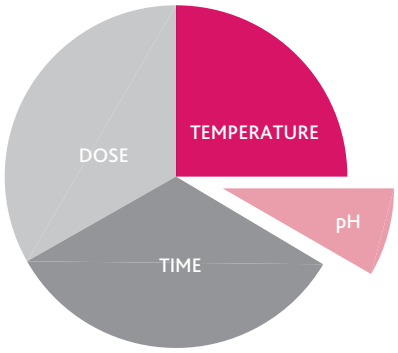
Q&A

ENZYMES

Enzymes occur naturally in grape berries and microorganisms in varying concentrations. Adding enzymes during vinification promotes clarification, extraction of skin compounds of interest, and optimizes pressing. Mastery of the use of enzymes allows for a reduction in the use of other products and eases winemaking downstream.

1. What are the main factors affecting enzyme activity and how do they relate to each other?



Enzyme activity rates are influenced by temperature, contact time, dose rate, and pH. Cooler temperatures require higher dose rates, or more time. When time is short, warmer temperatures and/or higher dose rates are required. To minimize dosage, allow warmer temperatures and/or more time.
Another key factor for enzymes is ethanol. Pectin chains will curl up when alcohol levels increase, making the sites harder to access for enzymes. Thus, enzymes are more effective on juice than on wine.

Temperature
Enzymes are sensitive to temperature but do have a wide range for activity. In general, lower temperatures will slow enzyme activity, and higher temperatures will speed up activity. Enzymes are proteins, and will denature at higher temperatures, hence it is important not to add enzymes directly to must during or right after flash détente, allow the must to cool to 60°C (140°F) first. Certain enzymes are more active at different temperatures, for example, enzymes such as **LAFAZYM® 600 XL**^{ICE} can maintain high activity levels down to 5°C (41°F) while **LAFASE® XL EXTRACTION** can function up to 55°C (130°F), making it a good choice for thermo-vinified musts.

pH
Enzymes work well within normal winemaking pH ranges, 3.0 - 4.0. pH is considered a minor factor for enzyme performance, as most variation occurs at extremes, for example pH over 5.0. Special formulas, such as **LAFAZYM® 600 XL**^{ICE}, retain activity well below pH 3.0.

Time
Use enzymes as early as possible in winemaking as the pectin chain is easier to access while in the aqueous phase. If faster turnaround is needed, use more enzyme or warmer temperatures.

Dosage
Refer to the dosage instructions for the enzyme activity you want to achieve within the appropriate time. For most applications, too much enzyme will simply mean faster performance. Too little enzyme will make the process longer, or not happen at all. Beware of too high of a dosage of red extraction enzymes which could result in grapes turning into a soup-like consistency and/or extract too many phenolics.

2. Are there any interactions to avoid when using enzymes?

There are two ingredient additions that can negatively impact enzyme activity; bentonite and tannins. Both bentonite and tannin can bind with proteins in the juice/wine. Enzymes are proteins, so they have the potential of being removed or inactivated by bentonite and tannin additions. In juice clarification, it is important to allow at least 6 hours for the enzyme to depectinize the juice before the bentonite addition. In red musts, it is recommended to add the fermentation tannin first at the crush pad, then add the enzyme with the first tank mixing.

3. What can I use for difficult to clarify juices, like Muscat?

The ideal method to clarify muscat and other difficult varieties is to use enzymes at two stages. First, use a pressing enzyme directly added to fruit. After press, use a settling enzyme to aid in clarifying. Dosages may need to be increased for especially difficult conditions. **LAFASE® BOOST** can be added at the pressing or clarification stage to add breakdown of complex side chains found in difficult to clarify varieties that inhibit enzyme performance. If only one addition is possible due to production constraints, mix a pressing and clarification enzyme, and add both at clarification.

4. Does bentonite help juice settle with enzyme treatment?

Adding bentonite with an enzyme will deactivate the enzyme, so it is important to delay the bentonite addition to allow time for the

enzyme to work. Bentonite can assist in settling and give excellent juice compaction, especially with the calcium based bentonites. Be sure that enzymatic activity is completed as measured by the pectin test before adding bentonite to juice.

5. How do I apply enzymes directly to white grapes before pressing? How long should I leave the enzyme before pressing?

Enzymes can be diluted even further to be easily sprayed to evenly coat clusters in bins, on destemmed fruit, or while fruit is being loaded into the press. Enzymes can also be applied through a dose pump post destemming when being pumped to the press. The key is to evenly apply the enzymes to all the fruit. We recommend at least one hour of contact time to grape clusters prior to pressing.
Avoid loading the press fully before adding enzyme: the enzymes will form pockets and be less effective. A layering approach would work best.

6. How does the Pectin Test work?

It is important to know that your wines are fully depectinized at juice stage and this can be easily measured with the pectin test. The pectin test simply adds juice to acidified alcohol. After a certain elapsed time, the juice is observed, and the presence of flakes indicates pectins. The **LAFFORT®** Pectin Test Kit contains 10 test tubes, complete instructions, and a stopwatch. Validating that your juice is pectin-negative is critical for effective flotation.

7. What enzymatic characteristics are best for flotation?

The key to effective flotation is complete depectinization of the juice. When pectin is present, juice will not have a clean flotation or separation of solids. Depectinization of white and rosé juice can be problematic at low temperatures, low pH, and with grape varieties containing highly branched pectin chains. These difficult conditions often add up and can be aggravated by winery time constraints. For these reasons, it is ideal to use an enzyme that has high activity at low temperatures and low pH. **LAFASE® XL FLOT** is a liquid enzyme developed for rapid depectinization in low temperatures and can complete activity in less than 2 hours. In further difficult conditions, **LAFASE® BOOST** can be used in addition to aid depectinization.

8. How do enzymes increase press yields or lower the pressure requirements?

By using enzymes such as **LAFAZYM® PRESS** or **LAFASE® XL PRESS** on whole cluster grapes the enzymes can begin breaking down the pectins allowing for increased yields at lower pressures, and/or higher total yields. Pressing enzymes increase the total free-run volume before needing to make a press cut and increase the total pressing volume.

9. What are the benefits of using enzymes in red wines?

Color and tannin extraction are the main benefits of using enzymes in red wines during fermentation. Additional benefits include faster settling and improved filtration later in the wine's life. Pectins can prevent wine clarification during aging, and pectins are much more difficult to breakdown in wine compared to juice. We observe more microbial issues in wines that have settling problems during aging. Review our list of enzymes to find one that fits your wine style. In general, **LAFASE® HE GRAND CRU** is for bigger structured red wines, and will extract more polysaccharides from the grape skin contributing to mouthfeel. **LAFASE® FRUIT** is formulated to focus on extracting anthocyanins, and aroma and flavor compounds for early release red wines. **LAFASE® XL EXTRACTION ROUGE** is a broad-spectrum liquid enzyme to increase yields, increase color and tannin, while also improve clarification.

10. Is there a sensory impact from enzymes, or are they just for processing?

Enzymes have a very wide range of activities beyond simply breaking down pectins. For red wines, **LAFASE® HE GRAND CRU** has hemicellulase & cellulase activities to break down the structural components of the grape cell wall, releasing juice, aroma, and color, and has additional side chain activity rhamnogalacturonase-II, which cleaves polysaccharides, resulting in more mouthfeel and sweetness. **LAFASE® FRUIT** has additional polygalacturonase to enhance cold soak maceration for improved aromas, and **LAFAZYM® THIOLS[+]** has secondary activities formulated for better thiol revelation in fermentation.
The ß-glucanase activity in **EXTRALYSE®** has two key properties. First it breaks down glucan chains in wines, especially those affected by *Botrytis cinerea*, leading to greatly improved filtration. Second, the activity improves the efficiency of autolysis during aging in wines aged on lees, leading to improved mouthfeel.

11. Is it best to add multiple enzymes at the same time or sequentially?

Enzymes typically have different functions and are used at different and specific stages of winemaking. Adding a pressing enzyme directly to grapes such as **LAFAZYM® PRESS** can improve yields at lower pressures. This can be coupled with addition of **LAFAZYM® CL** during settling, which aids in lees compaction improving clean juice yield. At fermentation **LAFAZYM® THIOLS[+]** can enhance thiol revelation and **EXTRALYSE®** can improve filterability. **LAFFORT®** technical representatives can assist in deciding where and when it is most beneficial to add enzymes for your specific stylistic and efficiency goals. For reds, adding an enzyme such as **LAFASE® HE GRAND CRU**, **LAFASE® FRUIT**, or **LAFASE® EXTRACTION ROUGE** can increase color, tannin, flavor extraction, while also reducing cold soak times, depending on which formula is best for your fruit. **EXTRALYSE®** can be used to improve filterability or increase autolysis during aging.

12. If I have botrytis on my grapes, what is the best enzyme treatment strategy?

Botrytis cinerea has two major negatives aspects in winemaking. The mold secretes long chain glucan molecules in grape juice making it highly viscous and difficult to filter, and contains laccase, an SO_2-resistant enzyme that causes browning in juice and wines. One of the first steps in **LAFFORT®**'s *Botrytis Infected Fruit Protocol* on pages 148 - 150 is to determine the level of laccase activity from the percentage of rot in the fruit. The next step is to add tannins based on the corresponding level to bind with laccase and prevent oxidative activity. ß-glucanase enzymes in **EXTRALYSE®** can then break down these glucans into shorter chains making them easier to filter.

13. What is the difference between Polygalacturonase (PG), Pectin Lyase (PL), and Pectin Methyl Esterase (PME)?

Grape juice contains pectin, comprised of a main chain, which may be esterified with methyl groups and multiple side branches and activities. The most important 'pectic' enzymes are Pectin Lyase (PL) and Polygalacturonase (PG). PL will cleave the pectin chain where there are methyl groups attached. PG only cleaves the pectin chain when there is no esterification with methyl groups.

Grape pectin structure changes with ripening towards the non-methylated structure because of the third 'pectic' enzyme, Pectin Methyl Esterase (PME). PME removes methyl groups from the pectin chain, and effectively helps the PG activity.

Each of the three major pectic enzymes also has exo- and endo-activities. The exo-pectinases will act on the ends of the chain, while endo-pectinases will act in the middle of the chain. A blend of exo- and endo-pectinases are important to efficiently break down pectin chains.

Commercial enzymes are composed of all three of the major pectic enzymes, with varying amounts of endo- and exo activities. The relative proportions of each activity mark its efficacy for application. For example, PL is very important in settling and flotation enzymes. PG is important in grapes with high maturity and in maceration of white and red musts.

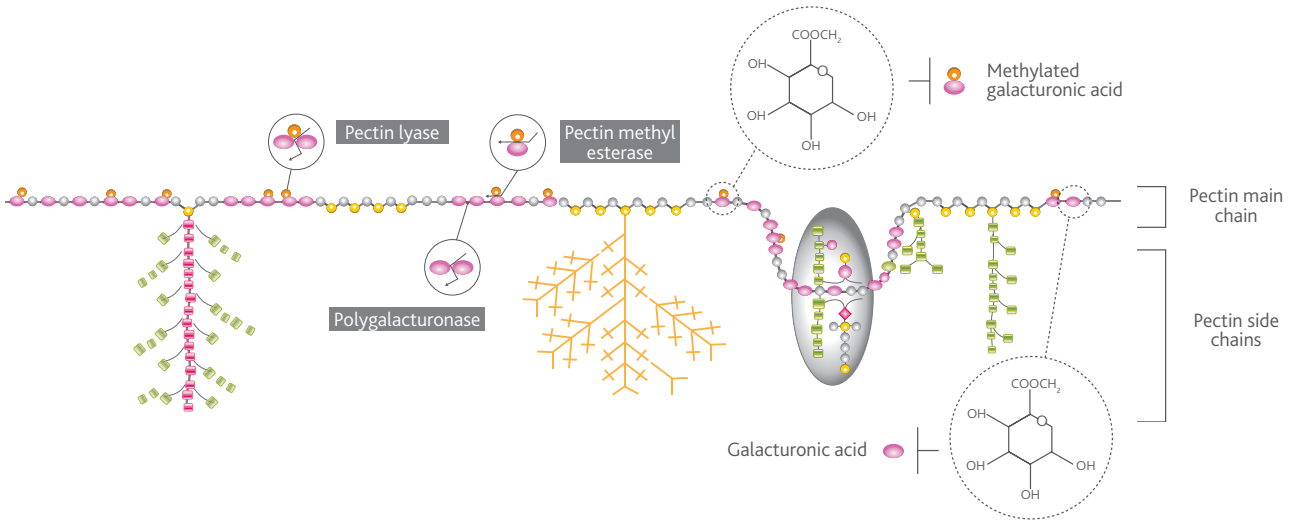
14. What is the difference between a primary activity and a secondary activity?

Pectic enzymes are produced primarily by fermentation of *Aspergillus niger* and *Aspergillus aculeatus* under specific conditions. The three primary activities produced include Polygalacturonase (PG), Pectin Lyase (PL), and Pectin Methyl Esterase (PME). Just as varying the temperature and nutrient regime in making wine with *Saccharomyces cerevisiae*, varying conditions of fermentation of *Aspergillus* species will produce different by-products, or side-activities. Some of the more widely found secondary activities include *Rhamnogalacturonase I* (RGI), *Rhamnogalcturonase II* (RGII), *Hemicellulase* (HE), *Cellulase* (CEL), and *Cinnamoyl Esterase* (CE).

As shown in the diagram below, pectin molecules can be highly branched with lots of "hairy" side chains attached, making it difficult for the main enzymes (PL, PG, PME) to break down the main chain. It is the action of the side activities that can remove the side chains so the main enzymes can have access to the pectin main chain.

Also, activities on side chains can have qualitative impacts, including *Rhamnogalacturonase II*, a specific enzymatic activity found in **LAFASE® HE GRAND CRU**, which cleaves off the rhamnogalacturonan II side chains and extracts polysaccharides. These side chains interact with tannins to build mouthfeel and structure.

PECTIN POLYMER SHOWING STRUCTURAL COMPONENTS AND SITES OF ENZYME ACTIVITY.



Pectinase enzyme activity in red wine grape processing is targeted towards degradation of the main backbone of the pectin molecule. The concerted activity of pectin lyase, pectin methyl-esterase and polygalacturonase break the main rhamnose-galacturonic acid chain resulting in enhanced extraction of both tannin and colored anthocyanin molecules. 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.

15. How is enzyme performance measured?

Enzyme production creates multiple main and side chain activities, and all **LAFFORT®** enzymes are blends of multiple production runs. Most commercial enzymes will have their activity rated as either Polygalacturonase or Pectin Lyase activity units, and these are generally rated on apple juice although **LAFFORT®** tests all enzyme activities on grape juice and wine.
Be sure to calculate dosage with activity, time, and cost to ensure you are getting the maximum value and benefit for your wines.

16. Are there any negative enzyme activities?

What makes one commercial enzyme better than the other is fitness for purpose, and whether they are sourced appropriately and purified, free of undesirable side activity leading to the production of vinyl-phenols or off-flavors. *Cinnamoyl Esterase* (CE) is produced by some species and strains of *Aspergillus*. CE can convert hydroxy-cinnamoyl esters in juice to hydroxycinnamoyl acid that Phenolic Off Flavor positive POF(+) yeast strains will metabolize into vinyl phenols, giving off aromas of paint, leather, and vinyl. It is important to use enzymes that are purified or have naturally low levels of CE, or use Phenolic Off Flavor negative POF(-) yeast strains. With low quality enzymes, there is a risk of developing negative compounds or faults in your wine.

ENZYME STORAGE AND PREPARATION

PRODUCT	STORAGE TEMPERATURE & PLACE	SHELF LIFE UNOPENED & OPENED	PREPARATION
Granulate enzymes	Cool, Dry, < 25°C (77°F)	Four years unopened, use within two months when opened.	Dilute 10:1 in water
Liquid enzymes	Refrigerated < 10°C (50°F)	Two years unopened, use within two months when opened.	Dilute 10:1 in water