Winemaking Enzymes

Analytical Services

Everything you wanted to know but were too busy to think about

On behalf of Laffort and Laffort Australia, enzyme range manager **Céline Fauveau Schaff**, technical manager **Alana Seabrook**, and managing director **Tertius van der Westhuizen**, have compiled a comprehensive look at enzymes.

Modern winemaking needs as well as recurring questions on how to compare enzymes called for an update on basic understanding of enological enzyme preparations. Deeper explanations of the complex composition of enzymatic preparations used in winemaking were long overdue. This article will try to re-establish the boundary between fruitful simplification and often damaging oversimplification. Nevertheless, enzymology remains a high-level scientific discipline of biology, the objective of this article is to provide basis that will allow winemakers to make educated choices.

Limits of science vulgarization

Enzyme preparations used in enology suffer from scientific over simplification that quenches the wine industry thirst for rational explanations.

The main problem with an over simplified approach is that the urge to make the scientific message clear often sweeps out capital details. Reduced to a pectinase activity, most enological enzymes appear to be the same. The understanding of their mode of action is truncated and choice in an overwhelming offer becomes difficult. Buyers fall back on an attempt at rationalization by comparing price / kg or price / activity level.

Enzymatic preparations for enology are complex

The enzymes used in enology are actually multi-activity cocktails. Their main component being the principal activity, but they also contain many secondary activities.

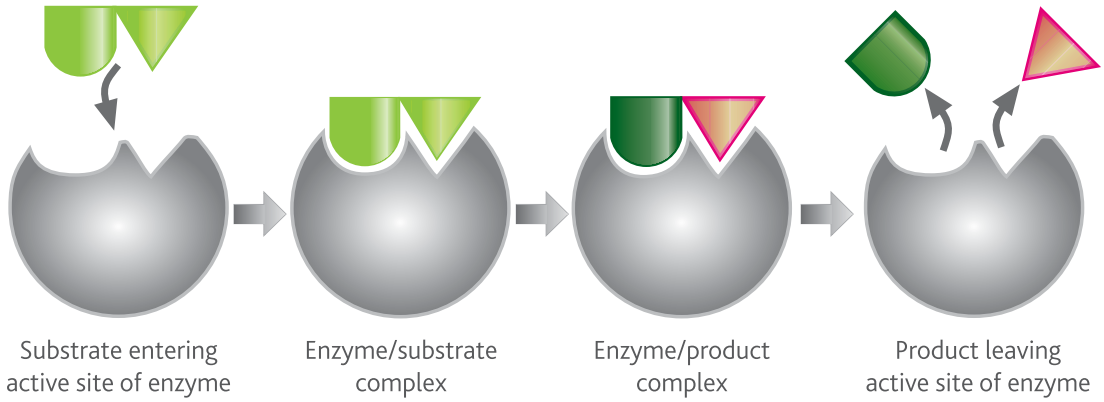

Figure 1: Commonly used, highly simplified representation of an enzymatic reaction
One of the winemakers' paradoxes lies in the fact that despite their everyday connection to their senses they give extra credit to a scientific theory classifying the rest "commercial speech". As a result many highly complex enzyme formulations are summarized in the most basic theory of enzymology: enzyme + substrate yields a product (Figure 1).

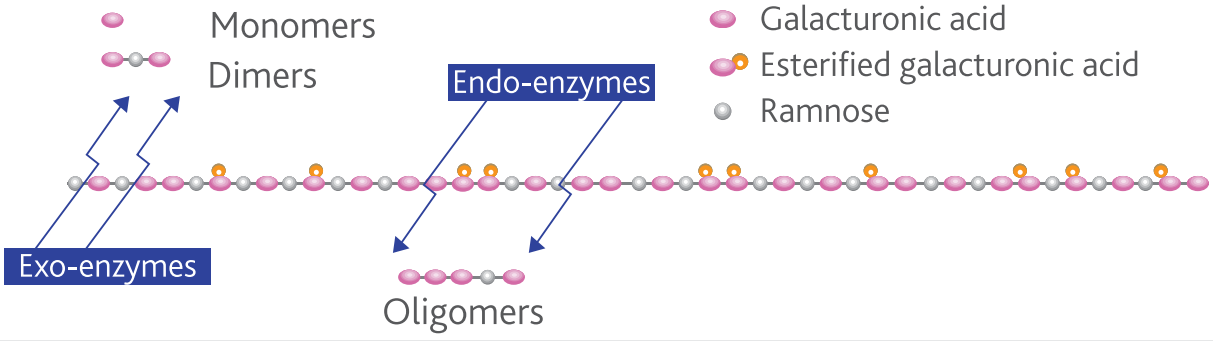

Figure 2: Endo and exo enzymes mechanism of action

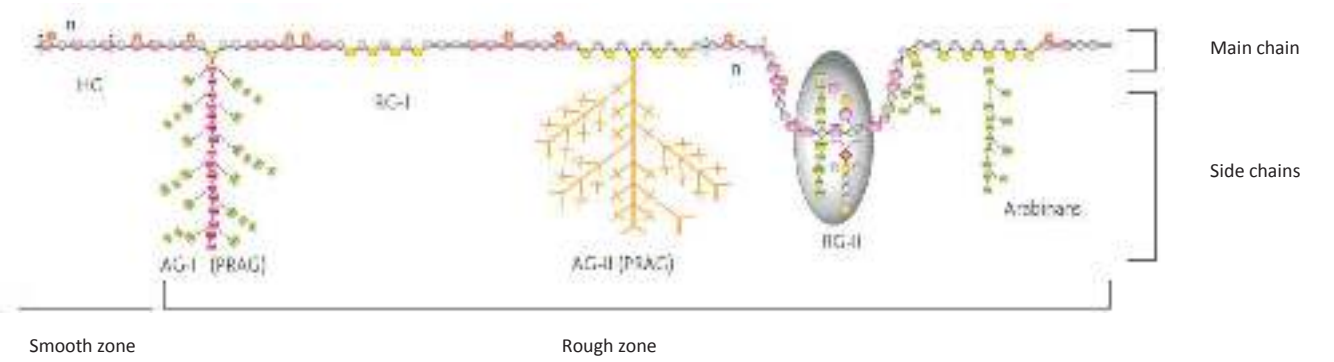

Figure 3a: Simple schematic representation of the various components of a pectin chain.
HG = Homo galacturonan; RGI and II = Rhamno glactutonans; AG I and II = Arabino galactans; PRAG = Polysaccharides rich in arabinose and Galactose

Enzyme cocktails are produced by living organism

The producing sources of enzymes for the industry are microscopic fungi (as yeast). The oenological enzymes of interest are derived from the *Aspergillus* and *Trichoderma* species. These species comprise of a large number of strains which exhibit a wide variety of skills to produce enzymes. Each strain produces a unique blend of activities. For example, a given strain of fungus used for the production of enzymes may have several dozens of genes encoding for enzymes that target hemicelluloses (Hatsch *et al*.2006).With their unique proprietary strains, manufacturers market their unique cocktails.

Among the numerous enzymes present in a cocktail are:

- Endo-enzymes, randomly cutting the pectin chain and releasing midsize polymers (Figure 2)
- Exo-enzymes targeting the ends of the chain and releasing mono and dimers (Figure 2)

De-substituting enzymes that "disconnect" the side chains. These ease the action of the previously mentioned. (Figure 3a and b)

Influence of the growing medium on the enzymatic cocktail

One study showed the strong influence of the production medium on the transcription of several genes coding for enzyme synthesis. For example, in the presence of xylan (a component of hemicellulose - Doco T., Williams P., Pauly M., O'Neill MA, Pellerin P. 2003), up to 30 xylanases can be expressed: endoxylanase, xylosidase,

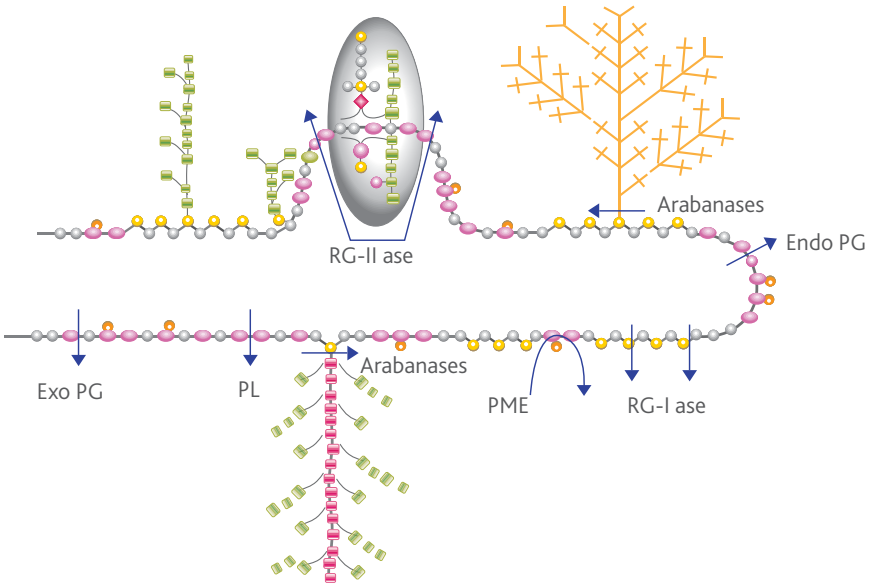

Figure 3b: main enzymatic activities responsible for pectin chain degradation

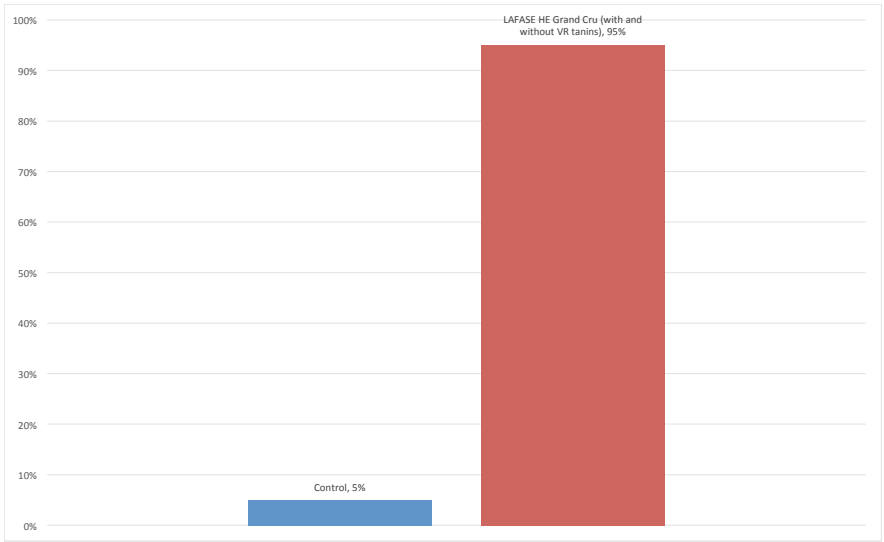

Figure 4 : Organoleptic impact of an enzyme treatment in red wines – 2014 sensory preference by a jury of 19 professionals
Figure 4 illustrates the preference of red wines treated with LAFASE® HE Grand Cru. This consistent tasting preference for enzyme treated wines is due to overall wine quality, increased mouthfeel and improved phenolic quality.

arabinofuranosidase, acetyl esterase, β-galactosidase, feruloylesterase... the impact of most of these activities in winemaking is yet to be studied. For the production of pectinases, it is the same. The fungus is able to mobilize a true enzymatic arsenal when in contact with a specific substrate. This arsenal unique to each strain is used by the fungus in nature during plant infection, to degrade complex molecules such as pectic polysaccharides and penetrate its tissues.

Principal enzymatic activities in enological preparations

The pecto-cellulosic cell wall membrane degradation: enzymatic chain reactions

Pectin composition varies with grape cultivar and its stage of development. For example, pectin methylation degree is inversely proportional to the stiffness of pectin, it varies during the grape ripening process. Given the diversity of substrates that constitute the plant cell walls, the synergistic action of a wide variety of enzymes is essential to ensure sufficient hydrolysis of the polysaccharide complex. If we take the simple example of polygalacturonase (PG), it hydrolyzes the homo galacturonan (HG) of the pectin main chain in un-methylated sites. Considering the fact grape pectins are methylated on average at 70%, the preliminary action of a pectin methyl esterase (PME) is necessary to remove the methyl group and allow PG to perform.

Secondary activities such as arabanases, galactanases or the rhamno galacturonases, releasing large complex polysaccharides side chains, clearing up the access to the main chain and therefore accelerating the work of other pectinases. (Figure 3b)

"Partners activities" or "essential activities" acting in support of the principal activities

These activities referred to as "secondary activities" are produced by the microorganism during the production cycle of the main activity. Many of these play a significant role in winemaking.

At times, acting in support of the principal activity to achieve the targeted benefit as it would be the case for cellulases or hemi cellulases in maceration. Other times, their action might be essential, their absence limiting the degradation of polysaccharides as it would be the case for arabanases and RGases (Rhamno galacturonases). It has often been observed that when these activities are lacking, a highly concentrated enzyme (in one single activity) was found to be ineffective as a result of not being able to access its substrate.

Unwanted "secondary activities"

Activities with an undesirable effect in winemaking are sometimes present in the enzyme cocktails. This is the case of cinnamoyl esterase, an activity formerly known as cinnamyl esterase (CE), depsidase or tannase. In 1992, P. Chatonnet *et al.* first identified this enzyme and the hydrolysis mechanism leading to the formation of vinyl phenols (unwanted odorous compounds in white wines). These findings were then confirmed in 1993 by Dugelay *et al.* From then on, Laffort has been offering purified enzymes, meaning enzymatic cocktails in which the undesirable activities are eliminated or kept at negligible levels.

Positive "collateral activities"

Also present in the cocktail are enzymes with empirically-observed collateral benefits. These organoleptic benefits are often the reason behind a winemaker's intuitive attachment to a particular enzyme preparation.

New scientific developments and findings

Too often any "unexplained" observation is confused for "un-explainable" and one starts questioning the facts. An attitude at the opposite of the empirical approach which methodically with confidence in one's ability to judge starts from an

accumulation of tangible observations to lead to knowledge.

Maceration enzyme impact on red wine's mouthfeel now explained

Despite many years of observations worldwide, the positive impact of LAFASE® HE Grand Cru on red wine mouthfeel was thought by many to be 'just a sales pitch' an addition to its more visible impact on color and tannin extraction. Well, anyone who needed more than tasting reports, this positive impact of enzyme treatment is now clearly established. In her PhD work Marie Agnès Ducasse (*Journal of Agricultural & Food Chemistry*" (2011.059. 6558-6567)) establishes the impact of enzymes on wine's polysaccharides composition, thus providing a scientific explanation to many years of observations of Lafase® HE Grand Cru impact on mouthfeel.

This work studies the impact on wine composition of several maceration / extraction pectolytic enzyme formulations. These results highlight differences between enzyme treated wines and a non-treated control, but also differences resulting from the use of different enzyme formulations. Each formulation generating polysaccharide residues of different structure as the specific product of the activities present in the cocktail.

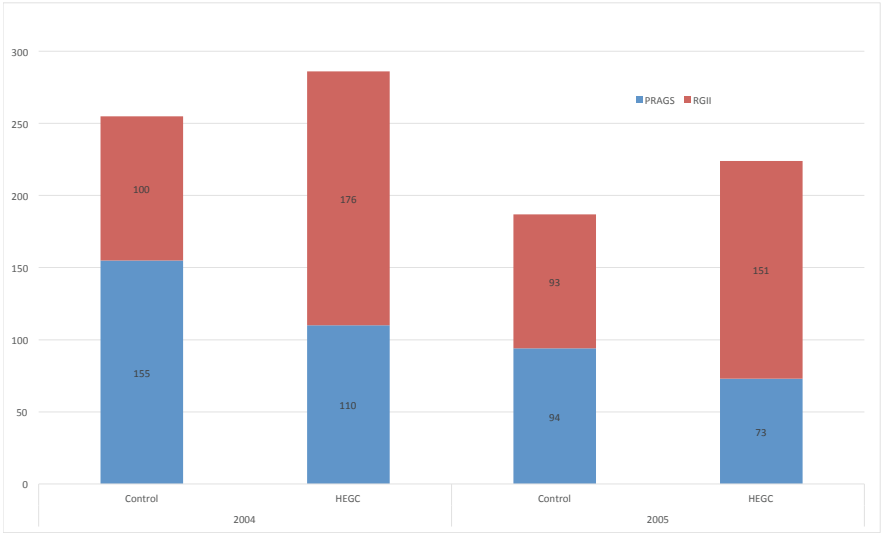

Figure 5 : Enzyme impact on red wines polysaccharide composition
Generally speaking, the use of pectinase results in a reduction of PRAGS and the absence of homogalacturonans residues (HG). These differences explain better clarity and higher filtration yields. A treatment with LAFASE® HE Grand Cru also allows, thanks to its RGIIase activity, releases into wines RGII a complex polysaccharide not degradable enzymatically.
The increased RGII concentration in wines treated with Lafase HE Grand Cru of RGII could explain their organoleptic superiority (Figure 4).

In addition to the visible technological differences consequential of a viscosity drop, these polysaccharides resulting from different pectin degradation modes potentially have a different impact on mouthfeel or wine colloidal stability.

The pectinases present in the commercial preparations display a wide ▸

Enzymes impact the mouthfeel of red wine.

variability of action modes and cleavage sites. As a result, structurally different polysaccharides are released in the wine. These variations in composition lead to visible technological differences such as viscosity, clarity but also differences in mouthfeel or wine colloidal stability.

How to compare and chose within a flourishing offer?

Whether used for clarification, maceration or filtration, an oenological enzyme preparation must have a diverse arsenal of enzymes to hydrolyze the various bonds constitutive of the pectin structure. However, although commercial enzyme preparations are formulated to contain necessary activities to achieve the promised result, we must keep in mind that many activities present in a preparation are not characterized. The expertise, experience and trust in the guarantees provided by your supplier must maintain a main criteria guiding your choice.

Two main types of enzymatic activity analysis

1. Industrial arbitrary global activity measurement units

These units are tools used by enzyme manufacturers for product standardization (FDU, AVJP). These units measure the synergistic performance of the various activities present in the enzyme cocktail. They provide an idea of the decrease in viscosity of a pectin sample solution. However, the measurements are performed on an apple pectin solution and are therefore not representative of grape pectin (by their degree of methylation) nor are they representative of enological conditions of pH and temperature.

YOU SHOULD KNOW

- Producer's arbitrary units are different and show no correlation (FDU, AVJP...)
- A high value of an arbitrary unit is not a guarantee of enological efficiency.

2. Units measuring the activity of a single enzyme

- The Katal (International SI unit) is the amount of enzyme that converts 1 mol of substrate per second. Enzyme activities are given in nano Katals (nKat).

- The specific activity:iIs the catalytic activity per protein mass unit (IU / mg solid enzyme). BGU for the beta glucosidase, PGNU for poly-galacturonase.

YOU SHOULD KNOW

- Two enzymes displaying a similar level of activity expressed in nKat or U/mg, may have very different behaviors under conditions of pH and temperature other than these of the analysis. They are referred to as isoenzymes = same enzyme but different pH range and temperature.
- Enological enzymes are cocktails in which each activity plays a role, acting in synergy with the other activities to reach the objective.
- Some activities referred to as "principal activities" are clearly identified and standardized; other known as "secondary" or "auxiliary" can be identified and measured but not standardized, and finally there is a pool of unidentified activities (active or not under oenological conditions).
- A high level of a single activity can be superfluous if the action of the enzyme is limited by the lack of "auxiliary" or partner activities limiting access to its substrate.

Trial is the way to compare

Like many aspects of winemaking, the use of enzymes is an art and a science, a combination of analytical results, observations, sensory analysis and experience. That is why after having pre-selected a formulation based on scientific knowledge, the development of a new enzyme systematically goes through a testing phase. At Laffort being a formulator of enzymes for the wine industry, we remain very close to our customers to build an experimental database that allows us to offer optimal formulations and recommendations for use in line with any winemaking technology.

Conclusion

Enzyme formulations available today for enology are subject to strict regulation and although several other activities are allowed (beta-glycosidase and beta-glucanases), this article focuses mainly on pectinases, their diversity and arsenal of auxiliary activities that accompanies them and ensures their effectiveness.

It should be clear from this article that there is no such a thing as 'a pectinase' on the winemaking product shelf but many different cocktails of different pectinases (with specific optimum pH and temperatures) accompanied by a myriad of other activities all leading to a unique degradation of the pectin molecule into various types of polysaccharides... resulting in significant processing and organoleptic differences.

In order to allow science to grow, enologists and winemakers play a crucial role in sharing their observations as well as technical and sensory results. The science of winemaking is in constant development and Laffort is the largest private investor in this area. With our strong commitment to research, and the financing of PhDs, we are now for example able to explain some bio chemistry underlying the variations in wines polysaccharide composition. This work is opening new perspectives to better explain the positive organoleptic impact of a unique cocktail of pectinases red winemaking: Lafase® HE Grand Cru. GW