Enzymes: Revisiting the Principles and Future Developments

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My first introduction to enzymes was in 1984 and it looked like this. This, supposedly, was the easiest way of representing the idea of a fast acting chain shortening organism, and it was certainly a very graphic and humorous way of representing an enzyme action. The computer game 'Pacman' was very popular at the time; this probably was part of the reason why this image was used. It was also believed to be an image that some detergent companies were using to graphically visualize the enzyme's activity.

**Catalytic effect**
To provide a more scientific explanation of enzyme activity and specificity, the Rohm company suggests the following:

> "Enzymes are compact, quite large protein molecules. On their surface, they have a point called the active centre which is capable of binding a substrate molecule. This is where an enzyme/substrate complex is formed. According to Kashland's theory of adaptation, during this process the enzyme and the substrate molecule may introduce such reciprocal change in the structure that a chemical reaction such as the rupture of a substrate bond is encouraged."

Thus a smaller amount of energy is required to complete the reaction. This goes some way to explaining how enzymes can achieve fast reaction rates at relatively low temperatures compared to chemical reactions (Figure 1).

**pH and temperature activity**
Commercial enzymes not only contain the active protein (2%–5%) but also the sugars, inorganic salts and preservatives to stabilise and standardise the specified activity of the final products. Very few liquid pectinases contain preservatives and, because of their proteinaceous nature, temperature and pH have a large influence on their relative activity. As a general rule, a pectinase has an optimum activity at a temperature of 55°C (Figure 2). Every 10°C drop in temperature halves the activity of the enzyme. In theory at least, one could use 8 times less enzyme if the must and juice could be processed at 55°C! Provided the quality were not affected.

The ionization state of the amino acids, which dictate the primary and secondary structures of the enzyme and therefore control its overall activity, is affected by pH. It is possible that a pH change, from say pH 4 to pH 2, may decrease the enzyme affinity for the substrate and therefore slow the reaction rate (Figure 2). Conversely, if the pH changed from say pH 4 to pH 7, the enzyme might become unstable again, thereby lowering the reaction rate.
Temperature stability
The main point is that the higher the temperature is, the higher is the rate of enzyme activity loss (Figure 3). During vintage, in some areas, the ambient temperatures could reach 40°C or higher, and if the product was not stored in a cool room the storage temperature could get even higher.

As an example, if an enzyme was stored for, say, 1 hour at 50°C, the enzyme cost could increase by 30%.

Transportation of the enzyme from supplier to winery can also be a problem since transit temperatures and time can also affect activity.

Most suppliers produce their products with a higher than specified activity to take into account minor temperature fluctuations and the effects of storage.

Liquid enzymes in general will lose about 10% of their activity per year if unopened and stored at less than 10°C. Powdered products are more stable with the expected loss of activity of between 5-10%.

Interestingly enzymes can also be frozen without any loss of activity on thawing.

Pectinase activities (pectin-02) and special effects
Generally speaking, the ‘pectinase enzyme’ purchased for clarification is a mixture of enzymes, each with a different type of activity capable of hydrolysing high molecular weight pectin. The major activities are:
• Pectin methylesterase (PE)
• Pectin transeliminase (PTE)
• Polyglacturonases (PG) both endo- and exo-

The PE de-esterifies the pectin and converts it to a low esterified polyglacturonic acid and methanol. This action of PE has little effect on the viscosity of the juice unless calcium ions are present. Then the viscosity would increase due to calcium ions cross-linking with the pectic acid.

The polyglacturonic chain is depolymerised in two different ways: (a) by hydrolysis and (b) by β-transelimination. The most important transeliminase enzyme is PTE (pectin lyase).

The most important hydrolysing enzyme is PG. There are two forms of PG, endo-PG and exo-PG. There are two forms of endo-PG, a liquefying type and a macerating type. The liquefying type preferentially hydrolyses the soluble pectin while the macerating type breaks down protopectin and can disintegrate fruit and vegetable tissue. If there is a high concentration of the ‘macerating’ type of endo-PG then there is the possibility of increased tissue break down, resulting in more cloud and more problems in settling out this cloud in the juice.

Effects of enzymes in winemaking
Pectinases in white wine
The juice from white crushed grapes contains cloudy material, protopectin, pectin, carbohydrates, flavonoids etc. The protopectin is converted into soluble pectin by the enzyme protopectinase present in the juice, and this causes an increase in the viscosity of the juice. The soluble pectin thus produced is negatively charged, as are the colloidal particles. This system is stable and the cloud will take a long time to settle out. The insoluble pectin also stabilises the cloud. The pectin forms a negatively charged complex with the colloidal material by enveloping the positively charged particles.

Generally pectolytic enzymes present naturally in the fruit are not in sufficient concentration to break down this pectin, especially with high pectin-containing varieties such as Sylvaner and Muscat. So additional pectinase enzymes must be used to break down the pectin that surrounds the complex, changing the charge and allowing rapid settling of the suspended matter. This system is further complicated by the carbohydrate and protein which is also present in the juice. A negatively charged complex can form between carbohydrates, proteins, and pectin which when acted upon by pectinase enzymes is converted to a positively charged complex. Because there will be both types of complex’s in the juice at any one time, flocculation will occur as the positive and negative particles coalesce.

Pectinases in red wine
As the cloudy juice is fermented with the skins in red wine-making, the action of enzymes are not as critical. However, with the positive aspects of enzyme addition, increased free run, colour extraction and stability, there are also some negative aspects. Some of the side activities (minor) can cause destruction of pigments possibly due to the degradation of antho-
PECTINASES, HYDROLYSIN AND ARABANASES are produced with pectinases, hemicelluloses and arabanases, allowing more juice to be extracted. These enzymes increase the amount of juice extracted. Rather than breaking down cell membranes, they allow more juice to be removed.

The other enzymes mentioned, namely proteinase, β-glucanase, hemicellulase, and β-glucosidase, are generally present as side activities to the pectinase enzyme.

### Glycosidases

Gewürztraminer exhibit the enzyme catalyses the hydrolysis of the terpene/sugar bond. In most red and white grape varieties significant amounts of these aroma compounds are bonded to two sugars.

### Glucose oxidase/Catalase

This has been used in wine making but the cost effectiveness is not apparent as the enzyme cost is high and the pH drop marginal. It catalyses the oxidation of D-glucose to gluconic acid. A reported benefit (Ough 1975)², especially in rosé and white wines, is that they reduce the undesired enzymatic browning and consequent changes in flavour and taste.

### Cellulase

Pure cellulases especially from the fungus Trichoderma can increase the amount of juice extracted. Rather than breaking down the cellular tissue, they increase the permeability of the cell membranes, thus allowing more juice to be extracted. Cellulases produced from the fungus A spergillus are often produced with pectinases, hemicelluloses and arabanases and are not as effective as the cellulase from Trichoderma.

### Proteinase

Generally speaking the proteins in wine can be likened to the tightly wound rubber strip around the soft centre of a golf ball. The proteinase enzymes find it very difficult to penetrate the dense mass and effect hydrolysis. It is only by heating the juice or wine to say above 50°C that the protein can be hydrolyzed as the protein mass becomes more susceptible to enzyme attack. So, if producing reds via thermovinification, proteinase enzymes could have a beneficial effect in reducing benzonite usage.

### β-Glucanases

β-glucanase is a slimy polysaccharide primarily associated with Botrytis cinerea infection of grapes. It has a similar action to pectin in that it can prevent flocculation of colloids by enveloping them and thus reducing filtration rates.

### β-Glucosidases

In most red and white grape varieties significant amounts of aroma compounds are bonded to two sugars. These aroma compounds especially the monoterpenes are important for the flowery, fruity aromas that Riesling and Gewürztraminer exhibit. The enzyme catalyses the hydrolysis of the terpene/sugar bond.

### New developments

Genetic engineering is also playing its part in enzyme technology. Before genetic engineering, enzyme manufacturers had to chemically mutate existing strains of fungi to determine if the new mutant strain gave better results than the existing strain. This was a very time consuming business and somewhat hit and miss.

Genetic engineering allows the enzyme manufacturer to ‘tailor’ make an enzyme more efficiently. Once the genetic code has been identified, the genetic engineer can either replicate that piece of genetic code, say up to 5 times, and put it back into the fungal fungus which will now produce 5 times more of that enzyme than before. Or he can remove that piece of genetic code so that enzyme is not produced. In this way it is possible to produce various individual pectinase activities and recombine them according to individual needs. The enzymes produced in this way are the same as non-GMO enzymes. The combination product only contains the desired enzyme activity and therefore has few if any secondary activities. These type of products will probably be available within the next 3–5 years. Single activity enzymes are available now to a limited degree and for very specific purposes.

These types of products, when they become available will benefit industry in general, however detailed knowledge of all of the substrates present will be necessary before a proper recommendation as to the type of enzyme required can be given so that the outcome is predictable.

Genetically modified enzymes are a possibility in the future but whether they can be commercially viable and get public and government acceptance is unknown.

### References


### General references

