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Wine enzymes – expand your knowledge

by Danie Malherbe & Céline Fauveau Schaff | 1 Dec, 2016 | Oenology research, Winetech Technical

One of the goals of this article is to try to provide winemaking professionals with a basis to make educated choices when selecting enzymes. We try to establish boundaries between fruitful simplification and damaging oversimplification.

Modern winemaking practices offer numerous products and processing aids to choose from for use at any stage of the winemaking process.

Would any winery use all these products Probably not. Choices are made taking into consideration harvest quality and the direction the wine will take, namely how quickly it will be released and consumed.

Enzymes are proteins which act as biological catalysts that facilitate and accelerate specific reactions. Each enzymatic activity is highly specific of one substrate (Figure 1). Pectinases in particular are common processing aids in winemaking.

Commercial winemaking enzyme preparations are obtained from microorganisms grown on a precise substrate under specific pH and temperature conditions in order to optimise the production of the desired enzymatic activities. Research in this field is active and continually expanding.

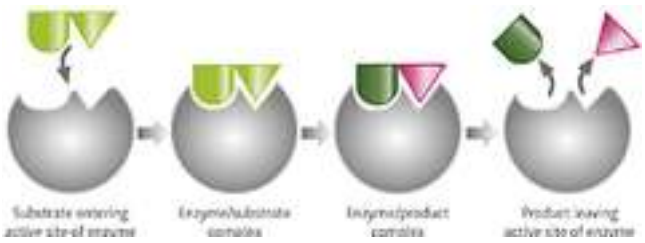

FIGURE 1. Enzyme preparations often suffer from overly simplified scientific communication, namely enzyme + substrate yields a product.

When asked to describe the specific purpose or use of a winemaking enzyme, the need to provide a simple, clearly understood explanation often leaves out important details. For example, reduced to a “pectinase”, most winemaking enzymes appear to be the same. This often results in winemakers making their choice based on price rather than product functionalities and production results.

Recurring questions about how to compare the various enzymes used in winemaking called for an update on the complex composition of commercially available preparations.

Enzyme formulations available for winemaking today are subject to strict regulation and although several activities are permitted (beta-glycosidase and beta-glucanases), this article focuses on pectinases, their diversity and the arsenal of additional activities that ensures their effectiveness.

So what are pectinases

To understand the answer to this question, you have to understand why pectinases are needed. Pectinases break down pectin which is a structural polysaccharide, constituted of a chain of various sugar molecules with a highly complex structure (Figure 2). Pectin is found in plants’ cell walls, including grape berries. It has various functions, the most important being to maintain the integrity of plant tissues.

A simplified representation of pectin consists of a main chain of galacturonic acid units, with ramifications or side chains of sugar molecules such as arabinose, galactose and rhamnose. Together with other polysaccharides such as glucans, cellulose and hemicellulose, grape pectin has a major influence on wine viscosity, clarity and filterability. In general, the longer the pectin chain, the more difficult the filterability of a wine.

From the above description of pectin and the fact that enzymes are highly specific of a single substrate (or hydrolyse only one specific type of linkage) it becomes obvious that in order to efficiently break down more than one substrate, more than one enzymatic activity is required. This multilevel breakdown calls for a cocktail of enzymes, where each enzyme has a precise role to achieve the destruction of a complex three-dimensional structure. Commercial winemaking pectinase preparations are in effect cocktails of various types of pectinases with

wide-ranging modes of action. As a result of the type of hydrolysis performed, structurally different polysaccharides are released. These impacts wine chemical and organoleptic characteristics.

Enzyme cocktails are produced by living organisms

Enzymes for the wine industry are produced by microscopic fungi. *Aspergillus* and *Trichoderma* fungi are cultivated under precise conditions that optimise the production of the desired activities. Both these microorganisms comprise a large number of strains. Each strain produces a unique blend of activities and may possess several dozen genes encoding for several dozen enzymes each targeting one substrate. In the same way a yeast producer cultures various strains of *Saccharomyces*, various strains of *Aspergillus* are used to produce the various enzyme preparations or natural cocktails. This is the first point of differentiation between commercially available pectinase preparations: Different fungal strains under different production conditions produce different combinations of enzymes.

Enzymes can be classified as follows:

- Endo-enzymes, randomly cutting the polymer chain and releasing midsize polymers (Figure 3). The endo-mode of action has a faster effect on viscosity reduction.
- Exo-enzymes targeting the ends of the chain and releasing mono and dimers (Figure 3).
- Desubstituting enzymes that “disconnect” the side chains, which is an important part of pectin breakdown. These enzymes facilitate the action of the endo- and exo-enzymes by creating a clear access to the main chain (Figures 2 and 3).

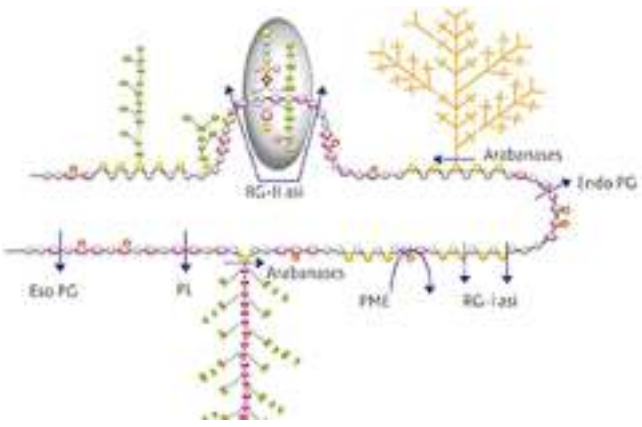

FIGURE 2. Simple schematic representation of the various components of a pectin chain. HG = homo galacturonan, RGI and II = rhamno glactutonans, AG I and II = arabino galactans and PRAG = polysaccharides rich in arabinose and galactose.

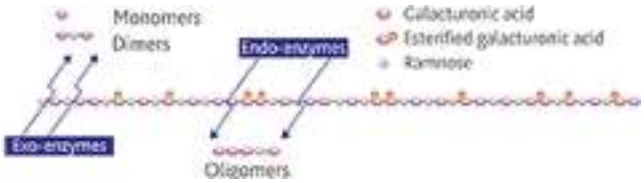

FIGURE 3. Endo- and exo-enzymes' mode of action.

A multi-activity cocktail

All enzymes have a principal or main activity, but they also contain a wide range of secondary activities. Pectinases are derived from *Aspergillus sp.*, often *A. 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, rhamno galacturonase and galactanase. All these enzymes exist as iso-enzymes (a different version of the same enzyme), identical in activity and mode of action, but performing at a different pH and temperature.

Main enzymatic activities in pectin degradation

Given the diversity of substrates that constitute the plant cell walls, the synergistic action of a wide variety of enzymes is essential to ensure satisfactory breakdown of the pectin molecule.

It is easy to demonstrate the complexity of the synergistic action of these enzyme cocktails. If we take the example of the polygalacturonase (PG) enzyme (Figure 4), it reduces the length of the pectin chain by breaking the link between two galactronic acid molecules. This reaction is only possible when galactronic acid molecules are unmethylated (do not contain a methyl group), in which case the help of another enzymatic activity, pectin methyl esterase (PME), is necessary. On

average 70% of grape pectins are methylated. PME removes the methyl groups from galactronic acid molecules, thus allowing PG to perform.

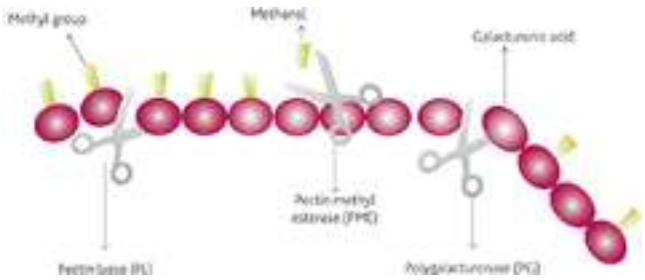

FIGURE 4. Main enzymatic activities responsible for pectin chain degradation

Partner secondary activities

Since we now understand the complexity of a molecule of pectin (Figure 2), it becomes clear that the abovementioned enzymes need to have access to linear portions of the pectin chain in order to work. To get access, additional partner activities are necessary. These activities, referred to as secondary activities, are produced by the microorganism during the production cycle of the main activity.

Many of these activities play a key role in support of the principal activity. These enzymes are arabanases, galactanases or rhamno galacturonases, and their function is to clean up the pectin molecule, thus clearing the access to the main pectin chain. This is done by releasing large polysaccharides (chains of various sugar molecules) that form the side chains that branch off the main pectin chain. It has even been observed that in the absence of some secondary activities, such as arabanases and/or rhamno galacturonases (RG-ases), polysaccharides degradation fails to occur, resulting in a pectin test remaining positive.

Unwanted secondary activities

Some activities have an undesirable effect, as in the case of cinnamoyl esterase, also known as cinnamyl esterase (CE), depsidase or tannase. This activity was identified in 1992. It catalyses the first reaction in the production of vinyl phenols. The second reaction is catalysed by wine yeast categorised as POF- (Phenolic Off Flavour) positive yeast strains. Vinyl-phenols are responsible for the loss of freshness, fruity character and, in worst-case scenarios, a medicinal smell in white wine.

These potential issues lead Laffort to develop and market purified enzymes or enzymatic cocktails in which undesirable activities are eliminated or kept at negligible levels.

Making sense of enzyme units

The "strength" of an enzyme is usually expressed as a measurement of its level of activity. Definitions and assay conditions vary from one supplier to the next and do not allow valid comparisons between products tested by different laboratories. Activities are expressed in two main unit types:

1. **Industrial global activity measurement units**

These units are tools used by enzyme manufacturers for product standardisation (FDU, AVJP, etc.). These units measure the synergistic performance of the various activities present in an 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 (the degree of methylation is different), nor are they representative of the oenological conditions of pH and temperature.

2. **Units measuring the activity of a single enzyme**

- The Katal (International SI unit) is the amount of enzyme that converts one mol of substrate per second. Enzyme activities are given in nano Katals (nKat).
- The specific activity is the catalytic activity per protein mass unit (IU/mg solid enzyme), for example BGU for the beta glucosidase and PGNU for polygalacturonase.

A practical example of the synergetic activity of an enzyme cocktail

In some cases winemakers are satisfied with colour and tannin extraction without the help of extraction enzymes. This position is not taking into consideration the added benefits of using macerating enzymes in the red winemaking process. These enzyme blends and the synergetic effect they have can easily result in:

- Increased wine yields with lower mechanical intervention.
- Better clarity.
- More stable colour.

- Better filterability or reduced need for filtration.
- Improved sensory effects.

Depending on your objective, various formulations of red winemaking enzymes can be used. Two enzymes formulations, Lafase® He Grand Cru and Lafase® Fruit, facilitate different wine styles with different targeted extraction. Each formulation generates polysaccharide residues of a different structure, resulting in a different impact on mouthfeel or wine colloidal stability (Figure 5).

In trials conducted with Lafase® He Grand Cru, a unique enzyme cocktail rich in rhamno galacturonase II activity, the wines were 60% richer in RG II (rhamno galacturonan II) after 20 months, compared with non-enzyme treated wines (Figure 6). In addition, reduced levels of PRAG (polysaccharides rich in arabinose and galactose) were measured, enabling enhanced wine clarification (Figure 6). An increase in colour intensity was also observed with more stable pigments and higher levels of condensed tannins resistant to sulphite bleaching.

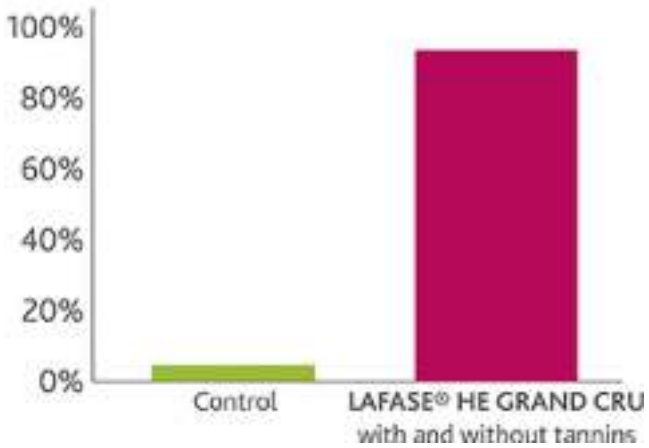

FIGURE 5. Organoleptic impact of an enzyme treatment in red wine – 2014 sensory preference by a jury of 19 professionals.

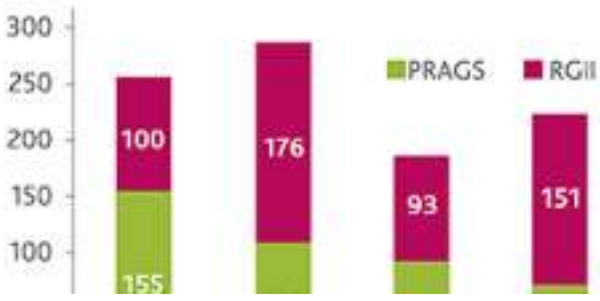

FIGURE 6. Enzyme impact on polysaccharide composition of red wine.

Conclusion

It should be clear from this article that there is no such thing as “one pectinase”. Instead, there are many different formulations of different pectinases, each with a specific and optimum pH and temperature. These are accompanied by a myriad of other activities, all leading to a unique degradation pattern of the pectin molecule. Each formulation generates polysaccharide residues with a different structure which has a different impact on mouthfeel or wine colloidal stability and results in significant processing and organoleptic differences.

Grape must and wine are complex environments for enzymes to work in, and any one or multiple factors can impact an enzyme’s efficiency. These include temperature, pH, enzyme dose, the amount of pectin that needs to be degraded, tannins and SO_2, to name but a few.

Make sure you select the correct enzyme and use the correct dose for the job. Check with your supplier how active its enzyme is and compare that with other enzymes you are considering. In so doing, you should be able to make a well-informed decision as to which enzyme best fits your winemaking process and the wine style you aim to produce. Laffort maintains a close relationship with its customers, allowing us to offer optimal formulations and up-to-date recommendations as to which enzyme works best for a specific winemaking process and condition.

Perform your own trials or taste the same wine made using various enzymes. Like many aspects of winemaking, the use of enzymes is a combination of analytical and sensory results, observations and experience.

Contact:

Danie Malherbe

danie.malherbe@laffort.com.

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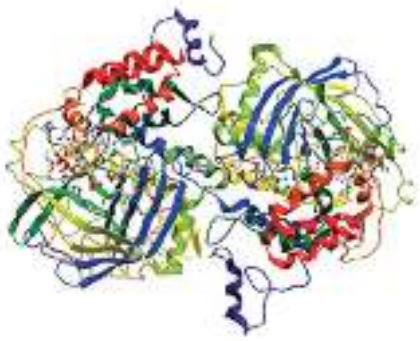

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