectinases produced by *Aspergillus niger* are active at wine pH (2.8 – 4), resistant to 500 ppm SO_2, resistant to 17% alcohol (sometimes higher in the case of glycosidases) and are also active between 41 – 149°F. Detailed application guidelines are given at the end of this booklet.



The grape cell wall consisting mainly of cellulose, hemicelluloses and pectins.

- ***Pectinases***

Pectinases are derived from *Aspergillus niger*. There are at least six different enzymes responsible for the breakdown of the pectin molecule. The main pectinases are: pectin lyase (PL), pectin methyl esterase (PME), polygalacturonase (PG), arabinanase, rhamnogalacturonase and galactanase. Many of these enzymes also exist as iso-enzymes (a different version of the same enzyme) that have different pH and temperature optima and affect different parts of the pectin chain. Therefore, the protein patterns obtained by electrophoresis are specific for a given enzyme preparation. Most of the enzymes hydrolyze the sugar chains according to an endo and exo mode of action, the endo mode of action being more efficient in terms of reaction speed and performance.

In the same way that yeast producers culture different strains of *Saccharomyces*, various strains of *Aspergillus* are used to produce different enzyme preparations. This is the first point of differentiation between commercial pectinase preparations, since different fungal strains will produce different combinations of enzymes.

- ***Hemicellulases***

The breakdown of hemicelluloses involves the action of, amongst others, at least three different enzymes: xylanase, galactanases and arabinanases. Technically, galactanases and arabinanases are also classified as pectinases since arabinan and galactan also form part of pectin side chains.

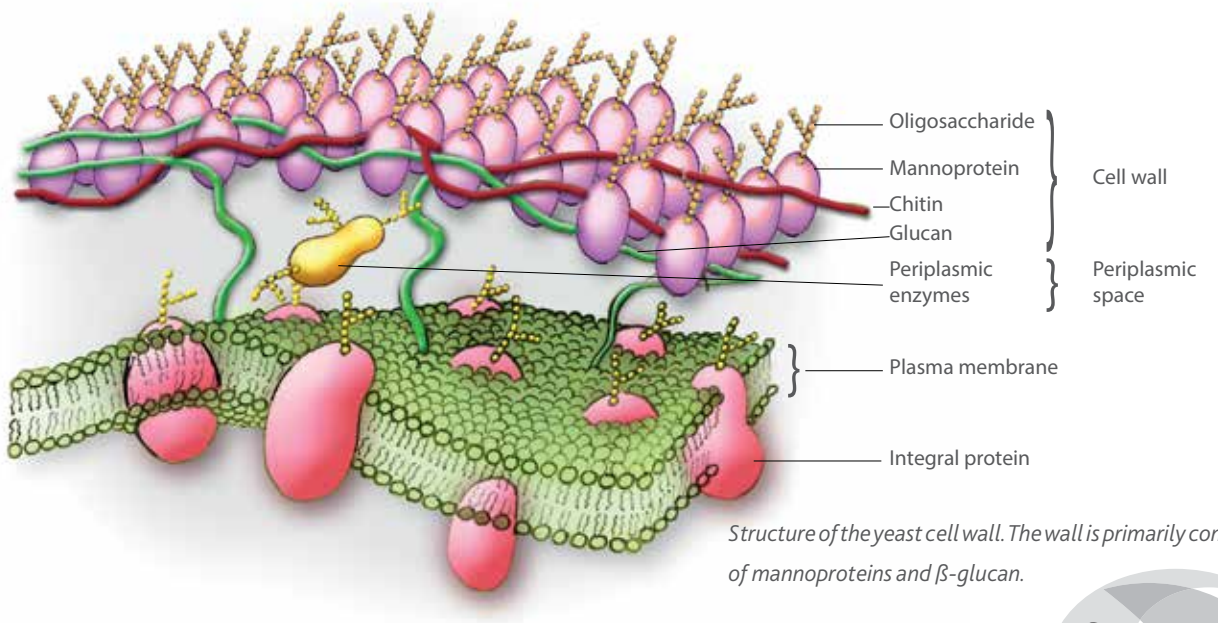
- ***Cellulases***

Cellulases are produced by *Aspergillus sp.* as a side activity of pectinases production. Typically cellulases are produced by strains of *Trichoderma longibrachiatum*. There are various enzymes involved in the breakdown of native cellulose. Their oenological role has not clearly been defined yet.

- ***Glucanases***

Glucanases for winemaking applications are produced by a different fungus than for pectinases – *Trichoderma harzianum*, as *Aspergillus niger* only produces ß 1.3-1.4 glucanase as a side activity and not ß 1.3-1.6 glucanase. When *Botrytis* infects grape berries, it secretes a significant amount of long chain glucan molecules (ß 1.3-1.6 glucan) into the grape juice. This glucan has a very high molecular weight and is responsible for very high viscosity. Wines produced from this grape juice will contain these long chain glucans, resulting in poor filtration and clarification (9). Treatment of the wine with glucanase containing enzymes can reduce the glucan chain length and thus improve the wine's filterability. An enzyme preparation known as **EXTRALYSE®** has been specifically developed for this application.

The same type of glucan secreted by *Botrytis* is found in yeast



Structure of the yeast cell wall. The wall is primarily composed of mannoproteins and ß-glucan.

cell walls (yeasts are considered the simplest form of fungi). This glucan can be liberated from yeast during fermentation and while on the lees. The release of glucans into the wine is accompanied by the release of various other wanted yeast compounds such as mannoproteins, amino acids, low molecular weight peptides and nucleotides, that can have a significant effect on wine mouthfeel. Pectinase/glucanase enzyme preparations such as **EXTRALYSE®** significantly enhance this yeast autolysis process and thus the release of these positive compounds.

- ***Glycosidases***

Glycosidases, like hemicellulases and cellulases, are by-products of pectinase production. Glycosidases are a group of enzymes responsible for releasing aroma compounds linked to sugar molecules. When these aroma molecules are linked to sugar molecules (called glycosylated aroma precursors) they are non-aromatic. Once the sugar molecules are removed they become aromatic. Monoterpenes (linalool, citronellol, nerol, geraniol,…) and C_{13}-norisoprenoid derivatives (ß-Ionone, ß-Damascenone) responsible for floral and fruity notes (rose, violet, citrus, …) are examples of grape varietal aroma compounds that occur in grapes in glycosylated precursor form. The main glycosidase enzyme is a ß-Glucosidase – an enzyme that removes glucose from the aroma compound. These enzymes are inhibited by high glucose concentrations and can therefore only be used towards the end of the alcoholic fermentation. Sweet dessert wines can benefit from the addition of glycosidases to lift their aroma: in this case a higher enzyme dose should be applied to balance the glucose inhibition. They can significantly enhance the aromatic profile of grape varieties containing mainly terpene aroma compounds such as Riesling, Gewürztraminer and Muscat. These aroma compounds are also present in smaller quantities in many other varieties (8).

The use of such enzymes on red wine is not advisable as it can destabilize red wine color, since anthocyanins are also glycosylated.

WHAT ARE PURIFIED ENZYMES?

Purification is one of the major advances in winemaking enzymes in recent decades. During enzyme production, the fungi produce a whole cocktail of enzymes, including various wanted and unwanted side activities. A "side activity" is defined as an enzyme produced in much smaller amounts than the main enzymes, which in this case are the pectinases. Hemicellulases are examples of wanted side activities and cinnamyl esterase is an example of an unwanted side activity. Depending on the application of the enzyme, ß-Glucosidase can be a wanted or an unwanted side activity.

- ***The importance of cinnamyl esterase free enzymes***

Cinnamyl esterase (CE) catalyzes the first reaction in the production of vinyl-phenols. This activity is always present in pectinase preparations (10), if not removed by a specific purification step. The second reaction, resulting in the actual production of vinyl-phenols, is catalyzed by wine yeast (decarboxylaze activity). Yeasts that have the ability to catalyze this reaction are categorized as POF (Phenolic Off Flavor) positive yeasts. Vinyl-phenols are responsible for the loss of fruity character and, in worst case scenarios, medicinal smells in white wines. Most commercial wine yeasts are POF positive, so it is important when using these wine yeasts that careful consideration be given to the choice of settling and white skin contact enzymes. Laffort's **LAFAZYM® CL**, **LAFAZYM® EXTRACT** and **LAFAZYM® PRESS** are examples of purified (cinnamyl esterase free) white wine enzymes, and are therefore safe for use with POF positive yeast strains.

LAFFORT also offers a range of POF negative strains, such as **ZYMAFLORE VL1®**, **VL2®**, **X16®** and **Alpha**.

In red winemaking, this same occurrence may result from the use of cinnamyl esterase contaminated enzymes. The cinnamyl esterase activity is not fully inhibited by wine tannins as previously thought, and therefore in the presence of non-purified enzymes, the concentration of vinyl-phenol precursors will increase. The danger with the formation of precursors of vinyl-phenols is, if *Brettanomyces* spoilage occurs, they will be first decarboxylazed into vinyl-phenols, (cinnamate

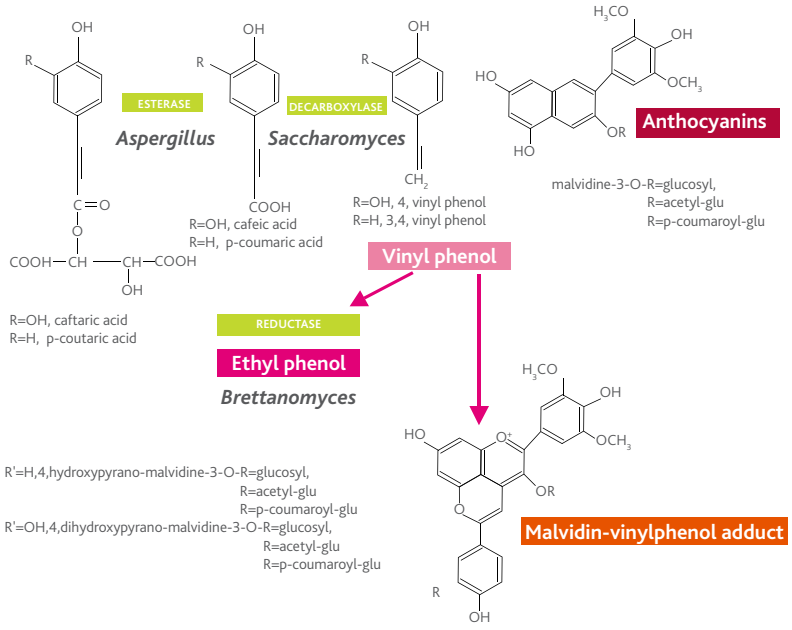
decarboxylase activity of *Brettanomyces*) and then reduced into ethyl-phenols (vinyl-phenols reductase activity only present in *Brettanomyces*). These ethyl-phenols have a much more intense medicinal/barnyard aroma than vinyl-phenols. The use of cinnamyl esterase contaminated enzymes in red winemaking can therefore lead to increased substrate for *Brettanomyces* off-flavor production.

We also have to note than vinyl-phenols formed during fermentation can combine with anthocyanin to produce malvidin-vinylphenol adducts of orange color (Ducasse, 2009 (14)), rendering them unavailable for *Brettanomyces*. However, vinyl-phenols formed a few months after alcoholic fermentation due to *Brettanomyces* spoilage, will most definitely be converted to ethyl-phenols, since very little monomeric anthocyanin remains to bind the vinyl-phenols.

Cinnamyl esterase free enzymes are therefore important in red winemaking. The use of purified (cinnamyl esterase free) enzymes such as **LAFFORT**'s **LAFASE® HE GRAND CRU** and **LAFASE® FRUIT** is therefore very important for red wine maceration.

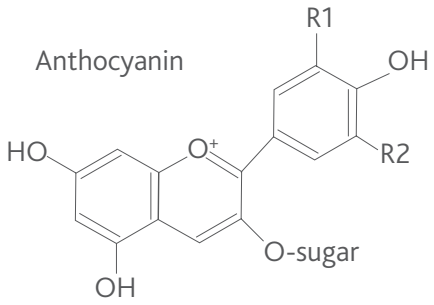
- ***The importance of glucosidase free enzymes***

ß-Glucosidase belongs to a group known as glycosidase enzymes that can free monoterpenes from their non-aromatic precursors, and targeted use of these enzymes after fermentation on specific grape varieties can, therefore, have a very positive outcome. However, ß-Glucosidase can also remove the glucose molecule that stabilizes anthocyanin, forming an unstable aglycon that spontaneously morphs/changes into a colorless form. This is why ß-Glucosidase activity is often referred to as "anthocyanase" activity. It is important that any enzyme used for the production of red or rosé wines should therefore not have any anthocyanase activity. **LAFFORT**'s **LAFASE® HE GRAND CRU** and **LAFASE® FRUIT** (red wine enzymes) are purified from this activity. **LAFAZYM® CL**, **LAFAZYM® EXTRACT** and **LAFAZYM® PRESS** (white wine enzymes) are also free of anthocyanase activity and therefore safe for use in the production of rosé wines.



	CONTROL	LAFASE® HE GRAND CRU	NON-PURIFIED ENZYME
Wine 1	107	nd	1904
Wine 2	172	261	737
Wine 3	65	57	563

A comparison between a control wine (no enzyme and no ***Brettanomyces*** *contamination) and two enzyme treated wines, contaminated with Brettanomyces. The values represent the concentration of ethyl-4-phenol (perception threshold - 400 µg/L). Source: Vincent Gerbaux, 2002 (11).*



R1, R2 = H, OH, OCH_3
Sugar= glucose, arabinose, galactose

A schematic diagram of an anthocyanin molecule stabilized by a sugar molecule.

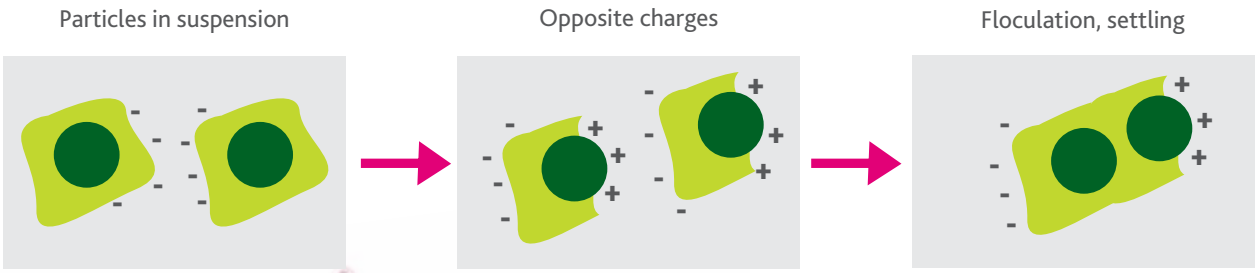
WHAT ARE THE DIFFERENT APPLICATIONS FOR ENZYMES IN WINEMAKING?

- ***Settling and flotation of musts***

Settling or flotation is a very important process in white and rosé winemaking as it enhances the aromatic finesse of wines. It is also essential to young red wine quality produced by thermo treatment. Grape macromolecules such as polysaccharides affect the colloidal make-up of wine by keeping grape solid particles in suspension. Degradation of pectins with pectinases will greatly diminish the colloidal load and lead to agglomeration of the particles. In the case of settling, sedimentation then follows since these compounds become too big to stay in suspension. The time it takes to achieve complete de-pectinization will depend on the quality of the enzyme used, dosage, settling temperature, grape variety and maturity level, as well as the physical treatment of the grapes prior to entering the settling tank. It could take anywhere between 1 – 8 hours to occur, or it may only happen partially if the enzyme quality and concentration is weak. The length of sedimentation will depend on the geometry of the tanks. In the case of flotation, all of the above factors will also determine the speed and efficiency of the process. Flotation is usually the clarification method of choice in wineries without sufficient cooling capacity for cold settling, or where producers want to save time by doing continuous flotation instead of overnight settling. Rapid de-pectinization is therefore essential for efficient results. Winemakers are advised to do pectin tests and measure clarity (NTU) to compare the performance of different supplier's settling / flotation enzymes.

Settling / flotation enzymes consist mainly of the three key pectolytic enzymes: polygalacturonase (PG), pectin lyase (PL) and pectin methyl esterase (PME), since it is adequate to quickly degrade the pectin structure of grape pulp cells. Other activities are present, but not in high concentrations and can influence the results.

Polygalacturonase (PG) activity – in particular endo PG - is important to hydrolyze pectin efficiently and reduce must viscosity rapidly. Pectin lyase (PL) recognizes pectin that has methyl groups attached to the sugar units making up the chain. It does not exist in grapes, and is only produced by fungi. As grapes ripen, grape PME activity increases, and as a result the methyl groups are removed from the pectin chain, forming methanol. The result is that and the exo PG, which cleaves the



MECHANISM OF ENZYMATIC SETTLING

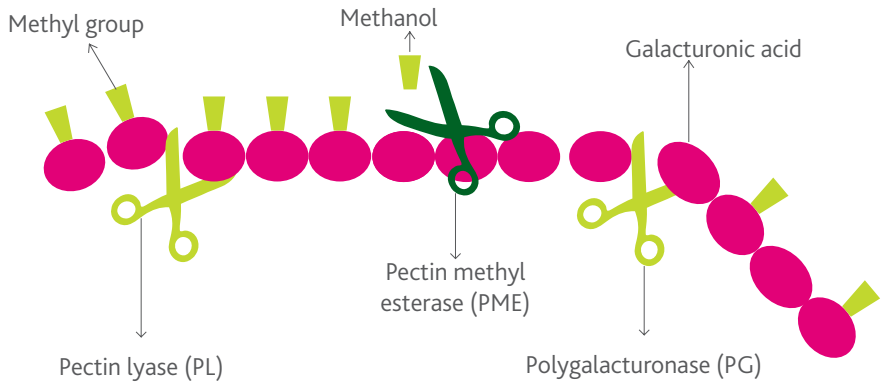
A schematic diagram illustrating the three steps in settling.

pectin chain between non-methylated sugar units, becomes active in addition to the endo PG. The balance between the various activities and their mode of action (endo or exo) as well as the concentration of the enzyme preparation will then greatly impact the difference in performance between various commercial enzyme preparations. This is why **LAFFORT** has chosen to increase the PG activity of its preparations for settling. In addition to a purified granulated preparation named **LAFAZYM® CL**, **LAFFORT** has introduced liquid enzyme formulations called **LAFASE® 600 XL** and **LAFASE® XL** for winemakers preferring to work with liquid products.

- ***Skin contact – white grapes***

The technique of skin contact on white grapes with enzymes is done for two reasons. The first is to increase both the quantity of good quality free run juice, as well as the overall quantity of juice per ton of grapes crushed. This second is to extract more varietal aromas from the grape skins and thus increase the aromatic potential of the wine. The grape quality and variety determine whether or not skin contact will be beneficial for wine quality. It is well documented that the use of specialized skin contact enzymes can increase juice yield and aroma extraction in shorter time. It is important to note that there can be significant differences between results obtained with different suppliers' enzymes. **LAFFORT**'s skin contact enzymes such as **LAFAZYM® PRESS** for direct pressing and **LAFAZYM® EXTRACT** for cold maceration are concentrated pectinases with many additional wanted side activities and none of the unwanted side activities. One requires a more concentrated pectinase "blend" of enzymes, with additional activities to achieve complete de-pectinization and extraction of valuable compounds when doing skin contact as opposed to doing only settling.

In the case of volatile thiol precursors, an important fraction is also localized in the skin as illustrated below for Sauvignon blanc grapes.



A simplified schematic diagram indicating where the main pectinases act on the pectin chain.

TO SUMMARISE: OUR ENZYME OFFER FOR CLARIFICATION APPLICATIONS

PROCESS	WINE TYPE	ENZYME	DOSAGE	EXPECTED OUTCOME
SETTLING AND FLOTATION	white and rosé	**LAFAZYM® CL**	5 - 20 ppm	*Lees compaction, high clarification yield .*
	white, rosé and red	**LAFAZYM® 600 XL**	0.5-2 mL/hL	*Fast depectinization, smooth operation.*
	white and red	**LAFASE® XL**	1-5 mL/hL	*Efficient depectinization.*

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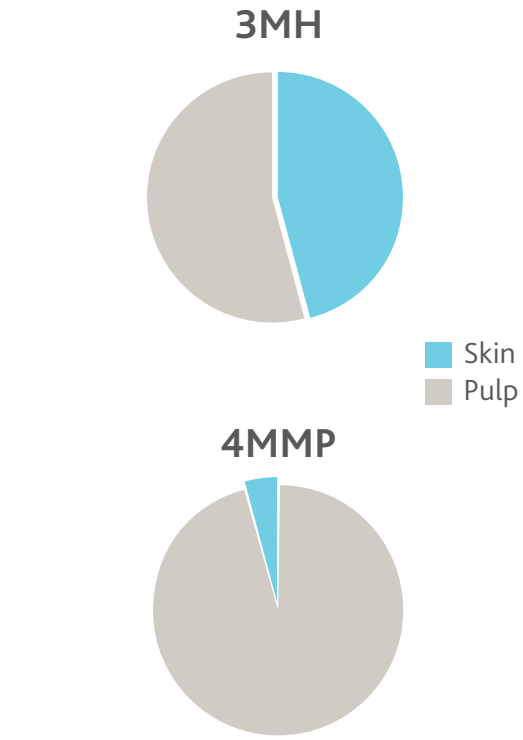
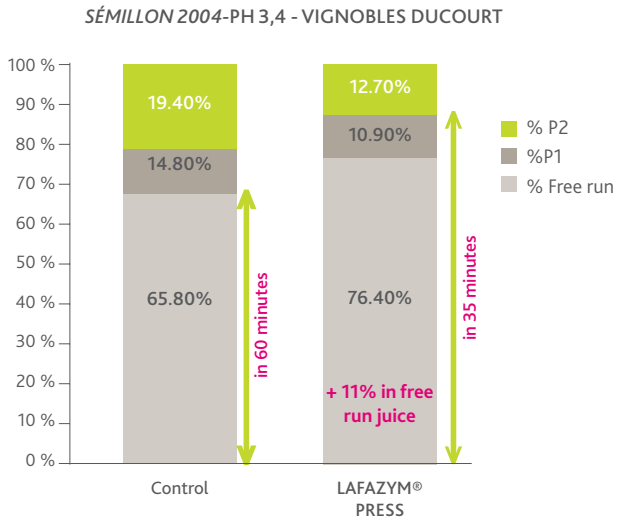


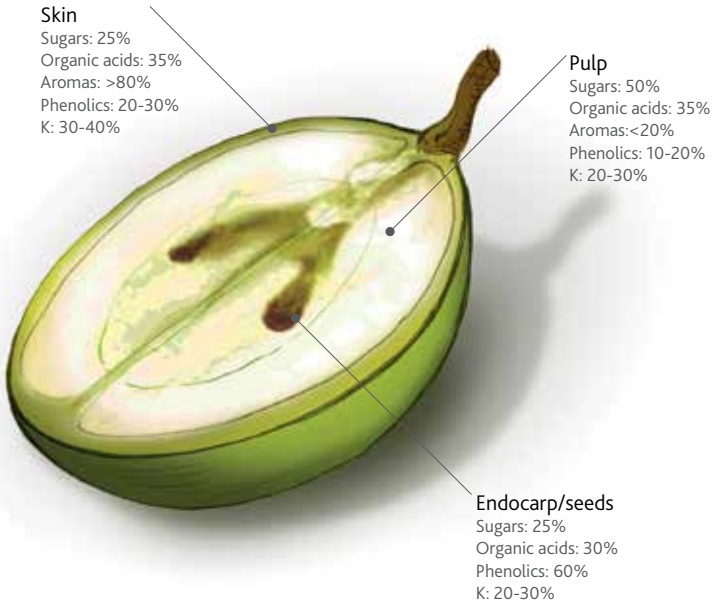
The distribution of the volatile thiol precurors of 3MH and 4MMP in the grape skins and pulp. (3MH – 3 – mercaptohexan-1-ol, 4MMP – 4-Mercapto–4–pentan–2-one) Source: Peyrot des Gachons, 2000 (13)

The biggest measureable effect of enzyme addition is noticed on direct pressing, as enzymes drastically increase the fraction of free run quality juice, as seen in this case with **LAFAZYM® PRESS.**



The effect of the use of **LAFAZYM® PRESS**, *a specialized skin contact enzyme, versus the use of no enzyme on juice yield. The light grey represents the free run juice, the dark grey the juice fraction obtained after the first pressing and the green the juice obtained after the final pressing. It is evident that apart from a significant process time saving, an increase in quality free run juice is obtained, coupled with a decrease in the quantity of lower quality press juices (Semillon, 2004, pH 3.4 . Vignoble Ducourt)*

A schematic diagram illustrating the distribution of sugars, organic acids, aromas, phenolics and potassium (K+) in grape berries. It is clear from this picture that a rather large source of varietal aromas is derived from grape skins, thus the benefit of skin contact. Source: Fontes et al, (2011 (12))



TO SUMMARISE: OUR ENZYME OFFER FOR SKIN CONTACT - WHITE AND ROSÉ

PROCESS	WINE TYPE	ENZYME	DOSAGE	EXPECTED OUTCOME
DIRECT PRESSING	white and rosé	**LAFAZYM® PRESS**	**20-50g/ton**	*Free run and total juice yield increase, reduced operation time.*
SKIN CONTACT	Fruity whites	**LAFAZYM® EXTRACT**	**20-30g/ton**	*yield increase and maximized aroma precursors.*

- ***Skin contact - red grapes***

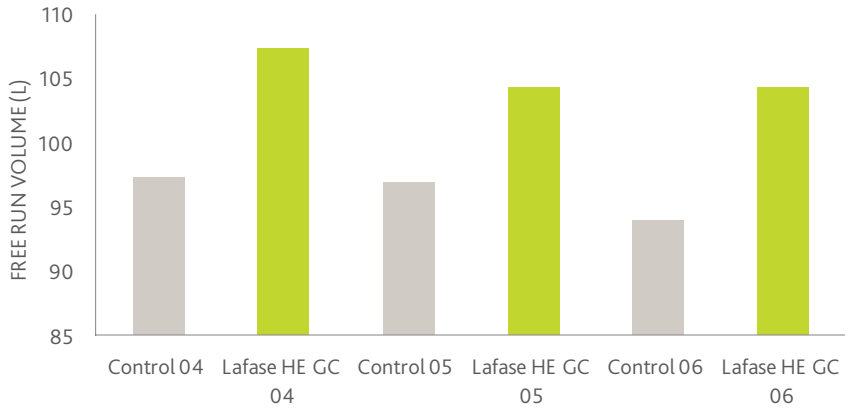
LAFFORT has invested much time and effort in recent years on increasing the performance of its red winemaking extraction enzymes. Part of this investment has been the development of a specialized experimental cellar in Bordeaux, where enzymes and other oenological ingredients can be tested under pilot plant conditions (170 kg grapes per vat). This experimental cellar provides a unique tool for the sensory evaluation and monitoring of enzyme trials from the start of vinification until finished wines.

The results of research have led to the development of a specialized **LAFASE® HE GRAND CRU** rich in rhamnogalacturonase activity. After 20 months, wines treated with **LAFASE® HE GRAND CRU** were richer in RG II (rhamnogalacturonan II) - 60% increase compared to non-enzyme treated wines - with reduced amount of PRAG (polysaccharides rich in arabinose and galactose), enabling good wine clarification prior to . Also, the increase in color intensity was due to derived pigments with condensed tannins that are resistant to sulphite bleaching as analyzed by Ducasse (14).

As for white skin contact enzymes, an important benefit of applying specialized macerating enzymes is the increased yield of free run wine. Both **LAFASE® HE GRAND CRU** and **LAFASE® FRUIT** result in yields of between 7 -10% higher free run wine volume.

Besides increasing the yield, there are numerous advantages associated with the use of macerating enzymes in red winemaking:

- Pectinases greatly facilitate color and tannin extraction, since anthocyanins and tannins are located in the skins of red grapes.
- Improved tannin extraction promotes better color stabilization compared to wines produced without the use of enzymes.
- It has been observed that press wine from musts treated with red wine macerating enzymes have a lower residual sugar than press wines made from musts without the addition of enzymes. This is very beneficial, as it can limit the potential growth of spoilage organisms such as *Brettanomyces* and spoilage bacteria.
- Since pectinases reduce the size of the polysaccharide chains in red wine, wine clarity and filterability is greatly improved.



*A trial conducted over a three year period (2004 – 2006) with a control (no enzyme added) and **LAFASE® HE GRAND CRU** (40 g /ton) treated wines. The graph indicates a significant increase in the quality free run wine volume in the **LAFASE® HE GRAND CRU** treated wines.*

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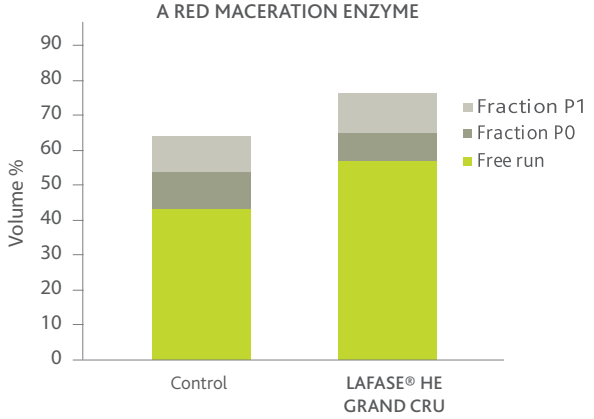
• Chain length reduction also leads to a decrease in PRAG (polysaccharides rich in arabinose and galactose) and an increase in RGII (rhamnogalacturonan II). The result is an improvement in mouthfeel and "sweetness."

• Winemakers report enzyme treated wines to have more pronounced fruit and softer tannins.

Many winemakers feel that they obtain enough color and tannin without the addition of enzymes. This is quite often the case with very dark colored, tannic grape varieties. However, one must not forget the other added advantages of using red wine macerating enzymes such as increased yield, clarity, color stability, filterability and sensory effects. Depending on the objective that one wants to achieve, different formulations of red wine enzymes can be used. The different formulations in the two enzymes **LAFASE® HE GRAND CRU** and **LAFASE® FRUIT** facilitate different targeted extractions and wine styles.

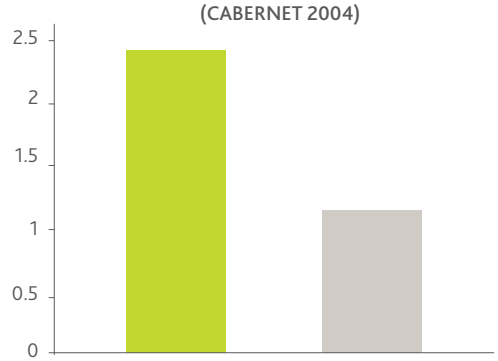
Due to the fact that enzymes are proteins, they can bind to wine tannins. The more tannic the variety, the more the enzymes will be inactivated. This is an important consideration in deciding a proper dosage. For this reason, red wine enzyme dosage recommendations are generally always higher than those of white wine enzymes.

VOLUME OF WINE WITH AND WITHOUT A RED MACERATION ENZYME



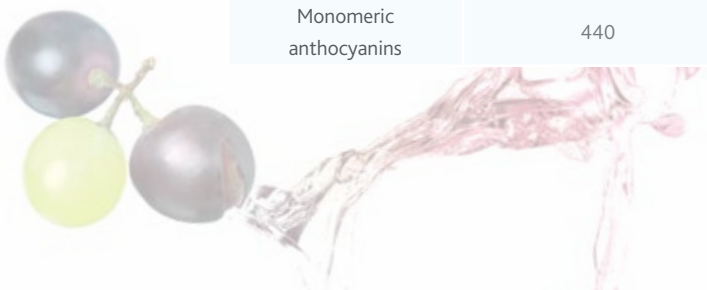
*Comparison of juice yields between a control (no enzyme) and **LAFASE® HE GRAND CRU**.*

DOSAGE RESIDUAL SUGAR IN PRESSED WINES (CABERNET 2004)

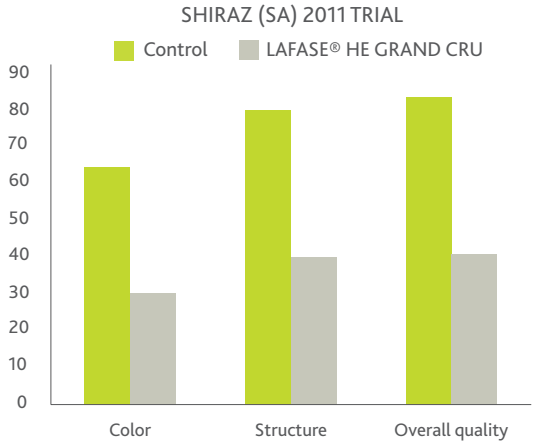


Press wines with maceration enzymes contain less residual sugar (0.8 g/L) than those with no enzymes (2.1 g/L) - prevention of the risk of contamination by microorganisms (experimental cellar).

ANALYZES AFTER MLF COMPLETION	CONTROL + COLD SOAKING	LAFASE® HE GRAND CRU	LAFASE® HE GRAND CRU + COLD SOAKING
Color index	0.89	1.18 (+32%)	1.17 (+32%)
DO 280 nm	43	50 (+16%)	50 (+16%)
Turbidity in NTU	44.6	14.2	11.9
Average polymerization degree (DPM)	5.4 +0.9	6.9+0.1	8.4 +0.8
Polymerised phenols	433	614 (+42%)	622 (+43%)
Total anthocyanins	477	527 (+10%)	559 (+17%)
Polymerised anthocyanins	37	46 (+24%)	49 (+32%)
Monomeric anthocyanins	440	481 (+9%)	510 (+16%)



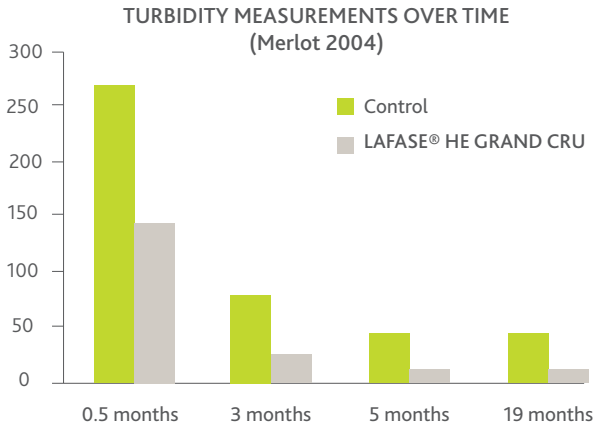
Experimental results comparing a control and **LAFASE® HE GRAND CRU** *with and without cold soaking (Merlot 2004)*



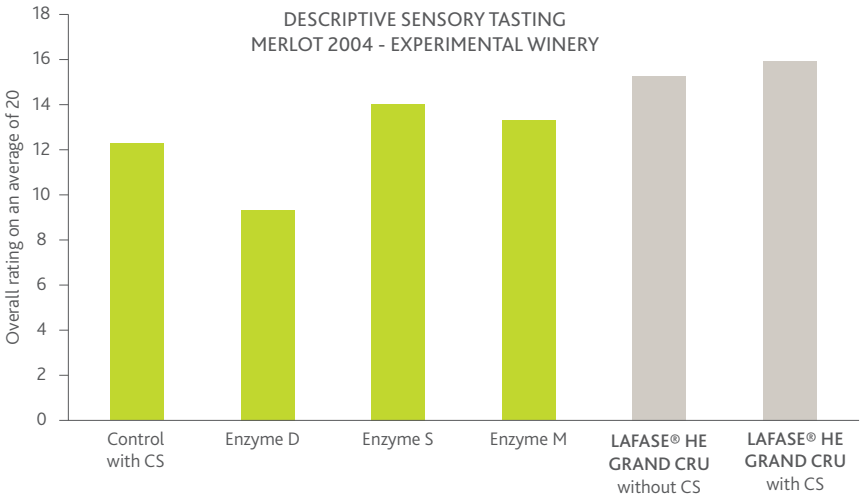
Results from a 2011 enzyme trial on Shiraz from South Australia. Winemakers were asked to score wines from 1 to 6, 1 being the highest score. Thus, the lower column indicates the most preferred. ***LAFASE® HE GRAND CRU*** *treated wines were preferred over no enzyme wines in terms of color, structure and overall quality.*

- ***Thermovinification***

There are various thermo treatment processes operating at different temperatures. Of late, large wineries are using thermovinification with flash-release technology. This involves red grapes being crushed, destemmed and heated to 185°F for two to five minutes and then cooled down under vacuum. Usually after the heat treatment, winemakers separate the juice from the grape solids either via flotation, centrifugation or filtration. To obtain a good clarification, enzymes are needed as time is essential for clarifying quickly in order to avoid the fermentation starting on very turbid juices. One can treat the juice with a specialized red wine settling enzyme. **LAFASE® THERMO LIQUID** is an example of a specialized red juice enzyme that is both purified from cinnamyl esterases and ß-Glucosidase (anthocyanase) activity. It is important to note that any enzymes added at crushing before the heat treatment would have been inactivated during the treatment, so therefore cannot aid the settling and clarification process.



A comparison between a control (no enzyme) and ***LAFASE® HE GRAND CRU*** *treated red wine. The* ***LAFASE® HE GRAND CRU*** *allowed for a rapid decrease of viscosity and turbidity. The result is increased clarification and improved pressing and filtration.*



A comparison between a control (no enzyme), ***LAFASE® HE GRAND CRU****, with and without cold soaking and various other red wine enzyme preparations on the market. The* ***LAFASE® HE GRAND CRU*** *wines were the preferred wines in terms of final wine quality. CS - cold soaking).*

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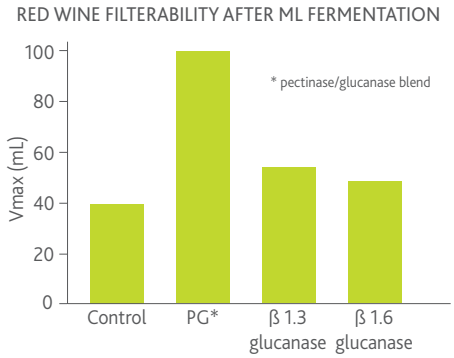
TO SUMMARISE: OUR ENZYME OFFER FOR RED GRAPES

PROCESS	WINE TYPE	ENZYME	DOSAGE	EXPECTED OUTCOME
SKIN CONTACT - COLD SOAK	Fruity reds	LAFASE® FRUIT	30-50 g/ton	Free run and total wine yield increase, reduced time, aromas extraction. Increase clarification and filterability.
LONG MACERATION	Full bodied reds	LAFASE® HE GRAND CRU	30-50 g/ton	High stable color and smooth structured tannins with easier clarification prior aging. Increase clarification and filterability.
SHORT MACERATION	Young red wines	LAFASE® FRUIT	30-50 g/ton	Improved yield, color and clarity. Increase clarification and filterability.
ON GRAPES PRIOR TO HEAT TREATMENT	Fruity reds	OPTIZYM®	20-40 g/ton	Reduce viscosity, improves performance.
ON MUST AFTER HEAT TREATMENT	Fruity reds	LAFASE® THERMO LIQUIDE	20-30 mL/ ton	Quick depectinization, higher must yield.

- ***Aging on lees and filtration***

The practice of wines after fermentation on either the gross or fine lees is very common. Scientists reported the release of various compounds such as amino acids, nucleotides, polysaccharides, mannoproteins and low molecular weight peptides into the wine. All these compounds have a direct or indirect positive influence on wine quality. Of particular interest are the peptides released as they have a profound effect on the perception of "sweetness" of the wine (umami taste), as well as a masking effect on wine astringency. However wineries often do not have the capacity or time to allow for long periods of on the lees to achieve the best results. The use of glucanase enzymes such as **LAFFORT EXTRALYSE®** can speed up the process drastically, as they act directly on the glucan chains in the yeast cell wall and favor the release of mannoproteins and peptides. A process that would normally take months can be shortened to weeks, allowing for earlier processing of more stable wines - resulting from the action of released mannoproteins which increases tartrate and protein stability.

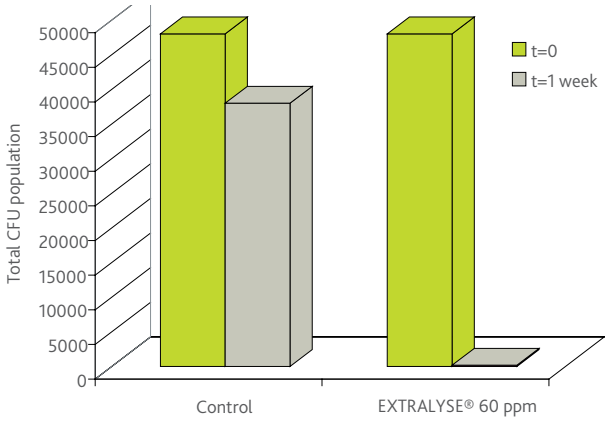
Glucanases are usually combined with pectinases, and this combination is particularly effective in improving wine filterability. *Botrytis* and *Saccharomyces* secrete long chain glucans into grape juice and wine, resulting in poor filterability that can also be worsened by the presence of residual pectins. The best results in improving filterability are achieved with a glucanase/pectinase combination rather than glucanase on its own, as demonstrated by Humbert- Goffard (15). **LAFFORT EXTRALYSE®** is a combination of ß 1.3 – 1.6 glucanase and various pectinases in specific ratios for preparing wines for filtration.



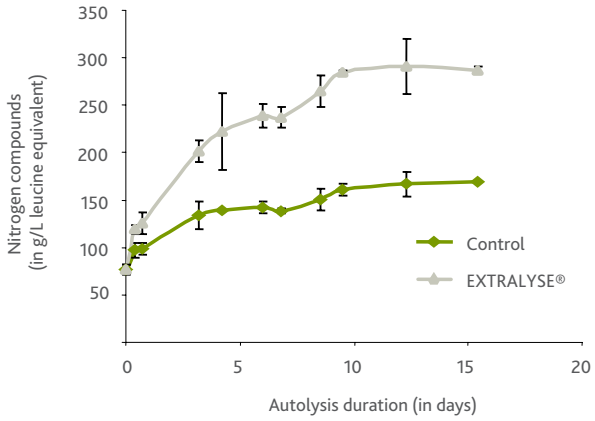
Filterability of a young red wine after specific enzyme treatments. The ß 1.3 glucanase and ß 1.6 glucanase tested separately both contributed to filterability improvement, but the best result was obtained with the glucanase/pectinase enzyme blend. Source: Humbert-Goffard et al.

EXTRALYSE® is applied on the yeast lees after the completion of primary fermentation if the objective is on lees. At that stage, the enzyme will benefit from the temperature. The minimum contact time depends on the dosage: : a minimum contact time of minimum three to four weeks with regular lees stirring is an average recommendation. If the objective is to improve filterability, a shorter contact time (five to seven days) is advisable.

With glucanase enzymes best results are obtained at wine temperatures above 41°F. To obtain an " on lees character" one can also treat the yeast lees on its own (as opposed to the whole tank of wine) with a higher dosage of enzyme at elevated temperatures to speed up the process.



***EXTRALYSE®** enables the rapid clarification of wines and a significant improvement in microbiological stability.*



*• During autolysis in a model environment, the **EXTRALYSE®** preparation releases twice as many nitrogen compounds, which are attributed to the organoleptic properties associated with maturation on lees, than natural autolysis without exogenous enzymes (Thesis Anne Humbert-Goffard, 2003,Faculté d'Œnologie de Bordeaux II).*

• *Aroma release post fermentation*

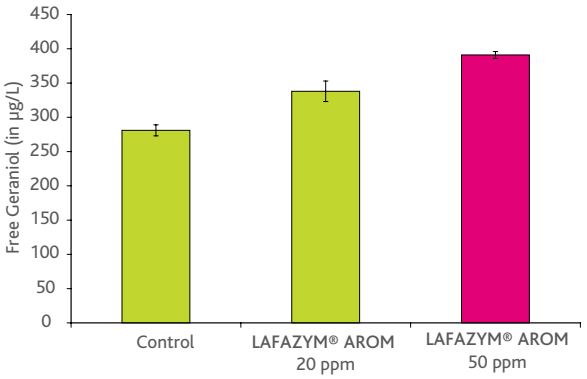
Glycosidases can release aroma precursors from the sugar molecules that render them non-aromatic, thereby greatly enhancing the aromatic potential of wines. Wines that benefit from this treatment are those made from monoterpene containing grape varieties, such as Riesling, Gewürztraminer, all the Muscat varieties and certain neutral varieties to some extent. Most of the aromatic potential of these grape varieties exist in the glycosylated form and is therefore not aromatic. These aroma precursors are converted to the free aromatic form over time via acid hydrolysis, but the process is very slow. The advantage of the slow process, however, is that it ensures longevity of these styles of wine. But for wines with short shelf lives, exogenous enzyme addition is key to the release of the aromas.

Glycosidases are inhibited by glucose and can therefore only be used on finished wines with a residual sugar of less than 20g/L. The

TO SUMMARISE: OUR ENZYME OFFER FOR ON LEES AND FILTRATION

PROCESS	WINE TYPE	ENZYME	DOSAGE	EXPECTED OUTCOME
LEES	white and red	EXTRALYSE®	60-100 ppm	Release of savory compounds, clarification, reduced time.
SEPARATED LEES TREATMENT	white	EXTRALYSE®	150-200 ppm	Enriched lees "milk" for fining.
FILTRATION	white, rosé and red	EXTRALYSE®	50 ppm	Young wine - efficient clarification prior , improved filterability, stable clogging index.

suggested dosage for **LAFFORT**'s **LAFAZYM® AROM** is 30 – 50 ppm. The time depends on the dosage used, but usually treatment does not last longer than one month. The enzyme activity must be stopped using 200 ppm bentonite. It is advised to monitor the SO_2 levels during the treatment and adjust it regularly. The use of **LAFAZYM® AROM** results in a very quick release of a large concentration of aroma. It can be advised that only part of the wine is treated for varieties such as Muscat and Muscat Gordo to serve as an "aroma reservoir", which can be used later on for blending purposes. It is imperative that the enzyme is removed first before the final blend is made.



*Results obtained with two different concentrations of **LAFAZYM® AROM** on a Muscat wine. The perception threshold of Geraniol is 130 µg/L.*

APPLICATION GUIDELINES FOR ENZYMES

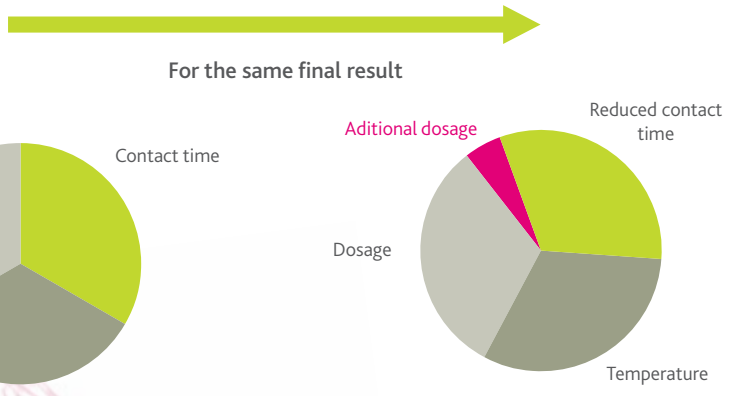
Enzymes should be added as early as possible in the winemaking process, i.e. at the grape reception (crusher) for skin contact, direct pressing, or when filling the tanks for red maceration. Some varieties (such as those with thick skins) are known to be difficult to process – Semillon, Ugni blanc, Muscat and Sylvaner. The use of enzymes on these grapes will therefore greatly improve their processing. A dosage increase of 30% is recommended for these varieties or for unripe grapes.

SO_2/pH: Enzyme activity is unaffected by SO_2 levels of up to 500 ppm, meaning that both enzymes and SO_2 can be added at the crusher. However a separate dosing pump should be used. Enzymes are efficient at all must/wine pH's.

Temperature: **LAFFORT** enzymes are active from 41° to 149°F, however the optimum temperatures are around 104 to 113 °F (pectinases, glucanases). Dosage is therefore crucial in each application. To speed up the hydrolysis, it is advised to double the dosage at low temperatures, or to increase the time of contact.

Bentonite: is the only inhibiting fining agent for enzyme activity, and should therefore always be added once enzyme activity is no longer desired. In case bentonite has been added, it is recommended to remove the bentonite lees prior to enzyme treatment.

Enzyme preparation: granulated enzymes should be dissolved in water or must prior to addition. They can be rehydrated in 10 times their weight (eg. 100g enzyme in 1000 mL water) prior to use. For convenience, **LAFFORT** enzymes are buffered, meaning that an enzyme solution can be prepared for the full working day and kept stable. Evenly spreading a diluted enzyme solution over a large volume of grapes is more effective than the spreading of granules.



A diagram showing the key parameters for enzyme activity. The dosage remains an efficient tool to adapt to extreme situations (unripe grapes, hard skins, low temperature, short contact time).

LAFFORT SPECIALIZED RED WINE ENZYMES

LAFASE® HE GRAND CRU

Application: Production of structured red wines rich in color and elegant tannins, destined for aging. Encourages extraction of small size polysaccharides (RGII) and improve capacities.

Type of formulation: Granulated purified enzyme, highly concentrated pectinase blend with positive side-activities.

Dosage: 30 – 50 g/ton.

LAFASE® FRUIT

Application: Production of fruity, rich in color, popular premium red wines, destined for early release on the market

Type of formulation: Granulated purified enzyme, highly concentrated pectinase blend with positive side-activities. Different formulation to **LAFASE® HE GRAND CRU** in order to achieve a more fruity wine style.

Dosage: 30 – 50 g/ton.

LAFASE® THERMO LIQUID

Application: Rapid settling of red musts after thermo treatment. Quick and efficient clarification for a large spectrum of temperatures (<149°F). Decreases viscosity of musts and wines.

Type of formulation: Liquid enzyme with extremely high polygalacturonase activity (PG>12500).

Dosage: 1 – 3 mL/100 kg of grapes.

LAFFORT ENZYMES - BASIC RANGE

OPTIZYM®

Application: Granulated enzyme for the production of bulk wines

Type of formulation: Concentrated pectinase blend.

Dosage: 30 – 50 ppm.

LAFFORT SPECIALIZED WHITE WINE ENZYMES

LAFAZYM® EXTRACT

Application: Skin contact on white grapes at low temperatures, for facilitating aromatic precursors and varietal aroma extraction (especially Sauvignon blanc). Allows reduced maceration times.

Type of formulation: Granulated purified enzyme, concentrated pectinase blend with positive side-activities.

Dosage: 20 – 30 g/ton.

LAFAZYM® PRESS

Application: Optimizes pressing by increasing free-run juice yields (white and rosé) and by decreasing the length of time and the number of pressing cycles.

Type of formulation: Granulated purified enzyme, highly concentrated pectinase blend with positive side-activities. Different formulation to **LAFAZYM® EXTRACT** (that is used for longer maceration), ideal for rapid maceration (time it takes to fill the press).

Dosage: 20 – 50 g/ton.

LAFAZYM® CL

Application: For must and wine clarification (settling and flotation).

Type of formulation: Granulated purified and highly concentrated pectinase blend.

Dosage: 5 – 20 ppm.

LAFAZYM® AROM

Application: for aroma release in white wines post fermentation (especially Riesling, Gewürztraminer and Muscat)

Type of formulation: Concentrated pectinase blend with ß-Glucosidase activity.

Dosage: 30 – 50 ppm.

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LAFFORT ENZYME FOR AND FILTRATION

EXTRALYSE®

Application: on yeast lees and filtration improvement. Accelerates yeast autolysis and releases larger quantities of molecules derived from the yeast, which provide roundness and suppleness in wines. Improves filterability, by degrading long chain glucan and pectin, that can block filters.

Type of formulation: Granulated, purified and concentrated pectinase / ß 1-3-1-6 glucanase blend.

Dosage: 60 – 100 ppm (on lees) - 50 ppm (filtration).

LAFFORT LIQUID WINE ENZYMES

LAFASE® 600 XL

A highly concentrated liquid pectinase preparation, naturally low in cinnamyl esterase (CE).

- Very suitable for color and tannin extraction in red musts.
- Improves clarity and filterability in red wines.
- Especially adapted for white musts from grape varieties that are difficult to settle such as Muscat varieties, Semillon and Sultana.

Dosage: 0.5 – 2 mL / hL

LAFASE® XL

An all-purpose basic pectinase preparation

- Quick and efficient depectinization.
- Improves pressing.
- Decreases wine viscosity.

Dosage:

1 – 3 mL/hL (white wines).

Increase the dosage to 5 m/hL for red musts with a high pectin content (difficult to filter wines).

PECTIN TEST

1. Add 2.5 ml of clear juice or wine in a test tube.
2. Add 5 ml of acidified alcohol (ethanol + 1% concentrated HCl).
3. Mix gently and let it stand before reading (5 minutes for juice and 10 minutes for wine.

The presence of pectin is confirmed by the presence of flakes. If pectin is completely hydrolyzed the liquid will be clear. If no flakes are observed after 10 minutes, the depectinization is complete. In presence of flakes, one should add more enzymes in the process.

Acidified alcohol preparation – Place 250 ml 96% ethanol in a flask. Add 2.5 mL of pure hydrochloric acid (HCl) at 37%. Mix gently. The solution is stable and enough for 50 tests.

GLUCAN TEST

Test sensitivity – 15 mg/L glucan.

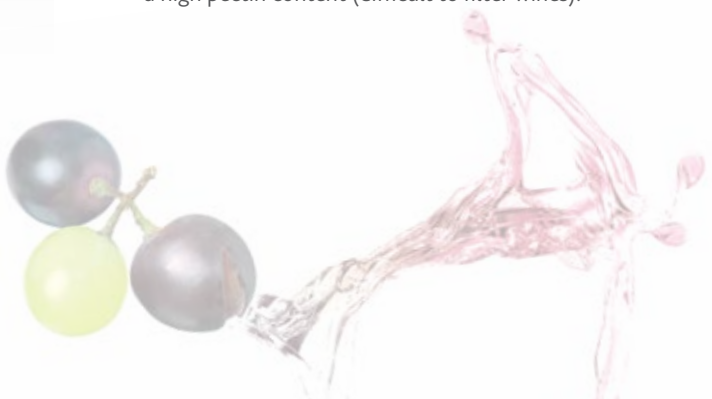
1. Add 5 mL of clear juice or wine in a test tube.
2. Add 2.5 mL of 96% alcohol and mix the two liquids.
3. If the glucan concentration is above 15 mg/L a filamentous precipitation will become visible.

BOTRYTIS TEST KIT

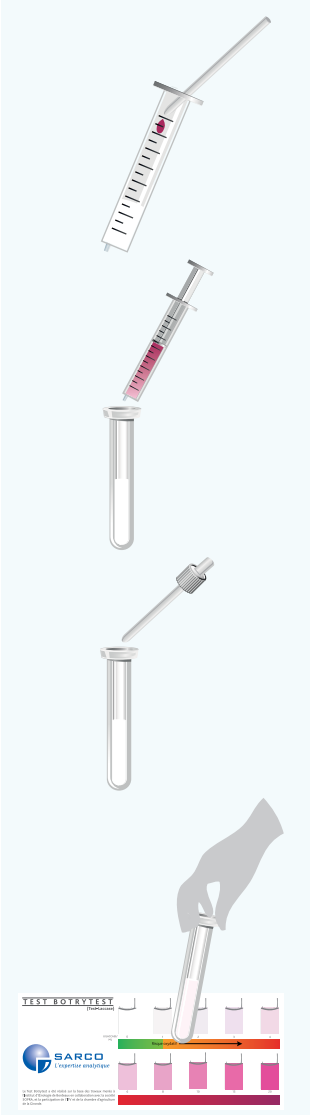
Preconditions: the analysis must be done on non-sulphited must or wine.

If necessary, the must/wine can be desulphited before analysis, by adding oxygenated water* (solution at 30%: 150 µL/100 mL of must/wine).

*Oxygenated water is not provided in the kit.



PROTOCOL FOR LACCASE ACTIVITY DOSAGE USING BOTRYTEST



1. Using the pipette supplied, put 5 mL of must or wine to be analyzed into the syringe.

2. Put the syringe on top of the tube and leave to percolate until the resin contained in the syringe is wet at its base (3 to 10 minutes). Then insert the plunger into the syringe and press lightly and slowly. Collect the first millimeter (up to the first marker) in the tube.

3. Add 1.4 mL of buffer solution (up to the second marker) then 0.6 mL of "BOTRYTEST laccase" reagent (up to the third marker on the tube).

   Mix the content by shaking the tube.

4. After exactly 3 minutes (use a stopwatch or timer), the laccase activity can be determined by comparing the color development with the color scale chart.

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REFERENCES

1. Canal-Llaubères, R.-M., 2010. Enzymes and wine quality. In: Managing wine quality Vol. 2, , p.93 – 132. Edited by Andrew G. Reynolds, Woodhead Publishing.

2. Amstrong W.P., 2012. Illustrations of molecular models: lock and key model of an enzyme. http://waynesword.palomar.edu/molecu1.htm

3. Saucier C., Roux D. and Glories Y., 1995. Stabilité colloîdale des polymers catéchiques. Influence des polysaccharides. Oneologie 95, Bordeaux. Tec et Doc Lavoisier, 395-400.

4.Doco T. et al (1995). Les polysaccharides pectiques de la pulpe et de la pellicule de raisin. Quel devenir pendant la phase préfermentaire? Rev. Fr. Oenol., 153, 16 – 23.

5. Dubourdieu D. and Riberau-Gayon P., 1981. Structure of the exocellular beta-D-glucan from *Botrytis cinerea*. Carbohydr.Res., 93, 294-299.

6. Dubourdieu D., Desplanques C., Villettaz J.-C. and Ribéreau-Gayon P., 1985. Investigations of an industrial ß-D-glucanase from *Trichoderma harzianum*. Carbohydr.Res., 144, 277-287

7. Llaubères R.-M., Dubourdieu D. and Villettaz J.-C., 1987. Exocellular polysaccharides from *Saccharomyces* in wine. J. Sci. Food Agric., 41, 277-286.

8. Canal-Llaubères R.-M., 1994. Enhancing the aroma of wines. Aust. Grapegrow. Winemak., 368, 49-51

9. Villettaz J.-C., Steiner D. and Trogus H., 1984. The use of glucanase as an enzyme in wine clarification and filtration. Am. J. Enol. Vitic., 35, 253-256.

10. Barbe C and Dubourdieu D., 1998. Characterization and purification of a cinnamate esterase from *Aspergillus niger* industrial pectinase preparation. J. Sci. Food Agric., 78, 471-478.

11. Gerbaux V., Vincent B. and Bertrand A., 2002. Influence of maceration temperature and enzymes on content of volatile phenols in Pinot noir wines. Am. J. Enol. Vitic., 53, 131-137.

12. Fontes N., Corte-Real M and Geros H., 2011. New Observations on the Integrity, Structure and Physiology of Flesh Cells from Fully Ripened Grape Berry. Am. J. Enol. Vitic., 62:3.

13. Peyrot des Gachons C., 2000. Recherches sur le potentiel aromatique des raisins de *Vitis vinifera* L.cv. Sauvignon blanc. Thèse de doctorat, faculté d'œnologie, Université de Bordeaux II Victor Ségalen.

14. Ducasse M.-A., Canal-Llaubères R.-M., de lumley M., Williams P., Souquet J.-M., Fulcrand H., Doco T. and Cheynier V., 2009. Effect of macerating enzyme treatment on polyphenol and polysaccharide composition of red wines. Food Chemistry, 118, 369-376.

15. Humbert-Goffard A., Basque E., Vatin L. and Canal-Llaubères R.-M., 2003. Rôle des preparations enzymatiques à base de ß-glucanases sur la mise au propre et la filtration des vins. Rev. Fr. Oenol., 201, 28-31.

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LAFASE® HE GRAND CRU & LAFASE® FRUIT improve:

- Color extraction and stabilisation
- Wine organoleptic qualities
- Quality wine yield
- Wine clarity and filterability

QUALITY

PURIFIED TO REMOVE UNDESIRED CINNAMYL ESTERASE AND ANTHOCYANASE

full bodied red wines

fruity red wines for early release

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