



USE OF ENZYMES IN JUICE AND WINE PRODUCTION

Learning Outcomes: Several issues need to be considered and understood when choosing a commercial enzyme preparation including the wine type, processing conditions, and desired effect. The factors impacting enzyme activity including of pH, temperature, and contact time are reviewed. Knowing the pH and temperature profile of the particular enzyme preparation determines the enzyme dosage and the cost to the winery. Decreases in pH, temperature, and/or contact time, along with increases in SO_2 may impact enzyme effectiveness.

Chapter Outline

Nature of Enzymes

Commercially Available Enzymes

Glucanases

Aroma/Flavor-Enhancing Enzymes: β -Glucosidases

Pectinases

Macerating Enzymes

Color Extraction and Stability

β -glucosidase

Collateral Activity: Anthocyanases and Cinnamyl Esterase

Lysozyme

Laccases and Tannases

Glucose Oxidases and Catalases

Developments in Enzyme Research and Use

Section 1.

Nature of Enzymes

Enzymes are biological protein catalysts that function to increase the rate of chemical reactions. This is generally on the order of millions of times faster than comparable uncatalyzed reactions. They do so by binding to one or more of the components of the reaction they catalyze, and lowering the activation energy needed to drive the reaction.

In addition to vastly accelerating the rate of reactions, enzymes are far more specific than other catalysts. Their exact function results from their unique sequences of amino acids, and the association or folding of the protein chain into the three-dimensional structure that defines the function of the specific enzyme. Enzymes used in the wine industry function by cleaving bonds between individual polysaccharide sugar units. These polysaccharides provide the structure for grape cells.

Enzymes and substrates are affected stereochemistry. This refers to molecular orientation. For example there are two forms of glucose, D and L one being the mirror image of the other. Almost all naturally occurring sugars are L. Two other stereochemical forms exist, alpha and beta used to describe the glycosidic bonds in cyclical sugars such as glucose. In general, enzymes that cleave beta bonds will not cleave alpha bonds, and visa versa.

Environmental conditions, both chemical and physical, including the following, have a significant impact on enzyme activity in juice and wine:

- enzyme storage conditions
- sulfur dioxide

- pH
- alcohol
- reaction temperature
- phenols
- fining agents
- contact time
- combination of physical and chemical properties of the juice/wine

Because of the nature of enzyme proteins they must be properly stored to retain activity. Even with optimum storage, products should be replaced each season. Examples of parameters affecting enzymatic response include high concentrations of sulfur dioxide, as well as other physical/chemical parameters of the matrix, such as temperature, pH, the presence (or increasing concentration) of alcohol and, in the case of red must, phenolic compounds. As such, it can be expected that wines with a high tannin concentration can result in increased enzyme binding and inactivation.

Enzymes are temperature dependant and operate within defined temperature ranges. Within that range, generally, for every 10°C increase in temperature, there is a doubling of enzyme activity. Heat or denaturants, such as alcohol, disrupt hydrogen bonding and the three-dimensional structure of the enzyme protein leading to loss of activity.

Changes in pH impact the ionization state of charged amino acids, which is important in substrate binding and/or catalytic conversion. Fining agents, such as bentonite, may also react with proteins, leading to denaturation and precipitation. Both bentonite and tannins may react with, and denature, processing enzymes. This is a primary reason why red wine processing frequently requires higher enzyme dosages than that needed for white juice/wine. Enological tannin addition must be used with an understanding of its ability to bind with added enzymes.

Fruit molds, yeast, and bacteria produce a variety of enzymes, including esterases, glucosidases and glucanases, as well as lipases. Vineyard molds, of several species, produce pectinases, as well as tyrosinases (phenoloxidases) and glucanases, which can have a significant impact on wine production, bringing about grape tissue deterioration, browning and, potentially, wine instability.

Commercially Available Enzymes

Applications of enzyme use in winemaking include:

- Extraction
- Clarification
- Stability
- Filtration
- Inhibition of gram positive bacteria.

Application range from enhanced juice yields and color extraction, to elevated varietal expression and selective control of lactic acid bacteria and physical stability. Most enzymes used in the wine industry are a group of enzymes. Enzyme products need to be used with a full understanding of the conditions listed above that impact their effectiveness.

For example, for optimum stability, enzyme preparations should be stored at approximately 40°F/4°C and not diluted (or rehydrated) until needed. Enzymes in red winemaking are generally added prior to fermentation. At this time red must, for example, contains a great quantity of free anthocyanin. Under vinification conditions, and in the presence of tannins, enzymatic activities do not last beyond the fermentation period. Recommended dosage rates are available from the manufacturer and may vary depending upon the activities of the product.

While the OIV Enological Codex authorizes the use of several enzyme formulations, the European Union Regulation, EC 1493/1999, authorizes only pectinase from *Aspergillus niger*, β -glucanase produced by *Trichoderma harzianum*, urease from *Lactobacillus fermentum*, and lysozyme.

Glucanases

Elevated levels of mold-derived polysaccharides from microbially-impacted fruit may be extracted into juice and subsequent wine. Of concern are the mid- to high-molecular weight β -glucans produced from *Botrytis cinerea* and other vineyard molds. When present in wine, β -glucan increases viscosity and, often, poses significant problems in clarification and filtration.

Structurally, β -glucans exists as a high molecular weight (800 kiloDalton) polysaccharide of β -1 \rightarrow 3-linked glucose units with β -1 \rightarrow 6-linked side chains (Dubourdieu and Ribéreau-Gayon, 1981). The presence of β -glucan in wine can be established by an alcohol denaturation test, during which a threadlike white precipitate unique to the glucan is formed (Zoecklein et al., 1995, 2005).

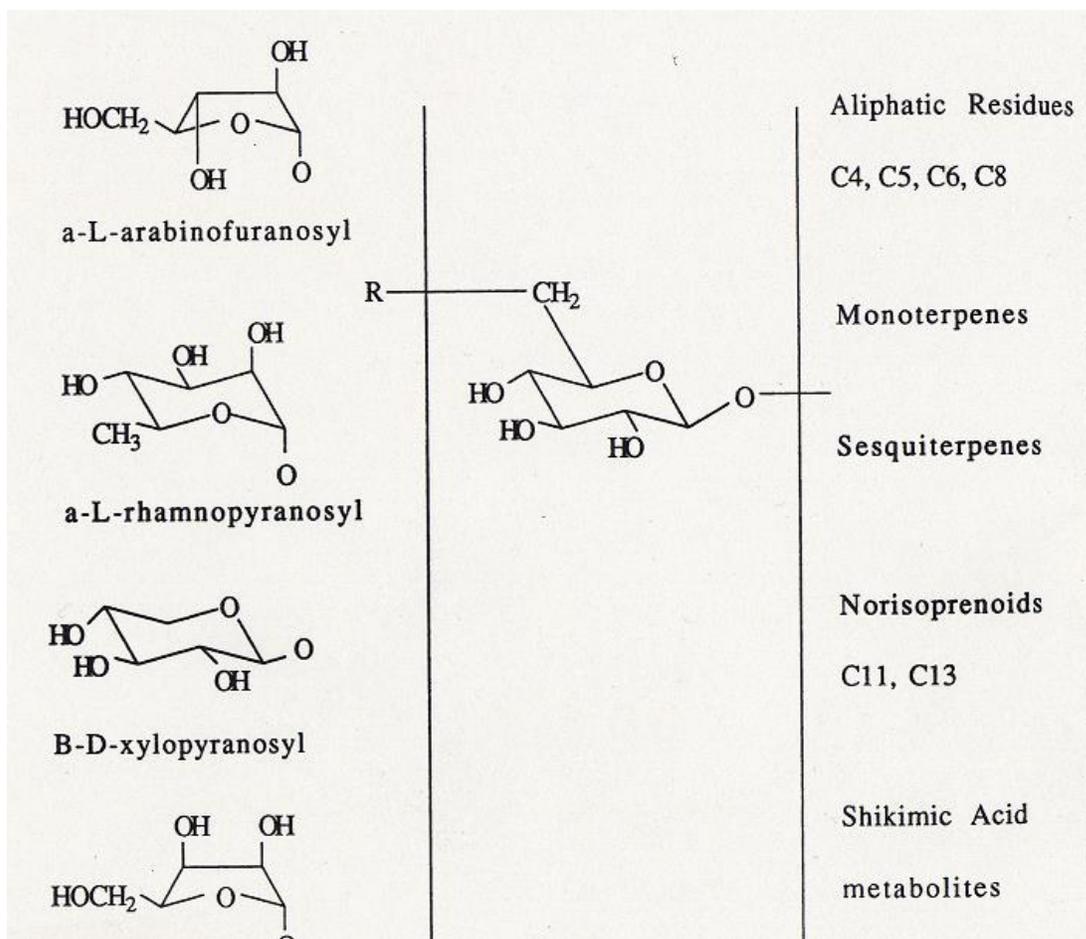
When elevated levels of glucan are detected or suspected, β -glucanases can be added to enhance juice clarification. Such formulations may also be used post-fermentation to improve filterability. When added to chilled juice, the dosage rate is generally increased to compensate for the inhibitory impact of low temperature and limited contact time. In Burgundy and many other regions around the world, wines are often aged on their lees in conjunction with the addition of glucanase enzyme, in an attempt to enhance the release of mannoproteins associated with the yeast cell capsule and enhance suppleness. In wine, the additional concern of inhibitory levels of alcohol applies.

Aroma/Flavor-Enhancing Enzymes:

β -Glucosidases

Aroma/flavor components, responsible for grape varietal character, exist in various proportions as free volatiles and their higher molecular weight (and flavor- or aroma-neutral) conjugated precursors (Fig.1). The aglycone or potentially volatile structure may vary. For example, glycoconjugate forms may contain terpenes in varieties such as Muscat, Riesling, Gewürztraminer, etc., such as geraniol, linalool, nerol, citronellol, or α -terpineol, linear or cyclic alcohol (hexanol, phenylethanol, or benzyl alcohol), C₁₃-norisoprenoid, phenolic acid, and/or volatile phenol.

Figure 1. Aroma and Flavor Precursors



Concentrations of precursors are typically higher than their free volatile forms, thereby indicating the potential for increased flavor/aroma upon release (Dimitriadis and Williams, 1984). Addition of β -glucosidases can enhance the aromatic profiles of some wines by hydrolyzing the β -1,4 bond, liberating the free volatile component from its conjugate.

Most research on glycosidically-bound aroma/flavor precursors has been conducted on monoterpenes which exist, primarily, as disaccharide glycosides, (namely α -L-arabinofuranosyl- β -D-glucopyranosides or α -L-rhamnofuranosyl- β -D-glucopyranosides, and a nonsugar portion).

Release of the free volatile from precursors may involve several enzymes, including β -glucosidase, α -arabinosidase, α -rhamnosidase, β -xylosidase, and β -apiosidase (Cummings, 1994; Plank and Zent, 1993; Williams, 1990). In this case, precursor hydrolysis is initiated by cleavage of the terminal sugar (α -1 \rightarrow 6 linkage) by either α -L-arabinosidase or α -L-rhamnosidase. The result is then subject to hydrolysis by β -D-glucosidase.

Most pectolytic enzymes have some β -glucosidase activity, which, as described in the above section, can catalyze hydrolysis. These have the greatest impact on high-terpene whites, such as Riesling, Gewürztraminer, and Muscats. However, for many white grape varieties pectolytic enzymes added pre-fermentation enhances wine aroma intensity. It should be noted that such formulations can also release glycosidically-bound phenols, as well. Such collateral activity may lead to increased levels of bitterness and/or astringency, as well as an enhanced aroma intensity.

The aroma-/flavor-enhancing portion of the enzyme formulation is inhibited by a high sugar concentration, as found in juice. It appears that some of the native enzyme survives fermentation and can react in the dry wine. As stated above,

there is also evidence that wines produced from juice or must treated with pectic enzymes that contain β -glucosidase can have more intense and complex aromas (Gunata et al., 1994).

The liberation of grape-derived volatiles can occur as a result of enzymes produced by yeasts. However, research relative to the effect of yeast strain on glycoside or conjugate hydrolysis during fermentation has shown limited differences among species/strains in terms of their ability to hydrolyze grape conjugates (Zoecklein et al.1999).

Thioaces

Winemakers tend to think of volatile sulfur compounds (VSCs) in negative terms because they are often associated with 'off' odors, such as hydrogen sulfide and mercaptans. However, several grape varieties, and their subsequent wines, are positively impacted by VSCs including Riesling, Gewürztraminer, Manseng, Chenin blanc and Sauvignon blanc wines.

Volatile sulfur compounds are, in part, produced from S-cysteine (a sulfur-containing amino acid) metabolism in the plant. A portion of each of these sulfur-containing compounds is in the free form, and a percentage bound to other components.

Yeast species and strains vary in their abilities to hydrolyze or release bound sulfur-containing compounds. In a study reported by de Barros Lopes (2004), commercial wine yeast strains differed by a factor of 20 in their release. Future research may provide exogenous thioaces that may aid flavor volatile release.



USE OF ENZYMES IN JUICE AND WINE PRODUCTION

Section 2.

Pectinases

Pectins comprise a group of plant structural polysaccharides (complex carbohydrates) made up primarily of galacturonic acid and its methyl esters. During most of the ripening cycle, pectins (and pectic substances) are present as insoluble elements contributing structure to the middle lamella between adjacent berry cell walls. With fruit maturation or disease, pectin is partially solubilized and fruit tissues soften.

Chemically, pectin exists as a linear polysaccharide of galacturonic acid linked via α -1,4-glycosidic bonds. Within the homogalacturonan backbone, galacturonic acid residues are, intermittently, replaced by rhamnose, forming a rhamnogalacturonan. Substitution serves as a starting point for branching of sugars: D-galactose, L-arabinose, and D-xylose. The frequency of substitution and branching serves to delineate the pectins. Depending upon the source, some of the carboxyl groups of galacturonic acid are esterified with methanol. The non-esterified galacturonic acid units can be either free acids (carboxyl groups) or salts of potassium or calcium called pectates.

Pectinase is a general name for a family of enzymes that attack and degrade the plant cell wall structural polysaccharide, pectin. Commercial pectinase

formulations include several linkage-specific components that (collectively) bring about more-complete degradation of grape tissue. Pectinases are added to juice and wines for various reasons, including the following:

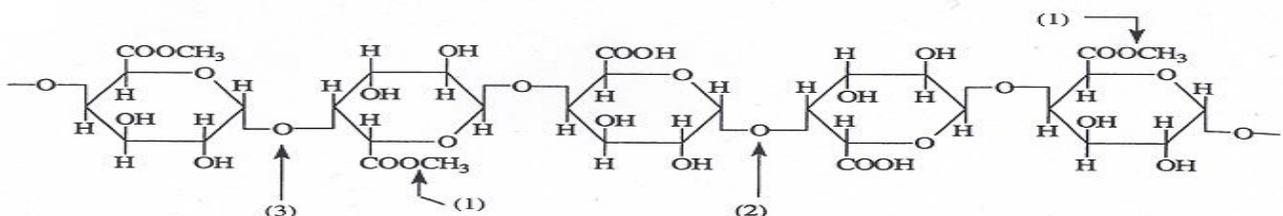
- increased yield
- aid in color extraction
- increased clarification rate
- increased filterability
- increased stability
- aroma enhancement

Most commercial pectinase preparations are derived from strains of *Aspergillus*, primarily *A. niger* (Canal-Llauberes, 1993). This species is accepted as GRAS (Generally Recognized As Safe) for the production of enzyme preparations by the United States FDA and TTB as well as the European OIV.

Commercial pectinases typically contain varying ratios of pectin methylesterase (PME), pectin lyase or transeliminase (PL), and polygalacturonase (PG), as well as associated collateral activity including β -glycosidase, cinnamyl esterase (CE), and anthocyanase.

Each of the individual pectolytic enzymes attacks the galacturonic acid backbone of the pectin molecule (Figure 2) differently. Methyl galacturonic acid is de-esterified by PME, producing a low methoxyl pectin, pectic acid, and methanol. Polygalacturonase hydrolyzes the α -1,4-glycosidic linkages within the galacturonan chain of low methoxy-pectin and -pectate, whereas pectin lyase brings about random hydrolysis within the chains of highly-esterified pectin.

Figure 2. Pectin, Showing Cleavage Points of Various Pectinases



Pectins can have a large and negative impact on juice clarification in high pectin grapes, rot-compromised fruit, and varieties such as *Vitis labrusca* and most French-American hybrids. During fermentation, both the heat produced and alcohol levels can help precipitate pectins. Most non-grape wines have a high pectin content, because of their native concentration and the fact that they are usually relatively low in alcohol.

Pectins and Filterability. Pectins and other polysaccharides can be associated with poor wine filterability and so-called white wine protein instability. Pectins, as deformable colloids, can easily plug filters. Additionally, what is commonly referred to as white wine protein instability is frequently a combination of proteins, tannins and polysaccharides. Several easy tests for the presence of pectin are available. See Zoecklein et al. (2005). Additionally, it should be noted that some enzymes produced from *A. Niger* should not be used prior to pressing to avoid a high level of non-soluble solids (see suppliers recommendations).

Macerating Enzymes

Formulations of macerating enzymes typically contain pectinase, cellulase, hemicellulase, and other carbohydrase activities. Compared with pectinases, utilization of macerating enzymes generally improves juice yields by degrading a broader array of structural polysaccharides that interfere with juice extraction, color, clarification, and filtration.

Cellulases and hemicellulases degrade cell wall polysaccharides, breaking down the entire structure and causing solubilization of the middle lamella. Due to this more complete degradation of cell wall and middle lamella components, macerating enzymes may improve press yields (especially with hard-to-press varieties) and/or lees settling and clarification rates, when compared with pectinases alone (Sims et al., 1988). Other benefits may include improved color

extraction in some red grape varieties, increased aroma and flavor, and improved body and structure impacted by a 10-30% increase in tannins and proanthocyanidins (Plank and Zent, 1993; Zent and Mama, 1992).

Color Extraction and Stability

Both color extraction and color stability are of importance in red wine production. Increases in color are due, in part, to improved extraction of the colored pigments brought about by the breakdown of pectin. However, not all enzyme formulations yield identical results. Suitability for color extraction depends on the ability to “loosen” the structure of the middle lamella. Protopectin is part of a three-dimensional hemicellulose network rich in galacturonic acid residues. Protopectinase activity results in the release of highly methylated esters (Felix and Villettaz, 1983). Besides increased color extraction, degradation of the protopectin allows juice to flow more freely from crushed grapes, resulting in increased juice yield.

Spectral color in wine is a function of these three elements:

- anthocyanin concentration
- polymeric pigments
- concentration of cofactors, or certain non-colored compounds, which bind with anthocyanins

Maceration enzymes are used in winemaking to increase the extraction of anthocyanin pigments and to aid pigment stability. Some phenols, including tannins, have the ability to polymerize, or associate, with themselves and other compounds, including anthocyanin pigments. As polymerization occurs, the molecule becomes larger. The number of subunits bound together is referred to as the DP number, or degree of polymerization.

In grapes and wines, anthocyanin pigments can be either free monomers, that is, unbound, or associated with other phenols to form polymers. Anthocyanin-tannin

polymerization occurs both in the fruit during maturation, and during processing and aging. Polymerization continues until an anthocyanin molecule binds the terminal end of the tannin chain, thus stopping the polymerization.

As such, the ratio of anthocyanins to tannins is important in impacting the extent of polymerization. This is highly crucial, because the polymerization affects two important red wine attributes:

- color stability
- astringency

Large tannin polymers provide a relatively large number of binding sites to interact with proteins, including salivary proteins. Red wines with an abundance of large polymers tend to lack softness, may lack color stability, and often possess a dry mouth sensation.

Smaller polymers, on the other hand, have fewer protein binding sites. As such, they produce less astringency, and provide a greater degree of soft tannins and more palate depth. The more anthocyanins, the shorter the resulting polymers and the finer the tannins. Smaller polymers lead to smaller colloids which have a softer mouthfeel. Additionally, these smaller pigment polymers provide a greater reductive strength.

Maceration enzymes are frequently used in red wine cold soak to increase the anthocyanin content relative to the tannin concentration. Anthocyanins are water soluble and, therefore, are extracted at a greater rate.

β -Glucosidase

Most pectolytic enzymes contain some level of β -glucosidase activity, as described above, which can catalyze conjugate hydrolysis, increasing aroma intensity. While these have the greatest impact on high-terpene whites, such

formulations can also release glycosidically-bound phenols, potentially leading to increased levels of bitterness and/or astringency, as well.



USE OF ENZYMES IN JUICE AND WINE PRODUCTION

Section 3.

Collateral Activity: Anthocyanases and Cinnamyl Esterase

Anthocyanases

Anthocyanases represent a group of fungal-derived enzymes that may also be present as contaminants in enzyme formulations. Anthocyanins are covalently linked with glucose. Hydrolysis of the glucose from the pigment molecule leads to destabilization and, subsequently, diminished color.

Cinnamyl Esterase

Collateral activity may be present in some pectinase formulations produced from *Aspergillus niger*, including cinnamyl esterase (CE). Cinnamyl esterase, in combination with yeast-derived cinnamyl decarboxylase activity, may be responsible for non-*Brettanomyces* phenolic off-flavors (“medicinal” or “phenolic”).

Formation of volatile phenols during fermentation is a two-step process: (1) cleavage of the ester linkage between tartaric acid and hydroxycinnamic acid by cinnamyl esterase, and (2) decarboxylation of the hydroxycinnamic acids by POF (phenyl off flavors)-positive strains of *Saccharomyces cerevisiae* and native strains, resulting in the formation of volatile vinylphenols. When present in low

concentrations, vinylphenols can have a very pleasant clove-like smell but, in higher concentrations, the effect on wine aroma is negative.

Substrates for the cinnamyl esterase hydrolysis include the tartrate esters of the hydroxycinnamic acids: coumaroyltartaric and feruloyltartaric. Quantitatively, these non-flavonoid phenolic esters comprise the major group of phenols in white grape musts. They are also present in reds where they represent a much smaller percentage of the phenolic pool. In that vinyl-phenols react with tannins in formation of a non-volatile product, they represent a greater concern in white wine. In reds, phenolic off-flavors are usually the result of ethyl-phenols and, thus, of *Brettanomyces* species growth.

The cinnamyl esterase secondary activity in red winemaking preparations can be beneficial. In the past, it was thought that the release by cinnamyl esterase of hydroxycinnamic acid would always lead to an increased production of ethylphenols, in the case of contamination with *Brettanomyces*. It is now well established that the vast majority of yeast strains marketed for winemaking transform hydroxycinnamic acids, the substrate of *Brettanomyces*, into vinylphenols. These vinylphenols react rapidly with the anthocyanin to help stabilize color.

In white wines, vinyl-phenols can cause undesirable odors. The occurrence can be avoided by the use of POF(-) yeast strains and enzymes with a naturally low cinnamyl esterase activity.

Lysozyme

Lysozyme is a naturally-occurring bacteriolytic enzyme that has application in controlling Gram-positive lactic acid bacteria and, thus, in the prevention of malolactic fermentation. Commercially, lysozyme is produced from egg white, although it is found widely in nature, including in tears and saliva.

Lysozyme, is a low molecular-weight protein which catalyzes hydrolysis of β -1,4 linkages between *N*-acetylmuramic acid and *N*-acetyl-D-glucosamine residues in the peptidoglycan fabric of the Gram-positive bacterial cell wall. Thus, lysozyme is active against *Lactobacillus*, *Pediococcus*, and *Oenococcus* spp., but not against acetic acid bacteria (*Acetobacter* and *Gluconobacter*) or yeasts, including *Saccharomyces* and *Brettanomyces* spp. (Mckenzie and Whilte, 1991).

Over the last decade, there has been interest in lysozyme as a supplement to sulfur dioxide for bacterial inhibition. Whereas the effectiveness of sulfur dioxide as a microbial inhibitor is pH-dependent, lysozyme activity is independent of pH. Further, the ability of the enzyme to inhibit malolactic fermentation is not influenced by sugar or alcohol concentration, and it may be effective against large (10^6 colony forming units/mL) populations (Bartowsky et al., 2004).

Lysozyme is generally added to grape juice that has a significant potential for developing high levels of *Lactobacillus* populations and, thus, an increased risk of volatile acid formation and stuck fermentations. It can also be used to delay the onset of MLF.

Commercial lysozyme is a finely granulated, microcrystalline powder prepared from egg whites. It is easily re-suspended in water, and is usually added to juice or wines at a rate of 100-500 mg/L (10-50 g/hL). It may be added to juices, wines, and sparkling wine cuvées. Such additions have not demonstrated a detrimental impact on aroma or sparkling wine *mousseux* characteristics.

Lysozyme is approved by the OIV, the European Commission, and the TTB for use in winemaking. There are several stability issues associated with use of lysozyme:

- As a protein, lysozyme is reactive with phenolics in red wines where it can, potentially, create instability. This results in a perceptible

reduction in wine tannins and color. Bartowsky et al. (2004) noted a 17% reduction in 520 nm absorbance (red wine anthocyanin absorption maximum) for Cabernet Sauvignon and Shiraz.

- In white wines, lysozyme may contribute to protein instability which is not easily corrected by bentonite.
- Bentonite will bind with a portion of the lysozyme added, and may reduce its concentration below that which is needed for bacterial control.
- Lysozyme is differentially effective against various lactic acid genera and species, and the activity may be transitory. Bartowsky et al. (2004) demonstrated that lysozyme retained a 75-80% activity in Riesling wine after six months. However, they also observed no detectable activity in Cabernet Sauvignon and Shiraz wines two days post-addition.

Laccases and Tannases

Laccases are similar to tyrosinase (polyphenoloxidase), except that they oxidize a wider range of phenolic compounds. Like other fungi, *Botrytis cinerea* produces laccase, pectolytic enzymes, and esterases. Laccase catalyzes phenolic oxidation, with the resultant polymerization responsible, in part, for browning.

Perhaps of greater concern is the oxidation of aroma/flavor compounds. Laccase is resistant to sulfur dioxide, cannot easily be removed with bentonite, and is active in the presence of alcohol, including in bottled wines (see module on Rots). In theory, tannases may find application in decreasing tannin levels in wine. However, the color stability of treated red wines is in question. Use of these enzymes is in the research stage and there is, as yet, no viable commercial use.

Glucose Oxidases and Catalases

Villettaz (1986) proposed using glucose oxidase and catalase in juice to convert glucose into gluconic acid, which cannot be metabolized by the yeast, resulting in

a wine of reduced alcohol and increased acidity. However, glucose oxidase reactions may lead to oxidation of other components, including flavor components.

Therefore, sufficient amounts of catalase are necessary to convert the hydrogen peroxide that is produced into water. This may be an alternative way of producing low alcohol wine, without the use of expensive equipment, such as reverse osmosis filters.

Developments in Enzyme Research and Use

During the past four decades, there have been dramatic changes in enzyme preparations used in the wine industry. These include both increased specificity and purity, as well as newly engineered products that allow the winemaker to use enzyme products for a specific processing need.

Some enzyme preparations are produced from genetically modified organisms (GMOs) which has created some philosophical concern with regard to their use. Enzymes, like other winemaking tools may detract from the concept of terrior winemaking for some while seen as desirable adjuncts to winemaking by others. Enzymes can increase yield. Enhanced processing efficiency with the use of enzymes may allow for a reduction in wine oxidation and cost of production.

There are a number of areas being investigated for improved wine quality and/or processing efficiency using enzymes, including the following:

- lyases for the release of volatile thiols for wines such as Sauvignon blanc
- esterases and alcohol acetyl transferase

- glycosidases, glucanases, and arabinofuranosidases for enhanced liberation of grape terpenoids
- bacteriolytic enzymes for reduced formation of biogenic amines, in addition to lysozyme
- stilbene synthesis and β -glucosidases for the increased production of resveratrol
- glucanases, pectinases, xylanases, and arabinofuranosidases for controlled yeast cell sedimentation and flocculation
- proteases for improved protein stability and clarification

Practical Summary of Winemaking Issues

- Enzyme activity is a function of several variables that may impact effectiveness, including pH, temperature, contact time, and juice clarity.
- Features such as sulfur dioxide level, use of bentonite, and tannin levels can also negatively impact enzyme activity.
- Enzymes are proteins and therefore heat labile and must be stored properly to maximize shelf life.
- Juice or wine temperature can dramatically impact enzyme effectiveness.
- Generally, for every 10°C increase in temperature, there is a doubling of enzyme activity.
- Several easy tests for the presence of pectin are available which should be conducted prior to filtration of wines suspected to be high in pectin.
- Always consult suppliers recommendations.



Study Questions

- 1. List important winemaking considerations when selecting a pectinolytic enzyme for cold settling of an aromatic white juice.**
- 2. Explain how the so-called flavor enhancing enzymes work. What are the practical considerations in selecting such enzymes?**
- 3. Explain the difference that are available among commercial pectinolytic enzymes.**
- 4. Many wines produced from rot-compromised fruit fail filterability tests. Why ? What methods should be used to determine the nature of the problem and correct**